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MASTER THESIS

"Method development for the determination of priority pollutants and emerging contaminants in river water samples by GC-APCI-QTOFMS"

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MASTER THESIS

Method development for the determination of priority pollutants and emerging contaminants in river water samples by GC-APCI-QTOFMS

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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Ανάπτυξη μεθόδου για τον προσδιορισμό ρύπων προτεραιότητας και αναδυόμενων ρύπων σε δείγματα νερού ποταμού με

GC-APCI-QTOFMS

ΕΛΕΝΗ ΠΑΝΑΓΟΠΟΥΛΟΥ

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ABSTRACT

The site of interest of this study is Asopos river which is located in Sterea Ellada, north of Athens, and passes through areas where 20% of total Greece industrial production takes place. The extensive installation of industries in the area near the river, and the uncontrolled disposal of industrial and agricultural wastes into the river, make the water quality of Asopos questionable.

Over the last decade, the development of high resolving power mass analyzers (HRMS) has contributed significantly to the expansion of environmental studies' interest from the restricted determination of priority pollutants to the wide-scope screening of emerging contaminants (ECs). In light of their potential risk to human health and the aquatic environment, action is urgently required. In the ECs analysis field, there is a clear trend toward LC-HRMS as the majority of ECs are more polar and less volatile. However, many high usage ECs, as well as priority pollutants, are volatile and thermostable, therefore GC-HRMS methods should be developed. 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.

The aim of this study was the development of a novel methodology for the determination of GC-amenable priority pollutants and emerging contaminants in river water samples from Asopos. Since the chemical domain of the analysis includes compounds with a wide variety of physicochemical properties, a generic sample preparation protocol should be followed. Several sample preparation methods were tested, including different extraction techniques and initial sample volumes. Solid phase extraction with C18 cartridges was selected for the pre-concentration of the analytes and the clean-up of the river samples.

The characteristics of the developed GC-APCI method, were evaluated with spiked samples of a representative group of compounds, for which reference standards were available. The validation dataset included chemicals from different classes of GC-amenable compounds, like polycyclic aromatic

hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and plant protection products (PPPs).

River water samples were collected from 2 sampling points, one close to industrial and agricultural activities and one close to the estuaries of Asopos. Two portable autosamplers were used in order to collect 24-hour samples from the river for 7 consecutive days during November 2018. Also, Weather conditions and the color of the river were recorded for each sampling day.

The resulting extracts from the sample's pretreatment were analyzed using gas chromatography coupled with an Atmospheric Pressure Chemical Ionization (APCI) source,with a hybrid quadrupole time of flight (GC-QTOF-MS).

Concerning the results of the current study, the number of the detected compounds and the total concentration of all analytes were significantly higher in the samples withdrawn close to the industrial and agricultural activities. Moreover the weather contitions maybe can affect the concentration and the transport of the analytes.The overall results will indicate the contamination degree of Asopos river basin due to the occurrence of GC-amenable priority pollutants and emerging contaminants.

SUBJECT AREA: Environmental Analytical Chemistry

KEYWORDS: Method Development, Priority pollutants, Emerging contaminants, River water, GC-APCI-QTOF

ΠΕΡΙΛΗΨΗ

Ο τόπος ενδιαφέροντος αυτής της μελέτης είναι ο ποταμός Ασωπός, ο οποίος βρίσκεται στη Στερεά Ελλάδα, βόρεια της Αθήνας, και περνά μέσα από περιοχές στις οποίες λαμβάνει χώρα το 20% της συνολικής βιομηχανικής παραγωγής της Ελλάδας. Η εκτεταμένη εγκατάσταση βιομηχανιών στην περιοχή κοντά στον ποταμό και η ανεξέλεγκτη διάθεση βιομηχανικών και γεωργικών αποβλήτων στο ποτάμι καθιστούν αμφίβολη την ποιότητα του νερού του Ασωπού.

Κατά την τελευταία δεκαετία, η ανάπτυξη αναλυτών μάζας υψηλής διακριτικής ικανότητας (HRMS) συνέβαλε σημαντικά στην επέκταση του ενδιαφέροντος των περιβαλλοντικών μελετών από τον περιορισμένο προσδιορισμό των ρύπων προτεραιότητας στον εκτεταμένο έλεγχο των αναδυόμενων ρύπων (ECs). Με την ύπαρξη της πιθανότητας να προκαλέσουν κίνδυνο στην ανθρώπινη υγεία και το υδάτινο περιβάλλον, απαιτείται επειγόντως δράση. Στην ανάλυση των αναδυόμενων ρύπων υπάρχει μια σαφής τάση προς την χρήση υγροχρωματογραφίας καθώ οι πλειονότητα αυτών των ουσιών είναι πιο πολικές και λιγότερο θερμικά ασταθείς. Ωστόσο πολλοί αναδυόμενοι ρύποι είναι πτητικοί και θερμοσταθεροί και επομένως πρέπει να αναπτυχθούν μέθοδοι αεριοχρωματογραφίας. Μέχρι σήμερα, οι λιγότερο πολικές και πιο πτητικές ενώσεις της κατηγορίας των αναδυόμενων ρύπων δεν έχουν μελετηθεί επαρκώς, επισημαίνοντας την ανάγκη δημιουργίας αποδοτικών ροών εργασίας αεριοχρωματογραφίας συζευγμένης με αναλυτές μάζας υψηλής διακριτικής ικανότητας.

Σκοπός αυτής της μελέτης ήταν η ανάπτυξη μίας νέας μεθοδολογίας για τον προσδιορισμό των ρύπων προτεραιότητας και των αναδυόμενων ρύπων σε δείγματα ποταμού από τον Ασωπό. Εφόσον το πεδίο της ανάλυσης περιλαμβάνει ενώσεις με διαφορετικές φυσικοχημικές ιδιότητες, μια γενικευμένη προκατεργασία δείγματος θα πρέπει να ακολουθείται. Πραγματοποιήθηκαν δοκιμές με διαφορετικές μεθόδους προκτεργασίας του δείγματος, συμπεριλαμβανομένων διαφορετικών τεχνικών εκχύλισης και διαφορετικών όγκων δείγματος. Για την προσυγκέντρωση των αναλυτών και

τον καθαρισμό των δειγμάτων νερου ποταμού, χρησιμοποιήθηκε εκχύλιση στερεάς φάσης με στηλάκια C18.

Τα χαρακτηριστικά της ανεπτυγμένης μεθόδου αξιολογήθηκαν με την χρήση εμβολιασμένων δειγμάτων με αντιπροσωπευτικούς αναλύτες από διάφορες ομάδες ενώσεων, για τα οποία υπήρχαν διαθέσιμα πρότυπα αναφοράς. Η επικύρωση της μεθόδου πραγματοποιήθηκε με πτητικούς αναλύτες από διάφορες κατηγορίες, όπως πολυκυκλικοί αρωματικοί υδρογονάνθρακες (PAHs), οργανοχλωριωμένα φυτοφάρμακα (OCPs), πολυχλωριωμένα διφαινύλια (PCBs), και φυτοφάρμακα (PPPs).

Τα δείγματα νερού ποταμού συλλέχθηκαν από 2 σημεία δειγματοληψίας, το ένα βρισκόταν κοντά σε βιομηχανικές και γεωργικές δραστηριότητες και το άλλο κοντά στις εκβολές του ποταμού Ασωπού. Δύο φορητοί αυτόματοι δειγματολήπτες χρησιμοποιήθηκαν για τη συλλογή 24 ωρών δειγμάτων από τον ποταμό για 7 συνεχόμενες ημέρες κατά τη διάρκεια του Νοεμβρίου 2018. Επιπλέον, οι καιρικές συνθήκες και το χρώμα του ποταμού καταγράφονταν για κάθε μέρα δειγματοληψίας. Τα εκχυλίσματα των δειγμάτων αναλύθηκαν χρησιμοποιώντας αεριοχρωματογραφία συζευγμένη με υβριδικό αναλυτή φασματομετρίας μάζας τύπου τετραπόλου χρόνου πτήσης (GC-QTOF-MS) με μια πηγή χημικού ιοντισμού με ατμοσφαιρική πίεση (APCI).

Σχετικά με τα αποτελέσματα της τρέχουσας μελέτης, ο συνολικός αριθμός των ενώσεων που ανιχνεύθηκαν καθώς και η συνολική συγκέντρωση των περισσότερων ρύπων ήταν σημαντικά υψηλότερες στα δείγματα που συλλέχθηκαν από το σημείο δειγματοληψίας που βρισκόταν κοντά στις βιομηχανικές και γεωργικές δραστηριότητες. Επιπλέον, οι καιρικές συνθέσεις μπορεί να επηρεάσουν τη συγκέντρωση και τη μεταφορά των αναλυτών.

Τα συνολικά αποτελέσματα θα παρουσιάσουν τον βαθμό ρύπανσης της λεκάνης απορροής του ποταμού Ασωπού λόγω της εμφάνισης πύπων προτεραιότητας και αναδυόμενων ρύπων στο περιβάλλον.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Περιβαλλοντική Αναλυτική Χημεία

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: Ανάπτυξη μεθόδου, Ρύποι προτεραιότητας, Αναδυόμενοι ρύποι,Νερό ποταμού, GC-APCI-QTOF

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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.

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Theoretical Part

CHAPTER 1
Introduction

1.1 Priority Pollutants (PPs)

Priority pollutants (PPs) are organic compounds that are well known for their activity as carcinogenic, mutagenic or highly toxic to living organisms. The U.S Environmental Protection Agency (EPA) published a list of these toxic chemicals for which the agency intends to promulgate discharge control standards [1]. Priority Pollutants refer to a list of 126 specific pollutants that includes heavy metals, organotins, chlorobenzenes, volatile organic compounds (VOCs), alkylphenols, polychlorobiphenyls (PCBs), pesticides, polycyclic aromatic hydrocarbons (PAHs), and polybromodiphenylethers (PBDs), phalates [2]. Priority hazardous substances are those priority substances which are toxic, persistent and liable to bio-accumulate (PBTs) and other substances or groups of substances of high concern. PBTs do not break down but persist in the environment and collect in animal and plant tissues, thus posing long-term risks to human health and ecosystems. As PBTs move up the food chain their concentration increase and the risk to humans and ecosystems increases accordingly. Controlling of emissions of very small quantities of these substances is therefore required [3].

1.1.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are the largest group of carcinogenic substances and they are made up of several hundred chemical substances (**Table 1**). PAH is formed when coal or hydrocarbons (e.g. various oils), are heated without sufficient oxygen so as to provide complete combustion to carbon dioxide. They are organic compounds which are mostly colorless, white, or light yellow solids [4]. Chemically the PAHs are comprised of two or more benzene rings bonded in linear, cluster, or angular arrangements. Polycyclic aromatic hydrocarbons have two or more single or fused aromatic rings with a pair of aromatic rings are often known as ‘‘small” PAHs, and those containing more than six aromatic rings are called ‘‘large” PAHs (**Figure 1**) [4].

Table 1: Sixteen polycyclic aromatic hydrocarbons [5].

Compound	MW (g/mol)	Molecular formula
Naphthalene	128.174	$C_{10}H_8$
Acenaphtylene	152.196	$C_{12}H8$
Acenaphthene	154.212	$C_{12}H_{10}$
Fluorene	166.223	$C_{13}H_{10}$
Phenantrene	178.234	$C_{14}H_{10}$
Anthracene	178.234	$C_{14}H_{10}$
Fluoranthene	202.256	$C_{16}H_{10}$
Pyrene	202.256	$C_{16}H_{10}$
Benzo[a]anthracene	228.294	$C_{18}H_{12}$
Crysene	228.294	$C_{18}H_{12}$
Benzo[b]fluoranthene	252.316	$C_{20}H_{12}$
Benzo[k]fluoranthene	252.316	$C_{20}H_{12}$
Benzo[a]pyrene	252.316	$C_{20}H_{12}$
Indeno[1,2,3-cd]pyrene	276.338	$C_{22}H_{12}$
Dibenzo[a, h]anthracene	278.354	$C_{22}H_{12}$
Benzo[ghi]perylene	276.338	$C_{22}H_{12}$

PAHs are formed from anthropogenic (e.g. emissions in the environment as a result of vehicle exhausts, asphalt pavements, unvented radiant and convective kerosene space heaters, heating appliances) and natural sources. They are known for their carcinogenetic and mutagenic properties and for being responsible of background level contamination in environmental matrices [5]. The biological effect of PAHs is linked to the plane structure of the molecule and its ability to affect DNA in the nucleus of cell. Most organisms can convert PAHs and often breakdown products can be more hazardous than the original substance. PAHs are fat-soluble, in some cases bioaccumulative and generally stable, which means that the compounds are difficult to break down and may be dispersed a long way in the environment. In aquatic environments, PAHs are connected with particles and later they transferred to sediments, where they can become very persistent. Therefore, aquatic ecosystems close to emission sources are in most risk. Many PAH compounds accumulate in organisms from the aquatic environment and they end up in food chain [4].

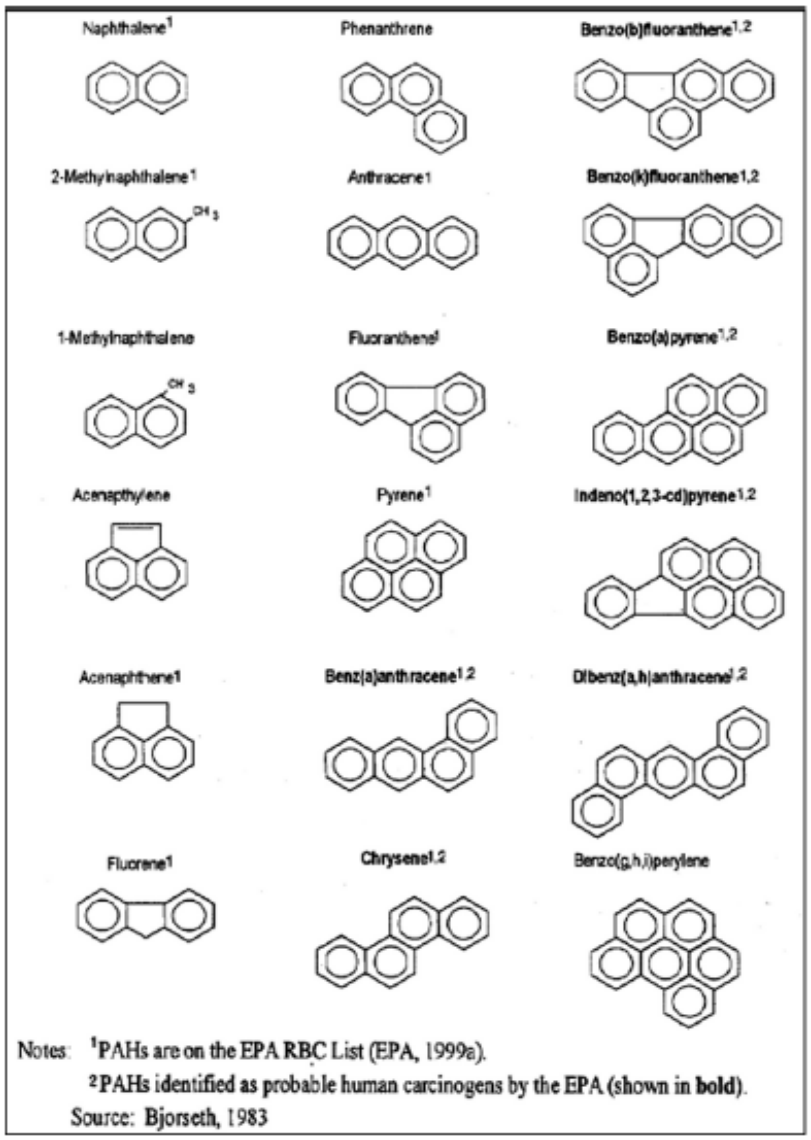

Figure 1: The most commonly analyzed PAHs [4].

PAHs also have various functions such as light sensitivity, heat resistance, conductivity emit ability, corrosion resistance, and physiological action. PAHs possess very characteristic UV absorbance spectra and this is useful in their identification. Most PAHs are also fluorescent, emitting characteristic wavelengths of light when they are excited [4]. PAHs are everywhere in the environment and commonly detected in air, soil, and water.

They are frequently measured in the atmosphere for air quality assessment, in biological tissues for health-effects monitoring, in sediments and mollusks for environmental monitoring, and in foodstuffs for safety reasons. In contemporary analysis gas chromatography (GC), rather than liquid chromatography (LC), is often the preferred approach for separation, identification, and quantification of

PAHs, mainly because GC generally affords greater selectivity, resolution, and sensitivity than LC [6].

The major source of PAHs is the incomplete combustion of organic material such as coal, oil and wood. PAHs are not synthesized chemically for industrial purposes. Nevertheless, there are a few commercial uses for many PAHs. They are mostly used as intermediaries in pharmaceuticals, agricultural products, photographic products, thermosetting plastics, lubricating materials, and other chemical industries [13].

However, the general uses of some PAHs are:

- Acenaphthene: manufacture of pigments, dyes, plastics, pesticides and pharmaceuticals.
- Anthracene: diluent for wood preservatives and manufacture of dyes and pigments.
- Fluoranthene: manufacture of agrochemicals, dyes and pharmaceuticals.
- Fluorene: manufacture of pharmaceuticals, pigments, dyes, pesticides and thermo plastic.
- Phenanthrene: manufacture of resins and pesticides.
- Pyrene: manufacture of pigments..

Other PAHs may be contained in asphalt used for the construction of roads, in addition to roofing tar. Furthermore, specific refined products, of precise PAHs, are used also in the field of electronics, functional plastics, and liquid crystals [4].

1.1.2 Organochlorine pesticides (OCPs)

Organochlorine pesticides (OCPs) are one of the most important persistent organic pollutants (POPs) (**Table 2**). During the last decades, their distribution, sources, transformation, toxicity and accumulation in the terrestrial and aquatic ecosystems have gained significant attention [7].

OCPs are semi volatile organic compounds. Due to the low water solubility, OCPs have a strong affinity for suspended particulates and subsequently settle down to sediments [8].

Table 2: Main Organochloride pesticeides [5].

Compound	MW (g/mol)	Molecular formula
Hexachlorobutadiene	260.744	C_4Cl_6
Dichlorvos	220.970	$C_4H_7Cl_2O_4P$
a-HCH	290.814	$C_6H_6Cl_6$
b-HCH	290.814	$C_6H_6Cl_6$
c-HCH	290.814	$C_6H_6Cl_6$
d-HCH	290.814	$C_6H_6Cl_6$
Hexachlorobenzene	284.766	C_6Cl_6
Heptachlor	373.300	$C_{10}H_5Cl_7$
Endosulfan sulphate	422.903	$C_9H_6Cl_6O_4S$
Aldrin	364.896	$C_{12}H_8Cl_6$
Dicofol	370.475	$C_{14}H_9Cl_5O$
Heptachlor Epoxide	389.299	$C_{10}H_5Cl_7O$
Endosulfan alpha	406.904	$C_9H_6Cl_6O_3S$
4,4'-DDE	318.018	$C_{14}H_8Cl_4$
Dieldrin	380.895	$C_{12}H_8Cl_6O$
Endrin	380.895	$C_{12}H_8Cl_6O$
4,4'-DDD	320.034	$C_{14}H_{10}Cl_4$
2,4'-DDT	354.476	$C_{14}H_9Cl_5$
4,4'-DDT	354.476	$C_{14}H_9Cl_5$
Isodrin	364.896	$C_{12}H_8Cl_6$
Pentabromobenzyl acrylate	556.668	$C_{10}H_5Br_5O_2$
Pentabromoethylbenzene	500.648	$C_8H_5Br_5$
Pentachlorobenzene	250.324	C_6HCl_5

These compounds pose great threats to ecosystems and human health. OCPs resulted in a variety of toxic and adverse health effects, including carcinogenesis, immunological and reproductive disorders in living organisms includeing humans and wildlife [7]. Although the application of these chemicals has been banned or restricted in many countries especially the developed ones, some developing countries are still using these compounds because of their low cost and versatility in industry, agriculture and public health [9]

Some of the main organochlorine pesticides (OCPs) are (**Figure 2**):

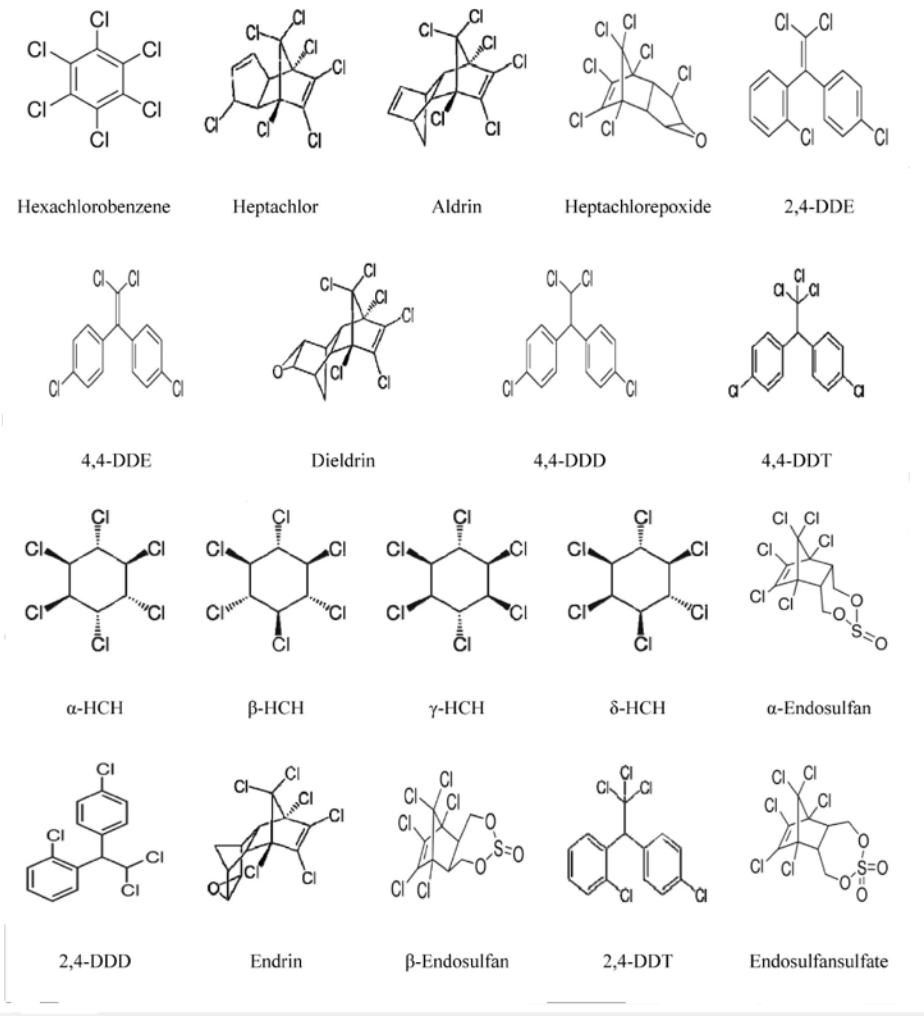

Figure 2:The most commonly analyzed OCPs [10].

Aldrin: It is a pesticide that has been used to control the population of insects and parasites, such as termites, grasshoppers etc.Its principal application was in agriculture and against termites, it is used for wood preservation and to combat any infestations [10].

Aldrin is readily epoxidised to dieldrin by both plants and animals. As a result, aldrin residues are rarely found in foods and animals, and then only in small amounts. It binds strongly to soil particles and is very resistant to leaching into ground water. Due to its persistence and hydrophobicity, aldrin is known to bioconcentrate, mainly as its conversion products. Aldrin is banned in many countries or its use is severely restricted.

Dieldrin: It is a non-systemic insecticide. It was used in agriculture against soil insects and tropical disease carriers. Its industrial uses include: protection of timber and timber structures from termites and covering of plastic and elastic

materials (eg telecommunication cables). Dieldrin binds strongly to soil particles and hence is very resistant to leaching into ground water. Volatilisation is an important mechanism of loss from the soil and, because of its persistence and hydrophobicity, dieldrin is known to bioconcentrate.

Endrin: It is a foliar insecticide used mainly on field crops, such as cotton and grains. It has also been employed as a rodenticide to control mice and voles. It is rapidly metabolised by animals and does not accumulate in fat to the same extent as other compounds with similar structures. It can enter the atmosphere by volatilisation, and can contaminate surface water from soil run-off. Endrin is banned in many countries or its use is severely restricted [10].

DDT : It was widely used during the second world war to protect the troops and civilians from the spread of malaria, typhus, and other vector borne diseases. After the war, DDT was widely applied on a variety of agricultural crops (mainly for cultivation of cotton) and for the control of disease vectors. It is still being produced and used for vector control. Growing concern about adverse nvironmental effects, especially on wild birds, led to severe restrictions and bans in many developed countries . DDT is still used to control mosquito vectors of malaria in tropical countries. DDT is highly insoluble in water and is soluble in most organic solvents. It is semi-volatile and its presence is ubiquitous in the environment. It is lipophilic and partitions readily into the fat of all living organisms and has been demonstrated to bioconcentrate and biomagnify [10].

Heptachlor: It is a non-systemic stomach and contact insecticide, used primarily against soil insects and termites. It has also been applied against cotton insects, grasshoppers, some crop pests, and to combat malaria.Heptachlor is highly insoluble in water, and is soluble inorganic solvents. It is quite volatile and can be expected to partition into the atmosphere. It binds readily to aquaticsediments and bioconcentrates in the fat of living organisms. Heptachlor is metabolised in animals to heptachlor epoxide, whose toxicity is similar to that of heptachlor, and which may also be stored in animal fat [10].

Hexachlorobenzene (HCB): It is a fungicide and first used for seed treatment, especially for control. It is classified as industrial POPs, as it is a by-product of

the manufacture of industrial chemicals including carbon tetrachloride, perchloroethylene, trichloroethylene, and pentachlorobenzene. It is a known impurity in several pesticide formulations, including pentachlorophenol and dichloram and may be present as an impurity in others. HCB is highly insoluble in water but soluble in organic solvents and quite volatile. HCB is a thermodynamically verystable compound and very resistant to breakdown [10].

1.1.3 Polychlorinated Biphenyls (PCBs)

Polychlorinated Biphenyls (PCBs) are among a group of man-made chemicals that are known as Persistent Organic Pollutants (POPs). PCBs were commercially produced world-wide on a large scale between the 1930s and 1980s [11]. PCBs belong to a broad family of man-made organic chemicals known as chlorinated hydrocarbons. The PCBs are mixtures of up to 209 different components (congeners), depending on the number and position of chlorines around the biphenyl ring [12] (**Figure 3**).

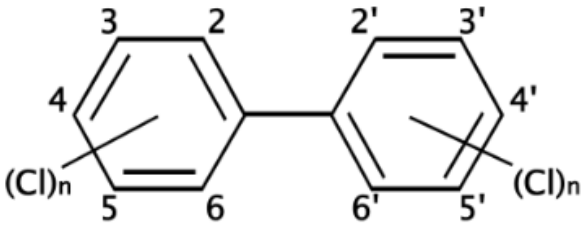

Figure 3: Chemical structure of PCBs [13].

They have a range of toxicity and vary in consistency from thin, light-colored liquids to yellow or black waxy solids. Due to their non-flammability, chemical stability, high boiling point and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including [12] :

- Electrical, heat transfer and hydraulic equipment
- Plasticizers in paints, plastics and rubber products
- Pigments, dyes and carbonless copy paper
- Other industrial applications

Some of the uses of PCBs have resulted in their direct introduction into the enviaronment.PCBs are classified as probable human carcinogens and

produce a wide spectrum of adverse effects in animals and humans, including reproductive toxicity, teratogenicity and immunotoxicity [13].

1.1.4 Polychlorinated naphthalene (PCNs)

Polychlorinated naphthalene (PCNs) are a group of 75 chlorinated naphthalenes, which are structurally similar to PCBs (**Figure 4**). Polychlorinated naphthalenes (PCNs) have been commercially produced and used mainly in electrical devices, but also for impregnation of wood, paper and textiles to attain waterproofness, flame resistance and protection against insects, molds and fungi [14].

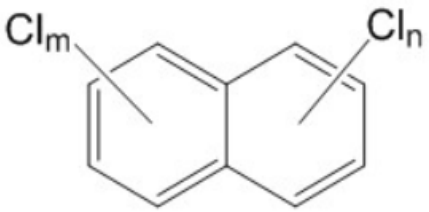

Figure 4: General structure of polychlorinated naphthalenes [15]**.**

Polychlorinated naphthalenes (PCNs) have been commercially produced and used in a variety of applications due to their dielectric, water-repellent, flameretardant, and fungus-resistant properties in combination with high stability and compatibility with other material. Besides the industrial production of PCNs there is also a release of PCNs to the environment via polychlorinated biphenyl (PCB) commercial products, in which PCNs are present as minor contaminants. PCNs consist of congeners with widely differing toxicological behavior. Some congeners have been identified as highly persistent and bioaccumulating, whereas most congeners seem to be quite readily metabolized. There are three known main sources of PCNs in the environment: the technical PCN formulations, technical PCB formulations, and thermal (e.g. combustion, roasting, metal reclamation) and other processes (e.g. chloro-alkali industry) in the presence of chlorine [16].

1.1.5 Legislation for Priority Pollutants

The strong presence of PPs in the environment and their negative health impacts on the quality of the aquatic world, the air and the health of all living

organisms has caused worldwide interest. Nowadays, a collective effort is being made to gradually reduce and eliminate these compounds from the environment. The purpose of legislation is to deal with problems generated by chemical pollution of surface waters. Pollution of waters poses a threat to the aquatic environment, with effects such as acute and chronic toxicity to aquatic organisms, the accumulation of pollutants in the ecosystem and the loss of biodiversity. Therefore, it is important to identifying the causes of pollution and avoiding, as far as possible, emissions of polluting substances in the most economically and environmentally friendly way [17].

In Europe, there are legislations for the monitoring of the quality of aquifer quality, such as the Water Framework Directive (WFD), which has been harmonized with the legislation of each European country. European Community (EC) policy regarding dangerous or hazardous substances in European waters was first adopted by means of a Council Directive on pollution caused by discharges of certain hazardous substances (Directive 76/464/EEC). An initial list of 33 priority pollutants (PPs) has been regulated in a number of specific directives by defining community-wide emission limit values and quality objectives in surface and coastal waters. As part of the ongoing restructuring of EC water policy, Directive 76/464/EEC has since been integrated into the Water Framework Directive (2000/60/EC) (WFD) [18], moreover, Directive 76/464/EEC will be fully repealed in 2013 and has now been codified as 2006/11/EC [19]. In particular, Directive 2000/60 / EC of the European Council of 23 October 2000 defines the priority hazardous pollutants, their degree of risk and the strategy to deal with of water pollution. In this way priority pollutants identified and among them, these which pose a significant risk to the aquatic environment. This Directive therefore aimed at eliminating priority pollutants substances and at obtaining concentration values as close as possible to the permitted limits [18]. For identification and categorization of the pollutants, COMMPS method (combined monitoring-based and modeling-based priority setting) was followed. This method was developed following the study and evaluation of monitoring data from water and sediment samples from all European countries [20]. Decision No 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy set out the first list of 33

substances or groups of substances that were prioritised at Union level for inclusion in Annex X to Directive 2000/60/EC. Some examples of the priority priority substances under consideration are alachlor, anthracene, atrazine and benzene.

A new Directive, published in December 2008 (Directive 2008/105/EC) [21], establishes limits, known as Environmental Quality Standards (EQS), for these 33 substances and for an additional 8 substances regulated under previous legislation. EQS are chemical standards set by the European Commission, following intensive studies concerning in particular surface water pollution. In particular, where the concentration of these substances exceeds a maximum scientifically acceptable limit, they are pollutants (they may be a priority substance or other pollutant) and pose a risk to both animal and plant life in aquatic ecosystems and to human health. Thus, EQS are a risk-based measure and based on these new "prohibited" pollutants [22]. The Water Framework Directive also calls for surface waters to meet good ecological status, which provides a measure of healthy ecosystems. To achieve this objective, Member States may need to ensure that additional pollutants of national relevance are controlled [23]. In pursuit of WFD objectives through identifying possible pollutant reduction measures, an accurate knowledge of these substances in the receiving aquatic systems, and more specifically in densely urban areas, has definitely proved necessary. Implementation of the European Water Framework Directive 2000/60/EC (WFD) [18] and its subsidiary directives requires Member States to improve their knowledge of priority substances within the aquatic environment. To achieve the stated objective, information on PP occurrence in surface waters, particularly within densely urbanized areas, needed to be collected [2].

In August 2013, with a new Directive 2013/39 / EC [17], which based on the existing legislation on priority pollutants and water protection, a new list of priority pollutants was issued in Directive 2000/60 / EC was adopted and in accordance with new EQS (EQS) standards have been adopted and some of the standards for existing substances have been revised to bring them into line with new data and scientific progress. The Stockholm Convention, which was held in Stockholm in May 2001 and was launched in May 2004 to protect human health and the environment [24]. Members undertook to ban the use, production

or import of the 12 substances included in the first list. In the list in May 2017, with an agreement of 181 members, 16 other substances were added and some others, such as Dicofol, are under consideration [25].

Particularly, cooperation between reference laboratories, research centers and environmental organizations plays a role in monitoring and exchanging data and knowledge on chemical compounds that can be considered as emerging environmental pollutants[26]. Such networks, such as the Norman network for Europe, create the conditions for the development of validated identification methods and the compilation and publication of emerging pollutant databases [27].

1.2 Emerging Contaminants (ECs)

Nowadays, some of the great challenges in the field of environmental analysis are the study of emerging contaminants behavior and the control of the risks associated to emerging contaminants [28]. Emerging contaminants have attracted wide attention from researchers and society over the world because these are chemicals that are not commonly monitored but which have the potential to enter the environment and cause known or suspected adverse ecological and human health effects. A significant number of ECs is present in the aquatic environment. According to the NORMAN network, at least 700 substances categorized into 20 classes, have been identified in the European aquatic environment [29].

Today, emerging contaminants encompass a diverse group of compounds, including:

- Pharmaceuticals
- Drugs of abuse
- Personal care products (PCPs)
- Steroids and hormones
- Surfactants and surfactant metabolites
- Perfluorinated compounds (PFCs)
- Organophosphate flame-retardants and plasticizers
- Brominated flame retardants – new groups in addition to PBDEs.
- Industrial additives and agents

- Gasoline additives
- Siloxanes
- Transformation products (TPs)

In addition, three new classes have to be added to the list of emerging pollutants: nanomaterials, 1,4-dioxane and disinfection by-products (DBPs) [28] [30]. The way that organic compounds enter the environment depends on their pattern of usage and way of implementation (e.g., disposal of municipal, industrial and agricultural wastes, excretion of pharmaceuticals and accidental spills) [28]. The main sources of emerging contaminants are untreated urban wastewaters and wastewater treatment plants (WWTPs) which normally are released into surface waters and then they end up into sediment, soil, groundwater and sea [31]. Nowadays, more than 700 emerging pollutants, their metabolites and transformation products, are listed as present in the European aquatic environment [26].

ECs have varied chemical properties and they are presented in complex matrices at very low concentrations. Despite their chemical properties, it is difficult for many compounds to estimate whether they will go into the solid phase or they will remain in the aqueous phase. Furthermore, the complexity of matrices and their low concentrations has led to the lack of efficient and standard methods for determination of ECs [32]. ECs are currently not included in routine monitoring programmes and their fate, behavior and ecotoxicological effects are often not well understood. Moreover, for emerging contaminants, there are no appropriate legislation in comparison with priority pollutants. Also, for most emerging contaminants there are not exist risk assessment and data ,so it is difficult to predict their effect in the aquatic environment [31]. Detection, identification and quantification of ECs and their transformation products in the various environmental compartments is essential for gaining knowledge on their occurrence and fate [29]. Therefore, one of the major trends in analytical chemistry is to develop fast and efficient procedures for the analysis of target and non-target organic compounds in complex matrices [30].

1.2.1 Pesticides

The development of agricultural activities during the last decades have greatly increased the quantity and improved the quality of food for the world

growing population. However, many agricultural activities rely heavily on the use of pesticides [33]. Pesticides are chemical compounds that are meant to control pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops [34]. The main source of pesticides in the aquatic environment is from agricultural activities. The term pesticide includes all of the following: herbicide, insecticides nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial and fungicide. The most common of these are herbicides which account for approximately 80% of all pesticide use. Most pesticides are intended to serve as plant protection products (PPP), which is a term used in regulatory documents, consists of several different components [35].The continuous use of pesticides raises the concern about which is the behavior, environmental fate and the potential adverse effects on organisms of these compounds once incorporated into the environment. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly [33].

Due to the lack of testing and production control, counterfeit and other illegal pesticides result in a range of serious risks that can be divided into three different categories [36]:

A. Risk to humans and animals from:

- Increased amounts of residues due to overdosing (inappropriate prescription of procedure)
- Occurrence of unknown and uncontrolled residues in plants, which can be harmful if consumed by people or animals
- Potential for direct adverse effect on sprayer operators
- Additional build-up of pesticide stockpiles

B. Risk to crops from:

- Decreased efficacy or no efficacy at all
- Potential phytotoxic effects of the pesticides or their impurities
- Improper use

C. Risk to the environment from:

- Toxic substances getting into the soil and underground water
- Potentially long-term contamination
- Potential contamination of adjacent crops
- Potential side effects involving beneficial organisms

1.2.2 Phosphorus flame retardants (PFRs)

Organophosphorus flame retardants (PFRs) are utilized as flame retardants, plasticizers, antifoaming agents, lubricants, and hydraulic fluids by diverse industries, including textiles, building materials, electronics, and chemicals [15]. Along with the gradual phasing out of brominated flame retardants, the consumption of alternative PFRs has increased dramatically (xiao)Flame retardants (FRs), which are chemicals added to materials both to prevent combustion and to delay the spread of fire after ignition, are used in polymers since the 1960s [37]. Most of the PFRs have a mechanism of action in the solid phase of burning materials (char formation), but some may also be active in the gas phase. Some PFRs are reactive FRs, which means they are chemically bound to a polymer, whereas others are additive and mixed into the polymer [38]. FRs may have different compositions. They may contain halogens (bromine and chlorine), phosphorus, nitrogen, metals, minerals based on aluminum and magnesium, or borax.

PFRs can be divided in three main groups. The first group contains the inorganic PFRs, including frequently used RP and APP. The second group consists of the organic PFRs. Three different general structures of these PFRs can be recognized: the organophosphate esters (OPEs), the phosphonates, and the phosphinates (**Figure 5**). The third group is the widely used group of halogenated PFRs [38].

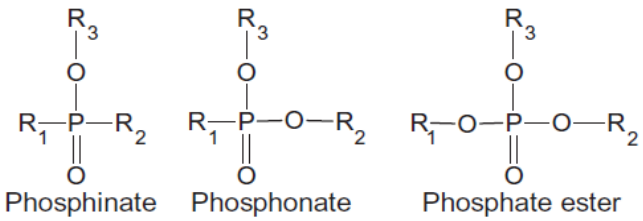

Figure 5: Three different general structures of the organic PFRs [39].

1.3 Asopos River

Asopos river rises in Viotia and flows into the South Evoikos gulf, about 60 km away north of Athens [39]. The Asopos basin, covering approximately 680 km^2 land and it is located in Central Greece. It flows from west to east direction and has a total length of 57 kilometers [40]. The river passes through Sykamino, Oropos, Schimatari and Inofyta, until it flows into the Evoikos gulf north of Oropos, in the settlement Halkoutsi (**Figure 6**).

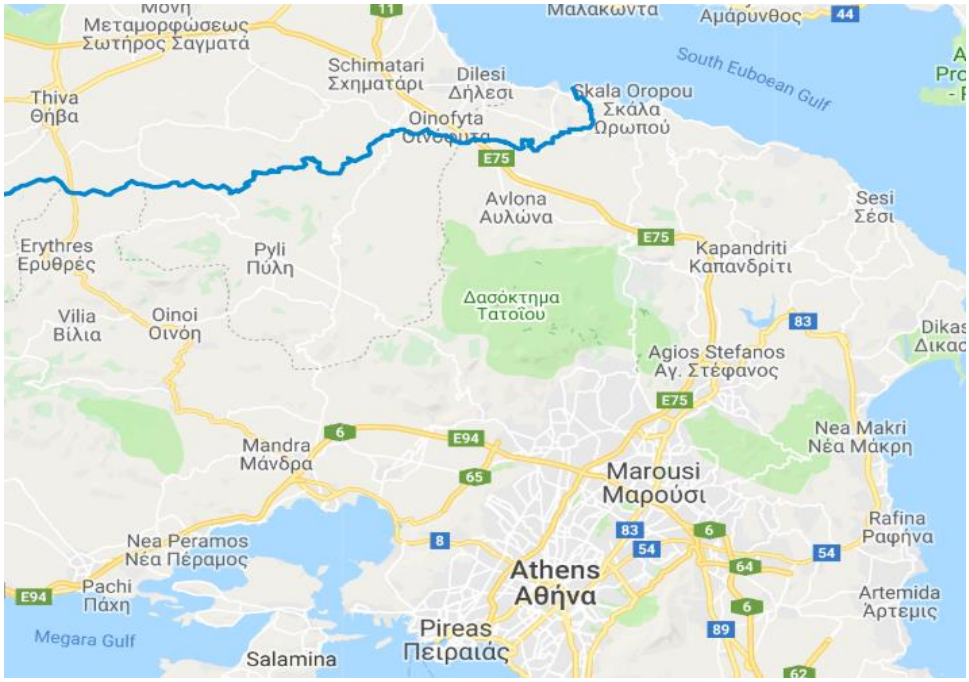

Figure 6: Asopos river and the area where it flows

Among the river human activities take place, mainly agriculture and industrial [41]. The river runs through areas where there are almost 20% of Greece's total industrial production, and today, river's waters receive waste from hundreds of industries [39]. The river passes through areas where 20% of total Greece industrial production takes place. The extensive installation of industries in the area near the river, and the uncontrolled disposal of industrial and agricultural wastes into the river, make the water quality of Asopos questionable [42].

1.3.1 The enviromental problem

The case of Asopos River pollution started about fifty years ago, when a Presidential Decree of 1969 allowed the establishment of industries in the wide area of Inofita-Schimatari. The presidential decree did not set limits to industrial

activities for the disposal of their wastes. The uncontrolled disposal of industrial waste in the surrounding aquifers threatened the quality of the waters of the Asopos River. The degradation of Asopos waters due to the intense industrial activity and the lack of appropriate measures and restrictions for the protection of the aquatic environment has so far been the subject of a study of various research groups. The results of these studies shows that, this environmental pollution needs further, more thorough, extensive and systematic research.

CHAPTER 2
Determination of PPs and ECs in River water samples- Literature Review

2.1 Introduction

Over the last decades, environmental pollution has become a matter of increasing concern due to the high number of both regulated and unregulated organic pollutants that can be present in environmental waters [43]. The detection of PPs and ECs in water samples is a promising approach to understand the overall contamination. There are many different anthropogenic pollutants which may be present in samples, typically at low concentrations. Multi-analyte methodologies must bedeveloped and applied in monitoring programs to provide a broad and realistic knowledge about water pollution in a rapid, sensitive and selective way. It is also crucial that the scope of these methodologies can be easily updated and extended, as new emerging contaminant are continuously being reported and are a matter of concern [44].

2.2 Sample treatment techniques

The determination of priority pollutants and emerging contaminants in environmental waters can not be performed without any sample pretreatment because they are too dilute and too complex [45]. Among extraction procedures employing different clean-up and pre-concentration techniques, it could be mentioned: liquid–liquid extraction (LLE), one of the oldest pretreatment procedure and commonly used because of its simplicity and low cost [46] and solid phase extraction (SPE), in which analytes can be adsorbed and extracted from complex matrices using a small amount of organic solvent [47].

2.2.1 Liquid Liquid extraction (LLE)

Liquid-liquid extraction (LLE) is based on the transfer of a solute from one liquid phase into another immiscible liquid phase according to differences in solubility [48]. There is a net transfer of one or more species from one liquid into another liquid phase, generally from aqueous to organic. The solvent that is enriched in solute is called extract. The feed solution that is depleted in solute is called the raffinate. Frequently, one of the solvents is water or an aqueous

mixture and the other is a non-polar organic liquid. As in all extraction processes, liquid liquid extraction comprises a step of mixing (contacting) followed by a step of phase separation. It is important to consider both steps in the selection of solvents and modes of operation.

Equilibrium is reached when the chemical potential of the extractable solute is the same in the two phases. Practically, this rule leads to the definition of a "distribution coefficient", K, as follows:

$$\mathrm{K} = \frac{\mathrm{C1}}{\mathrm{C2}}$$

where C1 and C2 are the equilibrium concentrations of the solute in the two phases, respectively. The distribution coefficient is an expression of the relative preference of the solute for the solvents [49]. LLE is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to countercurrent distribution equipment called as mixer settlers.

2.2.2 Solid Phase extraction (SPE)

Solid phase extraction (SPE) [50] is another approach that offers a number of important benefits. It reduces solvent usage and exposure, disposal costs and extraction time for sample preparation. Consequently, in recent years SPE has been successfully used for the separation and sensitive determination of metal ions, mainly in water samples. The principle of SPE is similar to that of liquid– liquid extraction (LLE), involving a partitioning of solutes between two phases (liquid and solid). However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix) and a solid (sorbent) phase. This sample treatment technique enables the concentration and purification of analytes from solution with sorption on a solid sorbent. The basic approach involves passing the liquid sample through a column, a cartridge, a tube or a disk containing an adsorbent that retains the analytes. After all of the sample has been passed through the sorbent, retained analytes are recovered upon elution with an appropriate solvent. An SPE method always consists of three to four successive steps (**Figure 7**).

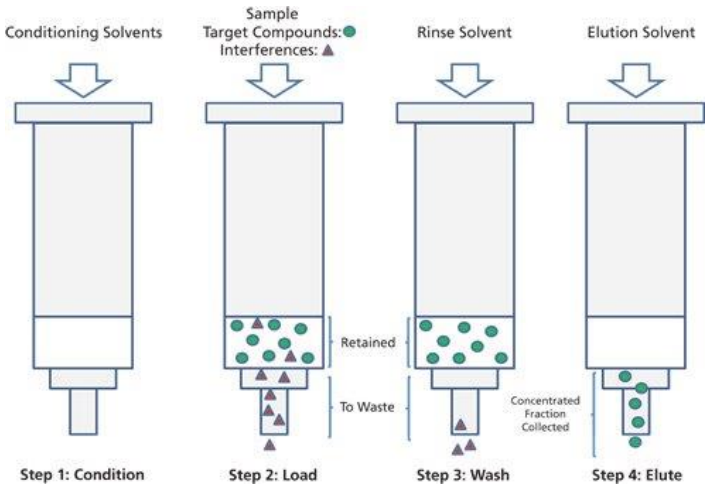

Figure 7: Steps of Solid Phase Extraction (SPE) [50].

First, the solid sorbent should be conditioned using an appropriate solvent. This step is very important, as it enables the wetting of the packing material and the solvation of the functional groups. In addition, it removes possible impurities, which contained in the sorbent or the packaging, removes the air present in the column and fills the void volume with solvent. The solid sorbent should not dry between the conditioning and the sample treatment steps, otherwise the analytes will not be efficiently retained and poor recoveries will be obtained. The second step is the loading of the sample through the solid sorbent. The sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system. The sample flow-rate through the sorbent should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration. During this step, the analytes are concentrated on the sorbent. The third step (which is optional) may be the washing of the solid sorbent with an appropriate solvent, having a low elution strength, to eliminate matrix components that have been retained by the solid sorbent, without displacing the analytes. A drying step, also suggested, especially for aqueous matrices, to remove traces of water from the solid sorbent. This will eliminate the presence of water in the final extract. The final step is the elution of the analytes of interest by an appropriate solvent. It is often recommended that the solvent volume be fractionated into two aliquots, and before the elution to let the solvent soak the solid sorbent [50].

The use of SPE procedures has been growing in the past few years due to their advantages offered for trace element determinations, namely conservation of species, good preconcentration factors (thus enabling the achievement of very low limits of detection), for automation, and possible online coupling to instrumental techniques.

2.3 Analytical Techniques

2.3.1 Gas Chromatography

Gas chromatography is one of most widely used techniques for qualitative and quantitative analysis. In gas chromatography, the components of a vaporized sample are separated by being distributed between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. In performing a gas chromatographic separation, the sample is vaporized and injected into the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase. In contrast to most other types of chromatography, the mobile phase does not interact with molecules of the analyte. The only function of the mobile phase is to transport the analyte through the column. Column temperature is an important variable that must be controlled. Thus, the column is normally housed in a thermostated oven. The optimum column temperature depends on the boiling point of the sample 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, it is often desirable to use temperature programming whereby the column temperature is increased either continuously or in steps as the separation proceeds [51].

There are two types of gas chromatography:

- gas-solid chromatography (GSC): the stationary phase is solid. The retention of analytes is results of adsorption/desorption steps with the solid phase.
- gas-liquid chromatography (GLC): the stationary phase is liquid that is held 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 referred as gas chromatography (GC).

A GC instrument (**Figure 8**), has simple components. Usually helium, hydrogen or nitrogen gas compressed in cylinders is used as the carrier gas (mobile phase). Flow of the carrier gas into a temperature controlled sample injection is controlled by pressure regulators and gas metering valves. A GC column is attached to the injection port and samples are introduced into the carrier gas steam at a temperature sufficient to insure vaporization of all components. Typically, the sample is introduced with a microliter syringe which is forced through a rubber septum at the injection port. A detector attached directly to the column exit monitors individual sample components as they are eluted from the column. The detector must be insensitive to carrier gas, while detecting sample components that are eluted. A recording of its response with time forms a chromatogram .

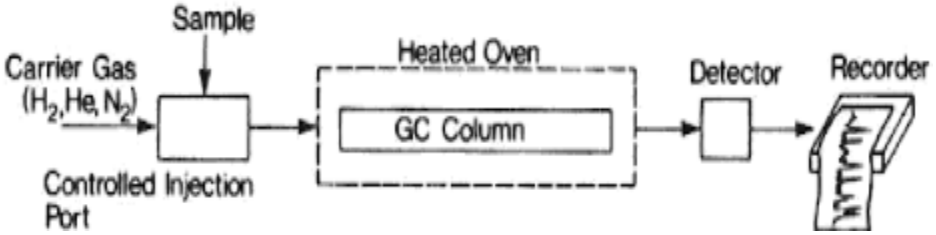

Figure 8: GC Instrumentation.

The chromatogram contains the analytical data for the components of a mixture. Qualitative information appears in the characteristic retention time of each component. Quantitative information is contained in peak area [51].

2.4 Mass Spectrometry

Mass Spectrometry is combined with chromatographic techniques and is a useful tool for pesticide residue analysis and in analytical chemistry in general. Mass spectrometry is based upon the in vacuum separation of ions, in the gas phase, according to their mass-to-charge (m/z) ratio. Mass spectrometry made use of a technique of ionization to ionize the substances to be analyzed. There are several types of ionization [52]. Mass Spectrometry can be divided into two groups, depending on the mass measurement: Low

Resolution Mass Spectrometry (LRMS) and High Resolution Mass Spectrometry (HRMS).

2.4.1 Ionization Techniques

2.4.1.1 Electron Impact Ionization (EI)

The starting point for a mass spectrometric analysis is the formation of gaseous analyte ions, and a mass spectrometric method is characterized by the ionization process. The appearance of mass spectra for a given molecular species is highly dependent on the method used for ion formation. These methods fall into two major categories: gas-phase sources and desorption sources. With a gas-phase source, the sample is first vaporized and then ionized. With a desorption source, the sample in a solid or liquid state is converted directly into gaseous ions. The most widely used source is the electron impact (EI) source. In this source, molecules are bombarded with a high-energy pack of electrons. This produces positive ions, negative ions, and neutral species. The positive ions are directed toward the analyzer by electrostatic repulsion. In EI, the electron pack is so energetic that produced many fragments. These fragments, are very useful in identifying the molecular species entering the mass spectrometer. Mass spectra for many libraries of MS data have been collected using EI sources [51].

2.4.1.2 Chemical Ionization (CI)

In chemical ionization new ionized species are formed when gaseous molecules interact with ions. Chemical ionization may involve the transfer of an electron, proton, or other charged species between the reactants. These reactants are the neutral analyte M and ions from a reagent gas. Assuming reasonable collision cross sections and an ion source residence time of 1 μs, a molecule will undergo 30–70 collisions at an ion source pressure of about 2.5 × 10^2 Pa. The 10^3 –10^4 -fold excess of reagent gas also shields the analyte molecules effectively from ionizing primary electrons which is important to suppress competing direct EI of the analyte. There are four general pathways to form ions from a neutral analyte M in CI (**Figure 9**):

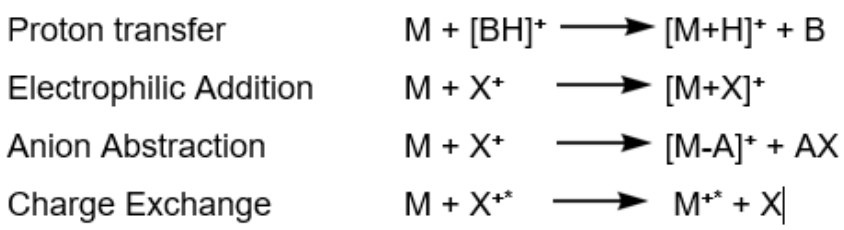

Figure 9: Main mechanism pathways of CI.

Although proton transfer is generally considered to yield protonated analyte molecules, $[M+H]^+$, acidic analytes may also form abundant $[M–H]^–$ ions by protonating some other neutral. Electrophilic addition chiefly occurs by attachment of complete reagent ions to the analyte molecule, e.g., $[M+NH4]^+$ in case of ammonia reagent gas. Hydride abstractions are abundant representatives of anion abstraction, e.g., aliphatic alcohols rather yield $[M–H]^+$ ions than $[M+H]^+$ ions. Whereas reactions 1–3 result in even electron ions, charge exchange (reaction 4) yields radical ions of low internal energy which behave similar to molecular ions in low-energy electron ionization [53].

2.4.1.3 Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric pressure chemical ionization (APCI) was first reported by Horning more than three decades ago [54]. Since then, the technique has been an invaluable tool in the development of interfaces for separation procedures, such as liquid chroma- tography, with mass spectrometry. Nowadays, APCI is an essential analytical tool for organic mass spectrometry [55].

Atmospheric pressure chemical ionisationis an analogous ionisation method to chemical ionisation. The significant difference is that APCI occurs at atmospheric pressure and has its primary applications in the areas of ionisation of low mass pharmaceutical compounds (APCI is not suitable for the analysis of thermally labile compounds). In APCI, the analyte solution is introduced into a pneumatic nebulizer and desolvated in a heated quartz tube before interacting with the corona discharge creating ions. It can be in two modes ionization modes positive or negative [56].

Ionization in positive-ion mode occurs by reaction of the analyte with protonated solvent molecules, generally giving rise to an abundant protonated analyte

molecule $[M+H]^+$ or adduct ions like $[M+NH4]^+$, if ammonium salts are added to the solvent. In the negative-ion mode, ions are generated by proton abstraction by oxygen ions O2* or by the formation of adducts with anions such as acetate or chloride present in the sample or solvent.

The corona produces primary N_2^{*+} and N_4^{*+} by electron ionisation. These primary ions collide with the vaporised solvent molecules to form secondary reactant gas ions - e.g. H_3O^+ and $(H_2O)_nH^+$. These reactant gas ions then undergo repeated collisions with the analyte resulting in the formation of analyte ions. The high frequency of collisions results in a high ionisation efficiency and thermalisation of the analyte ions. This results in spectra of predominantly molecular species and adduct ions with very little fragmentation [57].

Assuming nitrogen is the sheath and nebulizer gas with atmospheric water vapour present in the source, then the type of primary and secondary reactions that occur in the corona discharge (plasma) region during APCI are as shown in the scheme. The most abundant secondary cluster ion is $(H_2O)_2H^+$ along with significant amounts $(H_2O)_3H^+$ and H_3O^+. The reactions listed above (**Figure 10**) are ways to account for the formation of these ions during the plasma stage. The protonated analyte ions are then formed by gas-phase ion-molecule reactions of these charged cluster ions with the analyte molecules. This results in the abundant formation of $[M+H]^+$ ions [56].

Atmospheric pressure ionization has primarily been used to interface an MS with LC. However, this attractive ionization interface can also be applied to GC. The ionization mechanism employed by the APCI source is low- energy (soft), which generates spectral data typically rich in molecular or quasi-molecular ion information and ideal for compound confirmation [58].

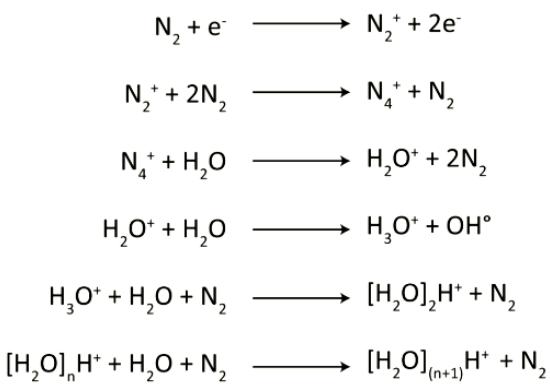

Figure 10: Reactions in APCI.

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 [58].

2.4.1.4 Electro Spray Ionization (ESI)

ESI is a process by which a solution is sprayed into a high electric field at atmospheric pressure. Charged droplets result from the nebulization of the solution in an electric field, with both solvent and analyte ions being detected. This is a widely applied technique. ESI is a liquid-phase ionization technique, which does not require the evaporation of a neutral analyte, but rather the formation of preformed ions in solution. Therefore, ESI is the method of choice for the ionization of analytes that would easily thermally decompose. In order to achieve preformed analyte ions in solution, the composition of the sample solution (or LC mobile phase) has to be adjusted in order to convert a neutral analyte into an ion in solution. Basic analytes, for example, are ionized by the addition of an acid to the solution. The electrospray process in greatly limited in terms of flow-rate that can be nebulized. The use of a nebulizing gas allows higher flow-rates to be used. However, higher flow-rates also require the use of heat, for example, by application of a concurrent, countercurrent, or cross flow of hot gas, for the desolvation of charged droplets in order to promote the

release of analyte ions into the gas phase. The high efficiency of the technique and its compatibility with LC and other liquid separation methods gave rise to much interest in the pharmaceutical applications of the technique. As the mass analyzer separates ions according to their mass-to-charge ratio (m/z) rather than their mass, both Sample solution Charged aerosol sample ions Mass analyzer Atmospheric pressure Vacuum Principle of electrospray ionization inside an atmospheric pressure ion source (**Figure 11**). As a result, in addition to increasing sensitivity, ESI effectively extends the mass range of analytes amenable to MS by more than an order of magnitude to beyond 150 kDa

An important issue in the application of ESI in the analysis of analytes in complex biological samples is the occurrence of ionization suppression or enhancement effects. These so-called matrix effects are due to influence of co-eluting matrix constituents on the liquid-phase analyte ionization and on the transfer of preformed analyte ions from the liquid to the gas phase [59].

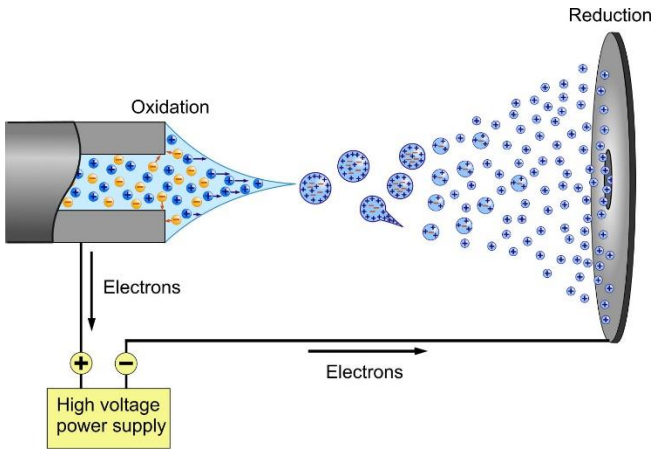

Figure 11: ESI Mechanism.

2.4.2 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 that have been used in pesticide residues analysis are Quadrupole, Triple Quadrupole, and Ion Trap.

2.4.2.1 Quadrupole (Q)

A quadrupole analyzer (**Figure 12**) uses a combination of radio requency 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. The single quadrupole is certainly the simplest, cheapest, most robust, and ubiquitous mass analyzer in research and development laboratories, but it suffers from a limited sensitivity, resolving power and mass accuracy [60].

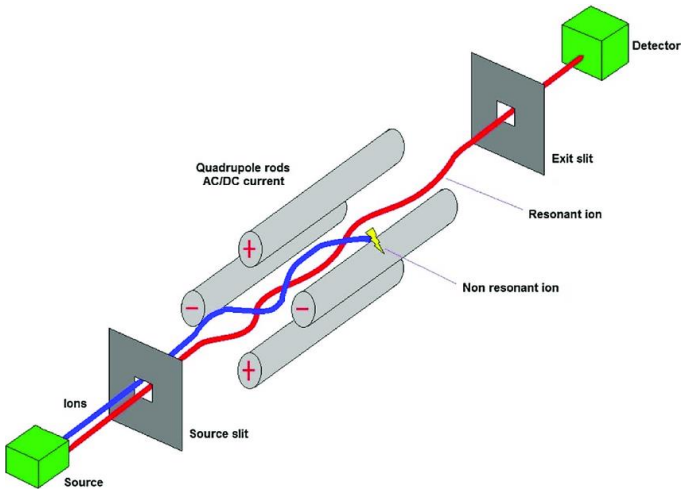

Figure 12: Quadrupole mechanism.

2.4.2.2 Triple Quadrupole (QqQ)

A QqQ mass spectrometer (**Figure 13**) offers MS/MS in which the first and third quadrupoles act as mass filters, while the second quadrupole 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 increased selectivity, improved S/N, lower limits of quantitation (LOQ), wider linear range, and improved accuracy.

In the advanced QqQ instruments, the basic linear quadrupole structure is modified with the curved quadrupoles, which offer longer flight paths, and thus, these systems could be used for more accurate (higher-resolution) selection of m/z. The unit mass resolution achieved by the typical quadrupole instruments corresponds to 0.7Da (full width at half maximum (FWHM)). However, with the advanced quadrupole instruments, the resolution up to 0.1Da (ultraselective) could be obtained [61].

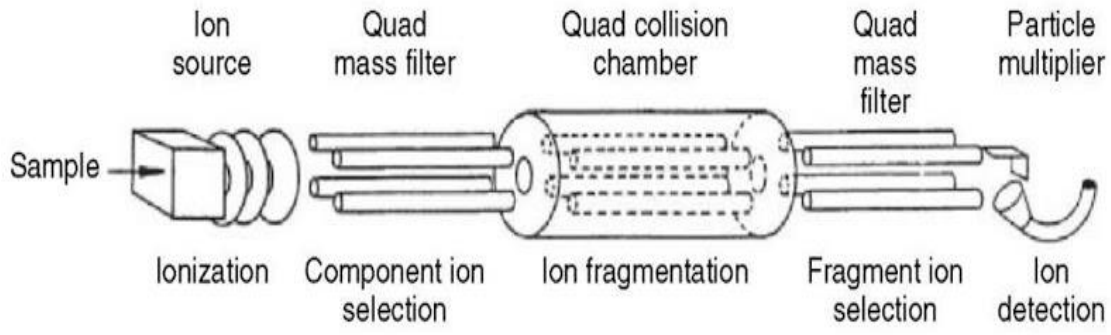

Figure 13: Linear QqQ instrument parts.

2.4.2.2 Ion Trap (IT)

An ion trap (**Figure 14**) may be described as a quadrupole that has undergone a solid of rotation. A typical ion trap comprises two endcap electrodes and a ring electrode, all of hyperbolic or hemispherical cross-section. The end-cap electrodes contain small-diameter holes for allowing ions to enter and leave the trap. Ions are confined inside the trap by a radio-frequency field of constant frequency but variable power. The ions may be detected, according to their m/z ratio, by applying voltages sufficient to eject them from the trapping field [62].

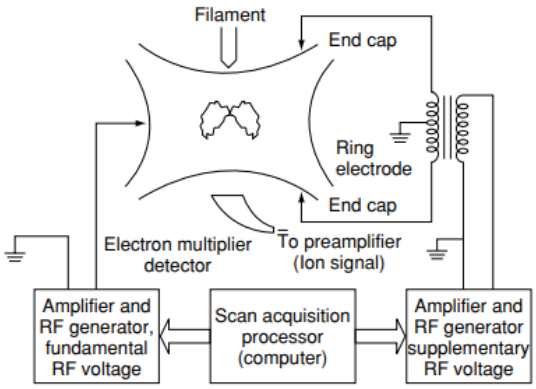

Figure 14: Schematic diagram of an IT.

2.4.3 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, co-elutting molecules with the same nominal mass can be identified. The HRMS instruments that have been used mostly in pesticides analysis are Time of Flight (TOF) and Orbitrap mass analyzers.

2.4.3.1 Time-of-Flight (TOF)

Time-of-Flight (TOF) (**Figure 15**) 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.

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. The only drawback to TOF analyzers is its limited dynamic range and quantitative performance [63] .

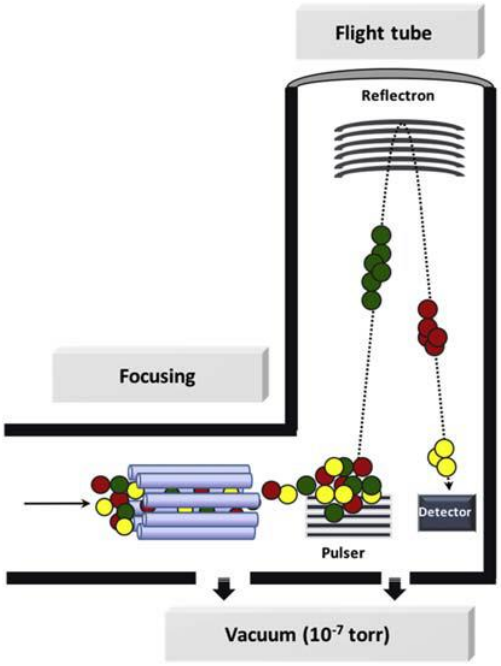

Figure 15: Scheme of TOF instrument.

2.4.3.2 Orbitrap

The Orbitrap mass analyzer consists essentially of three electrodes. Outer electrodes have the shape of cups facing each other and electrically isolated by a hair-thin gap secured by a central ring made of a dielectric. A spindle-like central electrode holds the trap together and aligns it via dielectric end-spacers. When voltage is applied between the outer and the central electrodes, the resulting electric field is strictly linear along the axis and thus oscillations along this direction will be purely harmonic. At the same time, the radial component of the field strongly attracts ions to the central electrode.

Ions are injected into the volume between the central and outer electrodes essentially along a tangent through a specially machined slot with a

compensation electrode (a “deflector”) in one of the outer electrodes. With voltage applied between the central and outer electrodes, a radial electric field bends the ion trajectory toward the central electrode while tangential velocity creates an opposing centrifugal force. With a correct choice of parameters, the ions remain on a nearly circular spiral inside the trap, much like a planet in the solar system. At the same time, the axial electric field caused by the special conical shape of electrodes pushes ions toward the widest part of the trap initiating harmonic axial oscillations. Outer electrodes are then used as receiver plates for image current detection of these axial oscillations. The digitized image current in the time domain is Fourier-transformed into the frequency domain in the same way as in FTICR and then converted into a mass spectrum.

The orbitrap analyzer offers very high resolving power in the range 100,000-240,000, and excellent mass accuracy below 1 ppm. Drawback of orbitrap analyzers is its low acquisition rate [60] [64].

2.4.3.3 Hybric Instruments

The coupling of two different analyzers is known as hybrid instrument. An example is QqTOF (**Figure 16**) where the first quadrupole is mass selective device, the second serves as a collision cell and the third is a TOF analyzer. 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.

For dissociation experiments, the most common activation method is collision induced dissociation (CID), where an inert gas is introduced into a collision cell where low energy (10-100 eV) and collisions occur between the precursor ion and the molecules of inert gas [60].

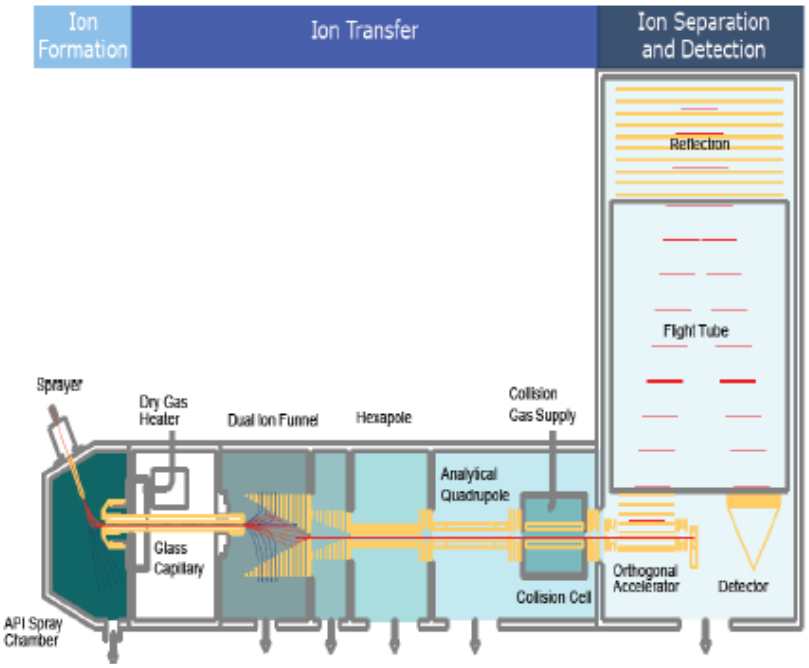

Figure 16: Scheme of QqTOF instrument (Maxis Impact, Bruker).

2.5 Acquisition Modes in HRMS

2.5.1 Data Depentent 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 [65].

2.5.2 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 (LE) 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 (HE) 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 [65].

2.6 Data Analysis Workflows in HRMS

After the sample preparation and the HRMS analysis, raw data can be treated with three different approaches, target, suspect and non-target screening. A systematic workflow for all three approaches is shown in **Figure17**.

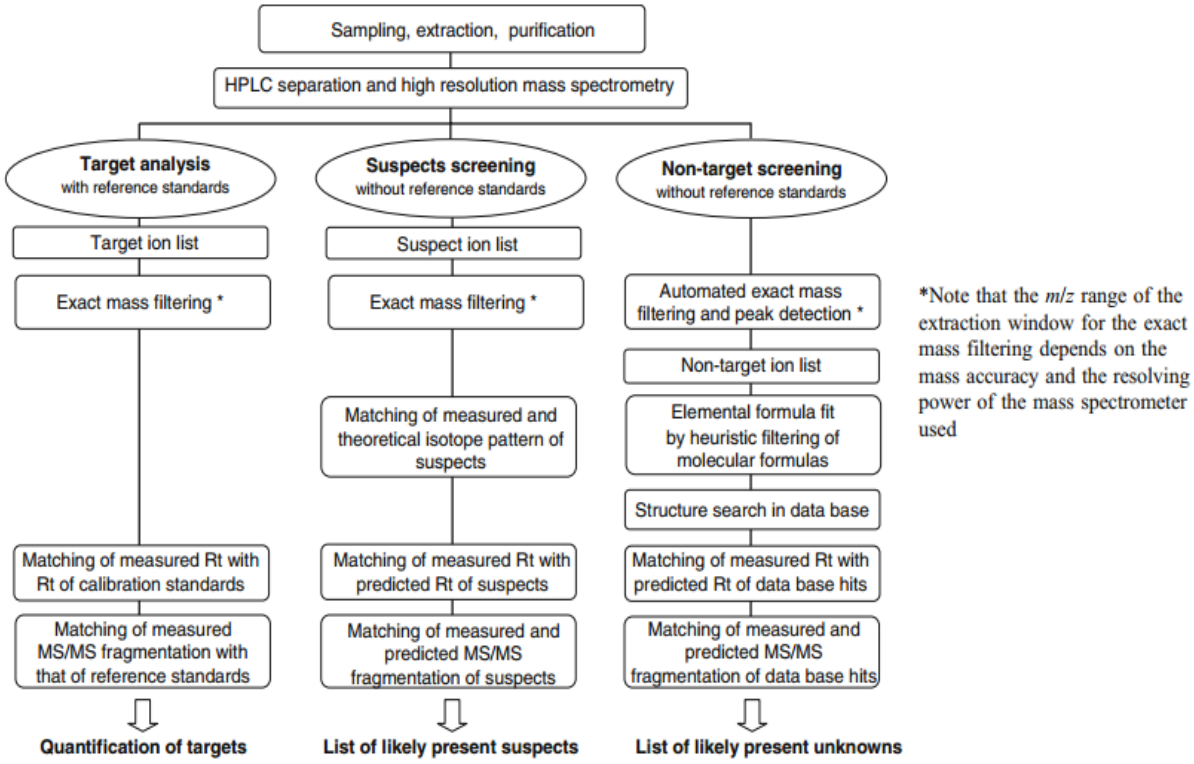

Figure 17: Systematic workflow for target, suspect and non-target screening.

2.6.1 Target Screening

In this approach, an in-house developed database is used for the screening of a large number of compounds. The information included in the

database is based on the analysis of the available reference standards [65]. The reference standard is necessary for comparison of the retention time, the MS spectrum profile (precursor ion, adducts, in-source fragments), as well as the MS/MS spectrum (fragment ions and ion ratios)[66]. Quantitation can be performed in full-scan mode, but requires greater effort than in LC-LRMS methods where Single Reaction Monitoring (SRM) mode is used [65] [66].

2.6.2 Suspect Screening

In this approach, a list of suspect compounds that are possible to be found in specific samples is built. The screening is based only on the exact m/z of the expected ions, which, in case of the ESI source, are usually the pseudomolecular ions $[M+H]^+$ and $[M-H]^-$, except for some compounds which exclusively show adduct formation. Molecular formula and structure are known, so this information can be efficiently used in the identification and confirmation process [67]. Absence from blank samples, mass accuracy, isotopic pattern, retention time prediction, ionization efficiency and information on fragment ions reported in the literature are parameters that can facilitate tentative identification of suspect candidates [67] [68].

2.6.3 Non-target Screening

In non-target methodologies, samples are searched for compounds 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 [65]. Except for the elucidation of unknowns, non-target screening is used for the identification of metabolites and transformation products, arising from in vivo and in vitro experiments, in-silico modeling and degradation laboratory studies [65] [69]. In this case, the number of chemically meaningful structures, which can be assigned to an unknown peak, is limited to structures that show a close relationship with the parent compound and also, an adequate control sample or time series is available [67].

2.7 Confidence in the identification procedure

2.7.1 Confidence in Target Screening

The confirmation of positive findings in target screening can be performed by attributing identification points (IPs). According to the 2002/657/EC guideline, 4 IPs are required for unequivocal confirmation, and for HRMS instruments with resolution higher than 10,000, the precursor ion earns 2 IPs and the product ions earn 2.5 IPs. This means that one single HRMS/MS transition can confirm the detection of a substance, which is risky when there are several co-eluting isomers [70]. Another fact is that resolving power may largely vary between HRMS instruments, which makes the definition of general criteria difficult More precise criteria for the use of mass accuracy and mass resolution have to be implemented to define clearly the requirements for a reliable confirmation in LC-HRMS [67].

2.8 Determination of organic compounds in water samples-Literature review

So far, there are a lot of different studies for the determination of different groups of organic coumpounds in water samples (surface water, underground water, drinking water) by LRMS, while few studies deal with the determination of priority pollutants and emerging contaminants in water samples by HRMS and especially there is a a lack of studies about GC-HRMS workflows. The development of high resolving power mass analyzers (HRMS) has contributed significantly to the expansion of environmental studies' to the wide-scope screening ECs.

An overview of the main analytical procedures for the determination of PPs and ECs (GC compatible compounds mainly PAHs, PCBs, OCPs) was performed. Details on the matrix, determinated compounds, methods of determination of the most commonly GC-amenable compounds, are presented in **Table 3**.

For the clean up of the samples and the pre-concentration of the analytes, solid phase extraction was used in most of the studies. Oasis HLB([71], [43], JRC technique) and C18 ([5],[9],[45],[73], [74], [75]) were the most widely used SPE cartridges.

Furthermore, the most studies in the following table refer to the determination of different groups of compounds, such as PAHs, OCPs, Pesticides, OCs. For this purpose different matrices (ground water, river water, drinking water, effluent water) were used. For the analysis of the samples gas chromatographic techniques were used couple with different detectors.

Table 3: Review of sample preparations compatible for GC-amenable compounds

Matrices	Compounds	Sample Prerartion	Analysis Technique	References
Ground water	Organic contaminants and TPs	SPE:OASIS HLB Contitioning: 3 mL MeOH,3 mL Water. Loading : 100 mL Sample Elution: 5 mL MeOH evaporation to 1mL +1 ml Ethyl Acetate evaporation to 100uL by gentle stream of nitrogen	GC–(EI)TOFMS GC–(APCI)QTOFMS	[71]
Surface water (River)	OCPs	SPE: C18-bonded phase containing 500 mg reversed phase octadecyl. Contitioning:5 mL Ethyl acetate, 5 mL MeOH,2x5 mL Water. Loading : 1 L Sample Elution: 6 mL Ethyl acetate + anhydrous sodium sulfate evaporation to 0.1mL by gentle stream of nitrogen	GC-ECD	[9]
Ground water	PAHs	10% (v/v) methanol was added to 200mL of water SPE: C18 of 500 mg Contitioning:5 mL Ethyl acetate, 5mL MeOH,5mL distilled water containing 2% (v/v) methanol Loading : 200 mL Sample Washing:5mL of HPLC-grade water Elution: 5x5mL Ethyl acetate evaporation and reconstituted in 0.25mL	GC–EI–MS	[5]
Ground water,Surface water, Effluent wastewater	Organic contaminants (pesticides, pharmaceuticals, personal care products, illicit drugs)	SPE:OASIS HLB Contitioning: 5 mL MeOH,5 mL Water. Loading : 250 mL Sample Elution: 10 mL MeOH 5ml of eluent evaporated to 1mL +1 ml Ethyl Acetate evaporation to 250 uL by gentle stream of nitrogen	UHPLC–ESI-(Q)TOF MS GC–APCI-(Q)TOF MS	[43]

Matrices	Compounds	Sample Prerartion	Analysis Technique	References
Drinkng water, Ground water, Surface water	Naphthalene, AcenaphthyleneAnthracene, Pyrene, Chrysene Benzo[k]fluoranthene, Acenaphthene Indeno[1,2,3-cd] pyrene, Fluorene, Phenanthrene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[a]pyrene, Benzo[ghi]perylene, Dibenzo[a,h] anthracene	PAHs extracted from the water sample by LLE with hexane. An internal standard mixture is added to the sample prior to extraction. The extract is concentrated by evaporation, and the residue taken up in a solvent appropriate for clean-up or GC analysis.	GC-Analysis	[72]
Ground Water	Organochlorine pesticides	SPE: C18-bonded phase containing 500 mg reversed phase octadecyl Contitioning:20 mL MeOH,10 mL Water .Loading the Sample Elution: 10mL Hexane evaporation to 0.2mL by gentle stream of nitrogen	GC-ECD	[73]
Water	EC-7 PCBs, Pesticides, HCBD,PAHs,EHMC,BHT,OPCs	SPE: HLB SPE Disk Contitioning:3x20mL Ethyl acetate, 3x20mL MeOH, 20mL Water Loading : 20 L Sample Elution: 3x20mL Ethyl acetate +evaporation to 0.1mL by gentle stream of nitrogen	HRGC-HRMS	JRC technical report for EMBLASS II project

Matrices	Compounds	Sample Prerartion	Analysis Technique	References
Water samples	Pesticides	SPE: Lichrolut C18(500 mg), Strata X(200 mg) Contitioning:10 mL CH_2Cl_2, 10mL ACN,10mL distilled water Loading : 1L Sample Elution: 5mL ACN,5ml MeOH evaporation til dryness	SPME-GC-MS	[45]
		SPE: Lichrolut C18(500 mg), Contitioning:10 mL CH_2Cl_2, 10mL MeOH,10mL distilled water Loading : 1L SampleElution: 5ml MeOH, 5mL ACN,evaporation til dryness		
Surface Water	Organochlorine pesticides	SPE: C18-bonded phase containing 500 mg reversed phase octadecyl Contitioning:20 mL MeOH,10 mL Water Loading the Sample Elution: 10mL Hexane evaporation to 0.2mL by gentle stream of nitrogen	GC-ECD	[74]
River Water	Phosphorus pesticides (OPs), Chlorine pesticides(OCs)	SPE: Contitioning:2x5 mL MeOH, 2x5 mL Water Elution: 10mL Ethyl Acetate	GC–(FPD,μECD)	[75]
Surface Water, Underground Water	Pesticide residues	SPE: C18-bonded phase disk and poly(styrene-divinylbenzene) disks Contitioning:10 mL Acetone ,10 mL MeOH Elution:2x 10mL of CH_2Cl_2-EthAc(1:1, v/v) The evaporation to 0.5mL by gentle stream of nitrogen	GC-MS GC-FTD GC-ECD	[76]

CHAPTER 3
Scope

The strong presence of PPs and ECs in the environment, both in the aquatic environment and in aquatic organisms, is now indisputable. Concerns about the effects on water quality, on the health of organisms but also on human health, have led environmental authorities to create programs and legislative frameworks for them. Analytical chemistry poses an important role in the process of identifying, controlling and quantifying these compounds in environmental samples. In recent decades, methods, with a wide range of treatment and analysis techniques, have been developed to simultaneously and quantitatively prioritize organic pollutants over a wide range of samples

So far, Asopos river had been studied from many research groups. The extensive installation of industries in the area near the river, and the uncontrolled disposal of industrial and agricultural wastes into the river, make the water quality of Asopos questionable. Since, there are not risk assessment data for the ECs, it is difficult to predict their effect in the aquatic environment and their fate. To the best of our knowledge, this is the first extended environmental monitoring study in Greece including target analysis, suspect & non-target screening of PPs & ECs.

Many high usage ECs, as well as priority pollutants, are volatile and thermostable, therefore GC-HRMS methods should be developed.

The aim of this study is the development of a novel methodology for the determination of GC-amenable PPs and ECs and the application of the method in river water samples from Asopos river basin.

The most challenging part of the method development is that this method should be ideal for the analysis of compounds with a wide variety of physicochemical properties. For this purpose, a generic sample preparation protocol should be followed. For the applicability domain of the developed method analytes from diferrent groups (PAHs, PCBs, OCPs, PPPs) are selected. Several sample preparation methods should be tested, including different extraction techniques and initial sample volumes.

The validation of the method is a critical and necessary process as it provides the reliability of reproducible and comparable results. For the validation of the method, performance characteristics (linearity, sensitivity, trueness, matrix effect and precission) should be evaluated with spiked samples of a representative group of compounds. Finally, the method wil be applied in real river water samples. Samples will be analyzed using GC-APCI-QTOFMS.

The total results will indicate the overall state of contamination of Asopos river basin due to the occurrence of GC-amenable PPs and ECs.

Experimental Part

CHAPTER 4
Materials and Methods

4.1 Chemicals & Reagents

For GC-APCI-QTOF system Hexane (Pesticide residue analysis grade) was purchased from Honeywell (New Jersey,USA) and Acetone (Pestipure grade) was ordered from Carlo Erba (Barchelona, Spain).

Distilled water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Moreover, Ethyl Acetate for analysis from Carlo Erba (Barchelona, Spain), Hexane (analytical reagent grade), Acetone and Isooctane from Fischer Scientific (Loughborough, UK), HPLC grade Methanol (MeOH) from Fischer Scientific (Loughborough, UK) and Dichloromethane (CH_2Cl_2) from Sigma Aldrich (Steinheim, Germany).

Regenerated cellulose syringe filters (RC, pore size 0.2 μm, diameter 15mm) were purchased from Phenomenex (Torrance, CA, USA). Glass microfiber filters (WHATMAN 934-AH) with a pore size of 47 mm were used for the first filtration of the samples. Furthermore, for the sample preparation Na_2SO_4 was purchased by Fluka Analytical (Buchs, Switzerland) and Silica gel, Pore Size 60 Å (0.060-0.200 mm) from ACROS Organics. As far as the SPE cartridges, Oasis HLB(200mg) from Waters (Milford Massachusetts, USA), and Isolute C18 EC (500mg) from Biotage (Ystrad Mynach, UK) were used.

Standards stock solutions of individual pesticides at a concentration of 1000 mg L^{-1} were purchased from Bruker Daltonik GmbH (Bremen, Germany). Standards of Hexachlorobutadiene, Dichlorvos, Alpha-HCH, Beta-HCH, Hexachlorobenzene, Lindane, delta-HCH, Heptachlor, Aldrin, Dicofol, Isodrin, Alpha-Endosulfan, Dieldrin, Endrin, 4,4'-DDT, 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, Endosulfan-sulfate 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. Standards of Polychlorinated biphenyls (PCBs) and Polycyclic aromatic hydrocarbons (16-PAHs) from Fluka-Sigma-Aldrich (Steinheim, Germany) were used. Also, a

standard solution of 16 PAHs certificated as CRM (Napthalene-d8, Acenapthylene-d8, Acenapthene-d10, Fluorene-d10, Phenathrene-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 CPAChem was used as internal standard. Working solutions of 1000 mg L^{-1} were prepared. The working solutions contained all the analytes for the method development and the method validation. The solutions stored at -20 °C.

4.2 Sampling and Storage

River water samples were collected from two sampling points (**Figure 18**). One sampling point was close to the estuaries of Asopos river (R1) and the other one was close to industrial and agricultural activities (R2) . The distance between two sampling point is 12 km.

Figure 18: Sampling points of river water samples.

Two portable autosamplers (one at each sampling point) were used in order to collect 24-hour samples for 7 consecutive days during the winter (November 2018) (**Figure 19**).

Figure 19: Autosampler near sampling point R2.

Seven river water samples were obtained from sampling point R1 and seven river water samples from sampling point R2. Weather conditions and the color of the river were recorded for each sampling day.

Each sample was approximately 5 L and it was transferred to the lab inside a cooler with containing ice. After the sampling, raw river water samples were kept in pre-cleaned glass bottles and they were stored in a refrigerator at 4°C until sample preparation. Before the sample preparation, river water samples were vacuum filtered through glass microfiber filters (WHATMAN 934-AH) with a pore size of 47 mm in order to remove suspended solids that may clog the adsorbent bed during SPE.

In **Table 4** the details of total river water samples with their analysis code are summarized.

Table 4: River water samples details with their analysis code.

No	Matrix	Analysis code	Type	Sampling place
1	River Water	R1_20/11	Water	Sampling point R1
2	River Water	R1_21/11	Water	Sampling point R1
3	River Water	R1_22/11	Water	Sampling point R1
4	River Water	R1_23/11	Water	Sampling point R1
5	River Water	R1_24/11	Water	Sampling point R1

No	Matrix	Analysis code	Type	Sampling place
6	River Water	R1_25/11	Water	Sampling point R1
7	River Water	R1_26/11	Water	Sampling point R1
8	River Water	R2_20/11	Water	Sampling point R2
9	River Water	R2_21/11	Water	Sampling point R2
10	River Water	R2_22/11	Water	Sampling point R2
11	River Water	R2_23/11	Water	Sampling point R2
12	River Water	R2_24/11	Water	Sampling point R2
13	River Water	R2_25/11	Water	Sampling point R2
14	River Water	R2_26/11	Water	Sampling point R2

4.3 Sample preparation

It is known, that one of the most important parts of an analysis is the sample preparation of the samples. The stages of each sample preparation protocol, affect the pre-concentration of the analytes and the clean-up of the samples.

4.3.1 Sample preparation tests

Based on the literature review (Chapter 2) regarding the determination of PPPs and ECs in river water samples, different sample preparation tests were performed in order to decide which sample preparation to follow. For the evaluation of the developed method analytes with different physicochemical properties were selected. These analytes were the 30% of the total analytes in our current database of the laboratory. The tests that were performed with regard to the extraction protocol as well as the pre-concentration of the analytes are described in detail below.

- **Extraction**

Three different extraction techniques were tested. The first test was based on ISO 28540:2011 method [72], a LLE protocol for the determination of PAHs in water samples. The potential scope extention of ISO 28540:2011 method to cover a wide variety of volatile and thermostable compounds was tested (Test 1). Moreover, the application of solid-phase extraction using two different sorbents, C18 EC (500 mg) (Test 2) and HLB (200mg) (Test 3) was evaluated.

Test 1

River water sample (1.25 L) was added in a separating funnel. After the addition of internal standards and standards for the spiked samples, 30 mL of Hexane were added in a separating funnel. The hexane layer was transferred into a 50 mL centrifuge tube. Sodium sulfate was added afterwards, to remove any remaining moisture (Centrifuge, 4000 rpm, 15 min). The dry extract was transferred into a round-bottom flask. The extraction was repeated twice with 30mL Hexane. The total volume of the extract was collected into the round-bottom flask and it was evaporated until approximately 2mL using rotary evaporator, at a temperature of 40 °C. It is important not to evaporate the extracts till dryness. It should be mentioned that between samples we evaporated some mL of hexane or acetone so as to clean the system.

For the clean-up of the samples, cartridges filled with Silica (1g) were used. The conditioning of the cartridges was performed with 3x5 mL Hexane:CH2Cl2 (50:50) mixture and 5 mL Hexane. Test tubes were placed under the cartridges. Sample was loaded and the round-bottom flask was rinsed with hexane which was added in to the cartridges. The elution of the analytes from the adsorbent material was performed by 10 mL Hexane:CH2Cl2 (50:50). The eluent was evaporated until 2 mL using a rotary evaporator, at a temperature of 40 °C . The extract was evaporated to dryness under a gentle nitrogen stream (at 40 °C) and was reconstituted to a final volume of 250 μL (Hexane). Worthmentioning is that before the evaporation with nitrogen, 20 μL isooctane was added as keeper [77] in each test tube. Keepers are substances (usually solvents) which are added to the samples to prevent analyte losses during solvent removal by evaporation . Finally, each extract was filtered through a 0.22 μm RC membrane filter into a 2 mL vial and it was ready for GC-HRMS/MS analysis.

Test 2

River water sample (1.25L) was cleaned-up and pre-concentrated using SPE. Internal standards and standards for spiked samples were added in the

samples prior to the sample preparation. Cartridges consisted of C18 end capped sorbent (500mg) were used. The conditioning of the cartridges was performed with 8 ml Ethyl Acetate, 8 ml Methanol and 8 ml Water. The cartridges were dried by passing air through them for 0.5 to 1 h (using vacuum on the SPE box, cartridges were visually inspected to check for their complete dryness). Before the elution step, 20μL isooctane was added as keeper in each test tube. The elution of the analytes from the adsorbent material was performed by adding 4 ml Ethyl Acetate, 3 ml Dichloromethane and 6 ml Hexane. The extract was evaporated to dryness under a gentle nitrogen stream (at 40 °C) and was reconstituted to a final volume of 250 μL (Hexane). Worthmentioning is that before the evaporation with nitrogen, 20 μL isooctane was added as keeper in each test tube. Finally, each extract was filtered through a 0.22 μm RC membrane filter into a 2 mL vial and it was ready for GC-HRMS/MS analysis.

Test 3

River water sample (1.25L) was cleaned-up and pre-concentrated using SPE. Internal standards and standards for spiked samples were added in the samples prior to the sample preparation. Cartridges consisted of Oasis HLB (200 mg) sorbent were used. The conditioning of the cartridges was performed with 6 ml Ethyl Acetate, 6 ml Methanol and 6 ml Water. The cartridges were dried by passing air through them for 0.5 to 1 h (using vacuum on the SPE box, cartridges were visually inspected to check for their complete dryness. Before the elution step, 20μL isooctane was added as keeper in each test tube. The elution of the analytes from the adsorbent material was performed by 9 ml Ethyl Acetate and 6 ml Hexane. The extract was evaporated to dryness under a gentle nitrogen stream (at 40 °C) and was reconstituted to a final volume of 250 μL (Hexane). Worthmentioning is that before the evaporation with nitrogen, 20 μL isooctane was added as keeper in each test tube. Finally, each extract was filtered through a 0.22 μm RC membrane filter into a 2 mL vial and it was ready for GC-HRMS/MS analysis.

- **Pre-concentration**

As far as the pre-concentration of the analytes was concerned, different initial volumes were tested so as to evaluate the influence of matrix effect to the determination of organic compounds. It was considered that with higher pre-concentration lower limits of detection could be reached. To determine to which extent is this valid, three different initial volumes were tested, keeping the final volume of reconstitution constant (250 μL). So, 10.000 times pre-concentration with 2.50 L initial volume, 5.000 times and 1.000 times pre-concentration with 1.25 L and 0.25 L initial volume respectively were tested.

4.3.2 Final sample preparation method

River water samples were collected and vacuum filtered through glass microfiber filters (WHATMAN 934-AH) with a pore size of 47 mm in order to remove suspended solids that may clog the adsorbent bed during SPE. Then, 1.25 L of each sample was pre-concentrated and cleaned-up using solid phase extraction (SPE) with C18 EC (500mg) cartridges. The conditioning of the cartridges was performed with 8 ml Ethyl Acetate, 8 ml Methanol and 8 ml Water. The cartridges were dried by passing air through them for 0.5 to 1 h (using vacuum on the SPE box, cartridges were visual inspected for complete dryness). Before the elution step, 20μL isooctane was added as keeper in each test tube. The elution of the analytes from the adsorbent material was performed by adding 4 ml Ethyl Acetate, 3 ml Dichloromethane and 6 ml Hexane. The extract was evaporated to dryness under a gentle nitrogen stream (at 40 °C) and was reconstituted to a final volume of 250 μL (Hexane). Worthmentioning is that before the evaporation with nitrogen, 20 μL isooctane was added as keeper in each test tube. Finally, each extract was filtered through a 0.22 μm RC membrane filter into a 2 mL vial and it was ready for GC-HRMS/MS analysis. In **Figure 20** the whole sample preparation protocol is summarized.

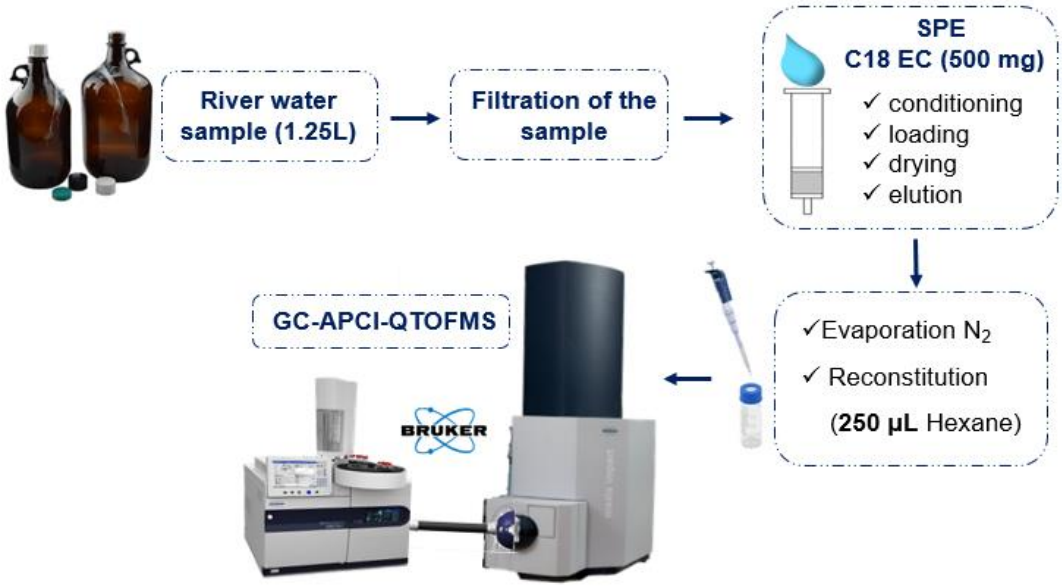

Figure 20: Final sample preparation.

4.4 Instrumentation-HRMS Analysis

The analysis of river water samples and the validation of the method were carried out utilizing a GC-APCI-QTOF system (**Figure 21**). The GC-APCI-QTOF system consisted of:

- a Bruker 450 GC
- a CP-8400 AutoSampler
- a hybrid quadrupole time of flight mass spectrometer (QTOF-MS) (Maxis Impact, Bruker Daltonics)

GC was operated in splitless injection 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. The injection volume was 1 µL. A Restek Rxi-5Sil MS column of 30 m (0.25 mm i.d. x 0.25 µm film thickness) was used with Helium as carrier gas in a constant flow of 1.5 mL min^{-1}.

The GC oven was programmed as follow: 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 was maintained at 280, 290 and 250°C, respectively.

The QTOF mass spectrometer was calibrated with Perfluorotributylamine (FC43) prior to each injection.

Bruker's software that was used for raw data analysis was DataAnalysis 5.1 and TASQ Client 2.1.

Figure 21: The GC-APCI-QTOF-MS system.

4.5 Method Validation

Validation of the method is a vital step after the development of a new methodology in the laboratory. This is a critical and necessary process as it provides the reliability of reproducible and comparable results. In order to validate a method, a number of performance parameters need to be tested and evaluated.

The method was validated for 130 analytes, which are the 40% of the total analytes in the current method. The selected compounds represented almost all the analyte classes included in the current method and had several physicochemical properties, so they eluted all over the chromatogram. The compounds of the validation dataset are shown in **Table 5** .

Validated parameters were:

- Linearity

Linearity of the instrument was studied for each compound by analyzing standard solutions in six different levels (10, 30, 50, 100, 200, 300 μg L^{-1}). Moreover linearity of the method was studied for each compound by analyzing spiked samples in six different levels (2, 6, 10, 20, 40, 60 ng L^{-1}). Calibration and standard addition curves were estimated using linear regression.

- Sensitivity

Concerning the evaluation of the sensitivity, limits of Detection (LOD) and Quantitation (LOQ) for instrument and method were determined theoretically. For this purpose, data from regression analysis calculating the standard deviation of intercept from calibration curves and spiked curves respectively were used. Trueness of the method was determined comparing the recoveries of 3 spiked levels (20, 40, 60 ng L^{-1}).

- Trueness

  Trueness of the method was determined comparing the recoveries of 3 spiked levels (6, 20, 60 ng L^{-1}).

- Matrix Effect

Matrix effect was determined by comparing the response of the analytes between matrix matched samples and standard solutions at three different concentration levels (6, 20, 60 ng L^{-1}).

- Precission

Precission was expressed as repeatability (% RSDr) and reproducibility (% RSDwR). Repeatability of instrument was determined by comparing % RSDr from the analysis of the same standard solution (100 μg L^{-1}). Repeatability of the method was determined by comparing RSDr from the analysis of three different spiked samples at three different concentration levels (6, 20, 60 ng L^{-1}).

Reproductivity of the method was determined by % RSDrw comparing six different spiked samples (6 ng L^{-1}) which were analysed in two different days.

Table 5: Validation Dataset.

Analyte	Group	Analyte	Group
Acenapthene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Cyanazine**	Plant Protection products (PPPs)
Acenapthylene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Cyproconazole**	Plant Protection products (PPPs)
Anthracene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Cyprodinil**	Plant Protection products (PPPs)
Benzo(a)pyrene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Deltamethrin**	Plant Protection products (PPPs)
Benzo(b)fluoranthene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Diazinon**	Plant Protection products (PPPs)
Benz(a)anthracene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Dichlofenthion**	Plant Protection products (PPPs)
Benzo(g,h,i)perylene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Dichlofluanid**	Plant Protection products (PPPs)
Benzo(k)fluoranthene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Dimethoate**	Plant Protection products (PPPs)
Chrysene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Ditalimfos**	Plant Protection products (PPPs)
Dibenzo(a,h)anthracene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Esfenvalerate_Fenvalerate Isom 1**	Plant Protection products (PPPs)
Fluoranthene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Esfenvalerate_Fenvalerate Isom 2**	Plant Protection products (PPPs)
Fluorene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Ethalfluralin**	Plant Protection products (PPPs)
Indeno(1,2,3-cd)pyrene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Ethion**	Plant Protection products (PPPs)
Phenanthrene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Ethoprophos**	Plant Protection products (PPPs)
Pyrene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Fenamiphos**	Plant Protection products (PPPs)
Naphthalene	Polycyclic Aromatic Hydrocarbons (PAHs)	**Fenitrothion**	Plant Protection products (PPPs)
PCB 101	Polychlorinated biphenyl (PCBs)	**Fenoxycarb**	Plant Protection products (PPPs)
PCB 138	Polychlorinated biphenyl (PCBs)	**Fenthion**	Plant Protection products (PPPs)
PCB 153	Polychlorinated biphenyl (PCBs)	**Fludioxonil**	Plant Protection products (PPPs)
PCB 180	Polychlorinated biphenyl (PCBs)	**Folpet**	Plant Protection products (PPPs)
PCB 28	Polychlorinated biphenyl (PCBs)	**Iprodione**	Plant Protection products (PPPs)
PCB 52	Polychlorinated biphenyl (PCBs)	**Malaoxon**	Plant Protection products (PPPs)
2.4-DDT	Organochlorine pesticides (OCPs)	**Malathion**	Plant Protection products (PPPs)
Dichlorobenzophenone	Organochlorine pesticides (OCPs)	**Methacrifos**	Plant Protection products (PPPs)
4.4-DDD	Organochlorine pesticides (OCPs)	**Methidathion**	Plant Protection products (PPPs)

Analyte	Group	Analyte	Group
4.4-DDE	Organochlorine pesticides (OCPs)	**Methomyl**	Plant Protection products (PPPs)
4.4-DDT	Organochlorine pesticides (OCPs)	**Methoprotryne**	Plant Protection products (PPPs)
Hexachlorobutadiene	Organochlorine pesticides (OCPs)	**Methoxychlor (DMTD)**	Plant Protection products (PPPs)
Dichlorvos	Organochlorine pesticides (OCPs)	**Metolachlor**	Plant Protection products (PPPs)
a-HCH	Organochlorine pesticides (OCPs)	**Metribuzin**	Plant Protection products (PPPs)
b-HCH	Organochlorine pesticides (OCPs)	**Monocrotophos**	Plant Protection products (PPPs)
c-HCH (Lindane)	Organochlorine pesticides (OCPs)	**Myclobutanil**	Plant Protection products (PPPs)
d-HCH	Organochlorine pesticides (OCPs)	**Omethoate**	Plant Protection products (PPPs)
Hexachlorobenzene	Organochlorine pesticides (OCPs)	**Paraoxon Eth**	Plant Protection products (PPPs)
Heptachlor Epoxide	Organochlorine pesticides (OCPs)	**Parathion-Ethyl**	Plant Protection products (PPPs)
Heptachlor	Organochlorine pesticides (OCPs)	**Parathion-Methyl**	Plant Protection products (PPPs)
Endosulfan alpha	Organochlorine pesticides (OCPs)	**Penconazole**	Plant Protection products (PPPs)
Endosulfan sulphate	Organochlorine pesticides (OCPs)	**Permethrin Isomer 1**	Plant Protection products (PPPs)
Aldrin	Organochlorine pesticides (OCPs)	**Permethrin Isomer 2**	Plant Protection products (PPPs)
Endrin	Organochlorine pesticides (OCPs)	**Phosalone**	Plant Protection products (PPPs)
Dicofol	Organochlorine pesticides (OCPs)	**Phosmet**	Plant Protection products (PPPs)
Dieldrin	Organochlorine pesticides (OCPs)	**Phosphamidon isomer 1**	Plant Protection products (PPPs)
Isodrin	Organochlorine pesticides (OCPs)	**Phosphamidon isomer 2**	Plant Protection products (PPPs)
Pentabromobenzyl acrylate	Organochlorine pesticides (OCPs)	**Pirimicarb**	Plant Protection products (PPPs)
Pentabromoethylbenzene	Organochlorine pesticides (OCPs)	**Pirimiphos ethyl**	Plant Protection products (PPPs)
Pentachlorobenzene	Organochlorine pesticides (OCPs)	**Procymidone**	Plant Protection products (PPPs)
Acephate	Plant Protection products (PPPs)	**Profenophos**	Plant Protection products (PPPs)
Acrinathrin	Plant Protection products (PPPs)	**Propazine**	Plant Protection products (PPPs)
Atrazine	Plant Protection products (PPPs)	**Propham**	Plant Protection products (PPPs)
Benfluralin	Plant Protection products (PPPs)	**Prothiophos**	Plant Protection products (PPPs)
Bifenthrin	Plant Protection products (PPPs)	**Pyrimiphos Me**	Plant Protection products (PPPs)

Analyte	Group	Analyte	Group
Bromophos Ethyl	Plant Protection products (PPPs)	**Quinalphos**	Plant Protection products (PPPs)
Bromophos Methyl	Plant Protection products (PPPs)	**Quinoxyfen**	Plant Protection products (PPPs)
Chlorfenvinphos mix Z&E isomer 1	Plant Protection products (PPPs)	**Quintozene**	Plant Protection products (PPPs)
Chlorfenvinphos mix Z&E isomer 2	Plant Protection products (PPPs)	**Simazine**	Plant Protection products (PPPs)
Chlorpyrifos Ethyl	Plant Protection products (PPPs)	**Spiroxamine Isomer 1**	Plant Protection products (PPPs)
Chlorpyrifos Methyl	Plant Protection products (PPPs)	**Spiroxamine Isomer 2**	Plant Protection products (PPPs)
Climbazole	Plant Protection products (PPPs)	**Tebuconazole**	Plant Protection products (PPPs)
Cyfluthrin Isomer 3	Plant Protection products (PPPs)	**Terbuthylazine**	Plant Protection products (PPPs)
Cyfluthrin Isomer 4	Plant Protection products (PPPs)	**Tetrachlorvinphos**	Plant Protection products (PPPs)
Cyhalothrin-lambda major	Plant Protection products (PPPs)	**Tetrasul**	Plant Protection products (PPPs)
Cypermethrin Isomer 1	Plant Protection products (PPPs)	**Triadimefon**	Plant Protection products (PPPs)
Cypermethrin Isomer 2	Plant Protection products (PPPs)	**Trifluralin**	Plant Protection products (PPPs)
Cypermethrin Isomer 3	Plant Protection products (PPPs)	**trans Chlordane**	Plant Protection products (PPPs)
Cypermethrin Isomer 4	Plant Protection products (PPPs)	**cis Chlordane**	Plant Protection products (PPPs)

4.6 Target screening of river water samples

A 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 compound, mainly plant protection products and it is continuously being updated. The database contained information about retention time, m/z and folmula for precursor,qualifiers and adducts as well as some chemical identifiers (e.g. CAS numbers).

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 ± 0.005Da.

4.7 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 theoritical (one that is recorded in the database) retention time (ΔRT: <0.1 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 5 mDa was considered as acceptable.

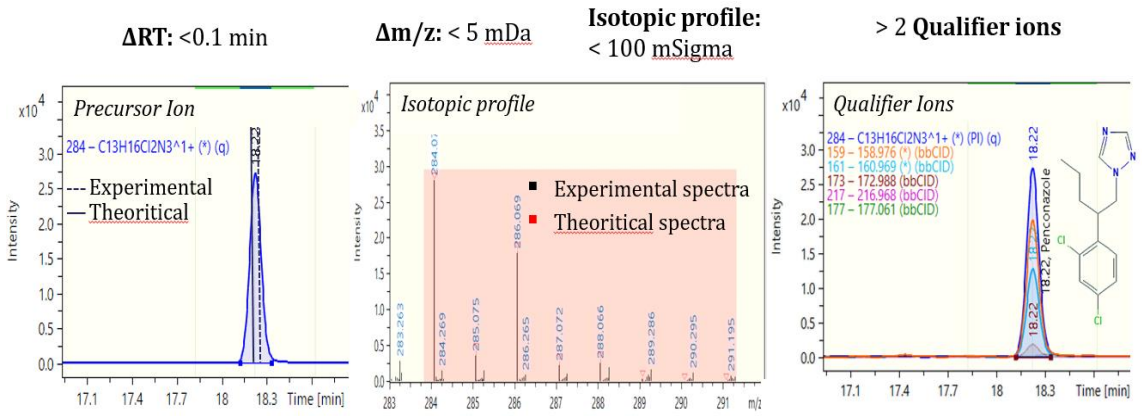

Figure 22: Evaluation of identification criteria for Penconazole.

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) appart from the precursor ion, was considered as an additional identification criterion. These four criteria are included in a score named MRSQ which is very helpful in the evaluation process of the screening results (**Figure 22**) exemplifies the evaluation of the aforementioned criteria for the identification of Penconazole. It is evident that this evaluation process, provides us high identification confidence for our final results in river water samples.

CHAPTER 5
Results and Discussion

5.1 General Observations

A generic method for the determination of GC-amenable PPs and ECs has been developed in the laboratoty of Analytical Chemistry in the National and Kapodistrian University of Athens. After the analysis of the tested samples using GC-APCI QTOF, we decided that SPE with C18 EC cartridges provides better results concerning the pre-concentration of the analytes and the clean-up of the river samples. Recoveries and matrix effects were determinated in order to conclude to the optimal sample preparation method. As regards the pre-concentration of the analytes, the initial volume of 1.25 L was selected. Moreover, the final method was validated for its performance characteristics (linearity, sensitivity, trueness, matrix effect and precission) for a set of 130 analytes. Finally, real river water samples were analysed using GC-APCI QTOFMS. The total results indicated the overall state of contamination of Asopos river basin due to the occurrence of GC-amenable PPs and ECs.

5.2 Internal Standard

The internal standard method was used to improve the accuracy of quantification. Different IS were used for each analyte. As internal standards, deuterium labeled PAHs standards, PCB 209, Triphenyl Phosphate (TPP) and 2,4,5,6-Tetrachloro-m-xylene were used. To evaluate if an internal standard correctly could be used for an analyte, the improvement of linearity, trueness and repeatability were investigated. Concerning the linearity evaluation, the correlation coefficient of each analyte's calibration curve (levels 10-100 μg L^{-1}) was compered. In first occasion for not using internal standard, the absolute areas was used and in the occasion of using an IS, the relative areas of analytes was used. The relative area was calculated by the below equation.

$$\boldsymbol{Relative\ Area} = \frac{\boldsymbol{Absolute\ Area\ of\ Analyte}}{\boldsymbol{Area\ of\ IS}}$$

It was observed that for all the analytes the correlation coefficient was improved by the use of IS. **Figures 23** and **24** illustrate the regression results for Deltamethrin, with and without the use of IS.

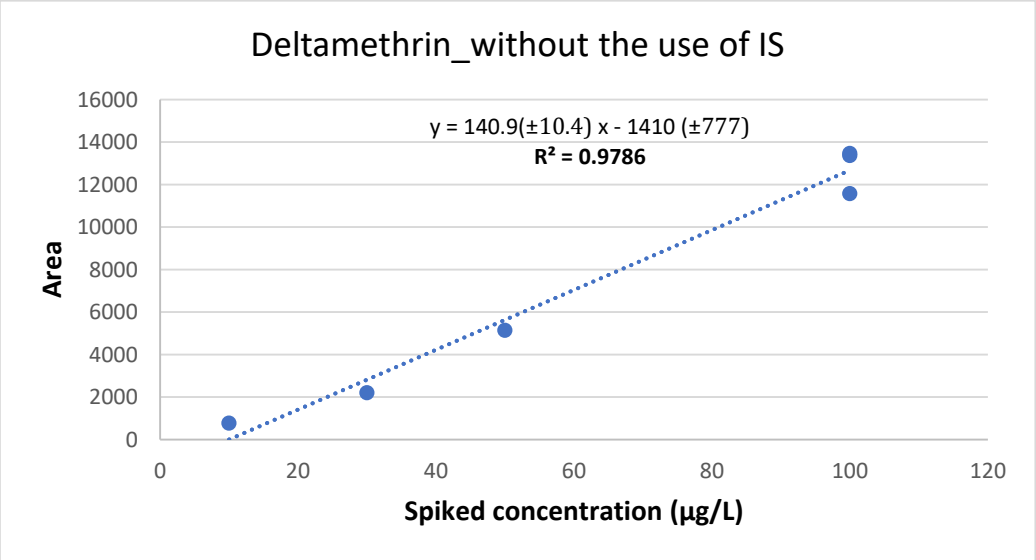

Figure 23: Calibration curve of Deltamethrin without IS.

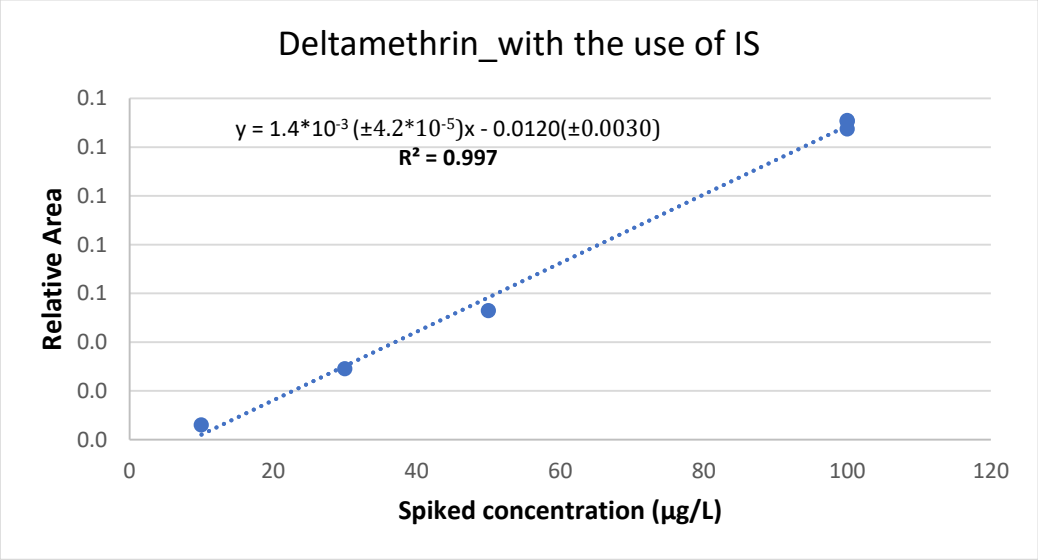

Figure 24: Calibration curve of Deltamethrin with the use of TPP (as IS).

5.3 Method Development

For the applicability domain of the developed method analytes with different physicochemical properties were selected. These analytes were the 30% of the total analytes in our current database in the laboratory.

- **Extraction**

Recoveries and matrix effects were calculated in order to conclude to the optimal sample preparation method.

Recovery rates

For the estimation of recovery, $\%R_{median}$ were calculated for each group of analytes (PAHs, OCPs, PCBs, PPPs) at each different extraction test. The overall results are shown at **Figure 25**. LLE provided better $\%R_{median}$ for PAHs as it was expected, since this method is the ISO method for the determination of PAHs. However, SPE provided satisfactory recoveries for most of the tested compounds with both sorbents (C18 and HLB). Moreover, LLE seems to provide low $\%R_{median}$ for plant protection products (PPPs). It is important to mention that PPPs are 80% of the total analytes in the current database and include many different sub-categories. Therefore, the final sample preparation method should be efficient for most of the tested compounds and especially for PPPs. For this reason, sample preparation using LLE was rejected. Also, the results of the other two cartridges which were used at solid phase extraction (SPE) were compered. Cartridge C18 EC provided better $\%R_{median}$ for most groups of analytes.

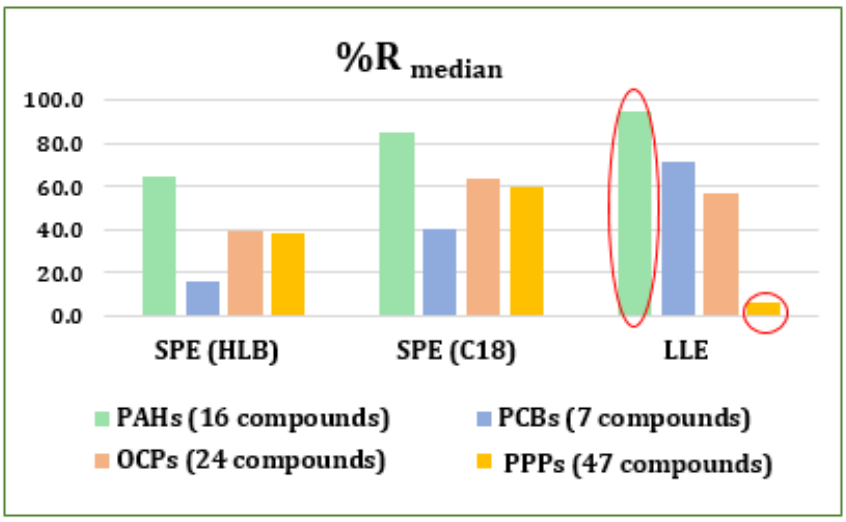

Figure 25: Median %Recoveries for the different extraction techniques that were tested.

Matrix Effect

Matrix effect (ME%) was considered as a vital characteristic of the method, thus it was evaluated in order to assess which exctraction technique was optimum. Matrix effect was calculated for all the analytes and the total results for each sample extraction technique are depicted in **Figure 26**. Ideally, the ME % is equal to zero meaning that neither ion suppression nor enhancement takes place. It has to be noticed that negative values of ME% indicate ion suppression while positive values ion enhancement.

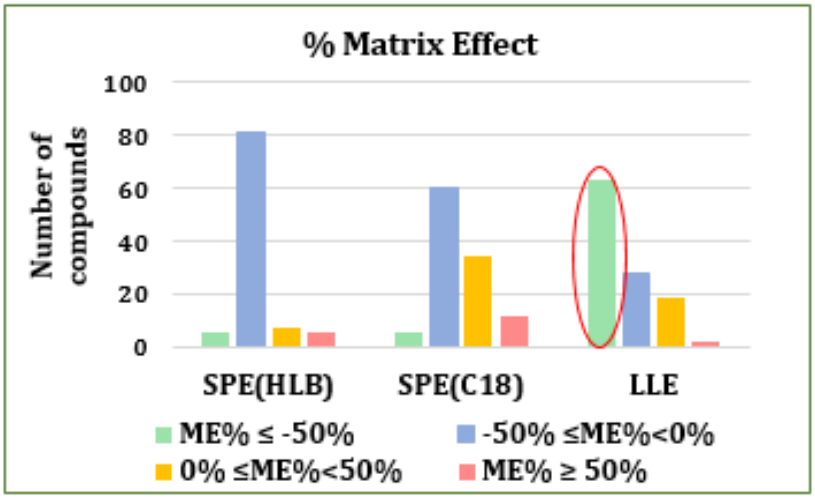

Figure 26: %Matrix Effect (%ME) for different extraction techniques.

LLE provided significant ion suppression for most of the tested compounds. On the other hand, SPE provided much lower matrix effect for most of the tested compounds, as it is indicated in **Figure 26** with the blue and yellow bars.

Taking into consideration both the recovery rate and the matrix effect results of the adove methods, the selected method was SPE with C18 EC cartridges (Test 2).

- **Pre-concentration**

As for the pre-concentration, with 10,000 times pre-concentration matrix effect was low (ME%<-50%) and there was significant ion suppression for most of the tested compounds. For this reason many of our spiked analytes couldn't be detected. With 1,000 times pre-concentration low limits of detection couldn't

be reached. As a results, the selected pre-concentration was 5,000 with initial volume 1.25 L.

5.4 Method Validation

In this study, the method was validated for 130 analytes, which constitute the 40% of the total analytes in the current database. These selected compounds represented almost all the classes of analytes in the database and covered a wide range of physicochemical properties.

5.4.1 Linearity

Linearity of the instrument was studied for each compound by analyzing standard solutions in six different concentrations levels (10, 30, 50, 100, 200, 300 μg L^{-1}). These standards were prepared in solvent (Hexane). In **Figure 27** is an example of instrument linearity of the analyte Benzo(g,h,i)perylene.

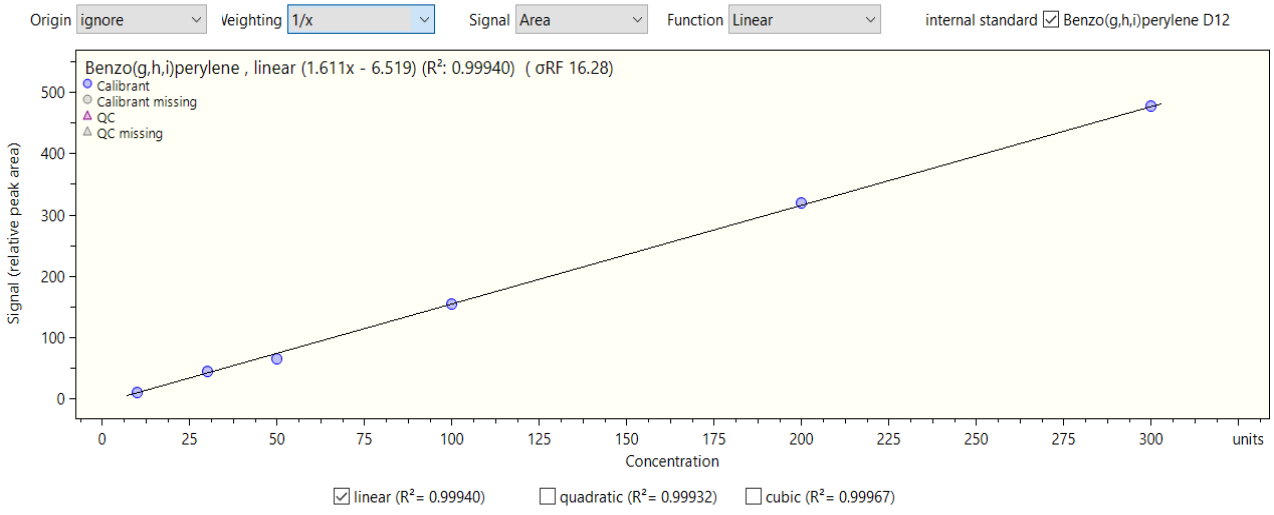

Figure 27: Linearity of the instrument for Benzo(g,h,i)perylene.

Moreover linearity of the method was studied for each compound by analyzing spiked samples in six different concentration levels (2,6, 10, 20, 40, 60 ng L^{-1}).

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

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

in which,

y: Relative peak area of each analyte

a: the slope

b: the intercept

C: concentration of analyte

S_b: Standard deviation of intercept

S_a: Standard deviation of slope

The correlation coefficients (R^2) for all calibration curves were also calculated. The values of all the above parameters are listed in **Table 6**.

The correlation coefficients (R^2) were above 0.985 for 80% of the tested compound in spiked samples. Moreover, the correlation coefficients (R^2) were above 0.990 for 90% of the tested compound in standard solutions.

Table 6: Calibratin curves for the instrument and the method using GC-APCI-QTOFMS.

Classification	Compound	I.S	River Water					Standards				
			a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
PAHs	**Acenapthene**	Acenapthene D10	0.0248	0.0010	0.277	0.051	0.993	0.02361	0.00057	-0.053	0.048	0.996
	Acenapthylene	Acenapthylene D8	0.02249	0.00042	-0.066	0.024	0.998	0.02108	0.00052	-0.048	0.039	0.998
	Anthracene	Anthracene D10	0.01397	0.00048	0.217	0.024	0.995	0.02627	0.00060	-0.039	0.045	0.998
	Benzo(a)pyrene	Benzo(a)pyrene D12	0.01560	0.00038	0.046	0.023	0.997	0.0297	0.0011	-0.201	0.086	0.993
	Benzo(b)fluoranthene	Benzo(b)fluoranthene D12	0.01274	0.00073	0.096	0.043	0.993	0.1216	0.0068	-1.49	0.57	0.978
	Benz(a)anthracene	Benzo(a)anthracene D12	0.01281	0.00066	0.064	0.042	0.989	0.0309	0.0010	-0.144	0.083	0.994
	Benzo(g,h,i)perylene	Benzo(g,h,i)perylene D12	0.02391	0.00057	-0.229	0.030	0.998	0.0318	0.0012	-0.136	0.094	0.992
	Benzo(k)fluoranthene	Benzo(k)fluoranthene D12	0.01692	0.00070	-0.020	0.035	0.993	0.0374	0.0014	-0.30	0.12	0.990
	Chrysene	Chrysene D12	0.01041	0.00069	0.099	0.035	0.983	0.02653	0.00088	-0.183	0.074	0.992
	Dibenzo(a,h)anthracene	Dibenzo(a,h)anthracene D14	0.01499	0.00075	0.074	0.048	0.990	0.0308	0.0011	-0.193	0.093	0.992
	Fluoranthene	Fluoranthene D10	0.0194	0.0014	2.131	0.073	0.986	0.02789	0.00095	-0.105	0.080	0.992

	Compound	I.S	River Water					Standards				
			a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
	Fluorene	Fluorene D10	0.01408	0.00066	0.656	0.040	0.989	0.02001	0.00037	-0.076	0.030	0.998
	Indeno(1,2,3-cd)pyrene	Indeno(1,2,3-cd)pyrene D12	0.01459	0.00078	0.178	0.038	0.986	0.02938	0.00063	-0.170	0.053	0.997
	Pyrene	Pyrene D10	0.01313	0.00080	0.442	0.043	0.989	0.02642	0.00066	-0.126	0.052	0.997
PCBs	**PCB 101**	PCB 209	0.0568	0.0022	1.69	0.12	0.995	0.0296	0.0027	1.65	0.21	0.960
	PCB 138	PCB 209	0.02254	0.00078	0.912	0.059	0.995	0.0232	0.0011	0.576	0.090	0.988
	PCB 180	PCB 209	0.01273	0.00077	0.557	0.045	0.993	0.0205	0.0013	0.464	0.077	0.992
	PCB 28	NO IS	327.9	7.6	-2713	441	0.999	0.0925	0.0080	1.76	0.46	0.985
	PCB 52	NO IS	110.294	0.022	-782.4	1.3	0.990	0.0506	0.0043	1.71	0.32	0.972
OCPs	**2.4-DDT**	Triphenyl phosphate	0.001102	0.000075	0.0102	0.0043	0.991	0.00642	0.00043	-0.018	0.025	0.991
	Dichlorobenzophenone	Triphenyl phosphate	0.00299	0.00016	-0.0159	0.0084	0.992	0.00542	0.00021	-0.019	0.017	0.991
	4.4-DDD	Triphenyl phosphate	0.00272	0.00017	0.0034	0.0094	0.988	0.01066	0.00039	-0.080	0.032	0.992
	4.4-DDE	Triphenyl phosphate	0.00186	0.00011	-0.0041	0.0057	0.990	0.00705	0.00028	-0.025	0.022	0.992
	4.4-DDT	Triphenyl phosphate	0.0001233	0.0000086	0.00241	0.00050	0.990	0.003185	0.000094	0.0052	0.0077	0.995
	Hexachlorobutadiene	2,4,5,6-Tetrachloro-m-xylene	0.00230	0.00018	0.021	0.010	0.988	0.01256	0.00050	-0.087	0.039	0.992
	Dichlorvos	Triphenyl phosphate	0.00365	0.00024	0.016	0.013	0.987	0.01004	0.00044	-0.050	0.025	0.996

Compound	I.S	River Water					Standards				
		a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
a-HCH	Triphenyl phosphate	0.001649	0.000091	-0.0100	0.0063	0.991	0.00345	0.00014	-0.014	0.012	0.990
b-HCH	Triphenyl phosphate	0.000449	0.000018	0.0030	0.0011	0.994	0.001872	0.000067	-0.0069	0.0055	0.992
c-HCH (Lindane)	Triphenyl phosphate	0.001207	0.000080	-0.0014	0.0047	0.991	0.002821	0.000092	-0.0062	0.0075	0.994
d-HCH	Triphenyl phosphate	0.000546	0.000026	-0.0015	0.0014	0.993	0.001821	0.000074	-0.0141	0.0058	0.992
Hexachlorobenzene	2,4,5,6-Tetrachloro-m-xylene	0.01571	0.00068	0.014	0.039	0.989	0.00920	0.00034	-0.032	0.028	0.992
Heptachlor Epoxide	Triphenyl phosphate	0.000648	0.000036	0.0032	0.0019	0.991	0.001992	0.000066	-0.0078	0.0056	0.992
Heptachlor	Triphenyl phosphate	0.000229	0.000022	0.0071	0.0012	0.974	0.001474	0.000058	-0.0042	0.0047	0.991
Endosulfan alpha	Triphenyl phosphate	0.000936	0.000017	-0.00047	0.00092	0.999	0.001657	0.000076	-0.0115	0.0057	0.992
Endosulfan sulphate	Triphenyl phosphate	0.000570	0.000016	-0.00193	0.00086	0.998	0.001686	0.000064	-0.0088	0.0051	0.993
Aldrin	Triphenyl phosphate	0.000297	0.000015	0.00074	0.00085	0.995	0.001591	0.000052	-0.0091	0.0044	0.993
Endrin	Triphenyl phosphate	0.000820	0.000028	-0.00004	0.0015	0.997	0.001166	0.000051	-0.0053	0.0041	0.990
Dicofol	Triphenyl phosphate	0.00316	0.00023	-0.016	0.015	0.978	0.00535	0.00020	-0.018	0.017	0.991
Dieldrin	Triphenyl phosphate	0.00177	0.00010	-0.0126	0.0063	0.978	0.002447	0.000071	-0.0013	0.0060	0.994
Isodrin	Triphenyl phosphate	0.000620	0.000032	-0.0054	0.0020	0.990	0.001859	0.000074	-0.0032	0.0061	0.991

	Compound	I.S	River Water					Standards				
			a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
	Pentabromoethylbenzene	2,4,5,6-Tetrachloro-m-xylene	0.00270	0.00036	-0.089	0.068	0.966	0.000489	0.000027	0.0031	0.0018	0.991
	Pentachlorobenzene	2,4,5,6-Tetrachloro-m-xylene	0.0147	0.0010	0.129	0.053	0.980	0.01729	0.00070	-0.097	0.057	0.990
Plant Protection Products (PPPs)	**Acrinathrin**	Triphenyl phosphate	0.000423	0.000015	-0.0016	0.0013	0.994	0.00393	0.00018	-0.041	0.014	0.989
	Atrazine	Triphenyl phosphate	0.0124	0.0011	-0.091	0.061	0.976	0.02300	0.00072	-0.094	0.061	0.993
	Benfluralin	Triphenyl phosphate	0.01534	0.00096	-0.161	0.049	0.984	0.02565	0.00084	-0.172	0.066	0.995
	Bifenthrin	Triphenyl phosphate	0.00612	0.00034	-0.047	0.018	0.991	0.0286	0.0015	0.00	0.10	0.992
	Bromophos Ethyl	Triphenyl phosphate	0.00407	0.00025	0.011	0.013	0.985	0.01368	0.00054	-0.090	0.044	0.991
	Bromophos Methyl	Triphenyl phosphate	0.00219	0.00021	-0.002	0.011	0.965	0.00498	0.00022	-0.036	0.017	0.991
	Chlorfenvinphos mix Z&E isomer 1	Triphenyl phosphate	0.001013	0.000055	-0.0044	0.0028	0.988	0.001470	0.000067	-0.0017	0.0050	0.992
	Chlorfenvinphos mix Z&E isomer 2	Triphenyl phosphate	0.00934	0.00043	-0.006	0.023	0.994	0.0222	0.0011	-0.177	0.080	0.991
	Chlorpyrifos Ethyl	Triphenyl phosphate	0.0206	0.0017	-0.139	0.083	0.966	0.0431	0.0017	-0.12	0.14	0.992
	Chlorpyrifos Methyl	Triphenyl phosphate	0.00430	0.00026	-0.036	0.013	0.981	0.00766	0.00034	-0.040	0.027	0.990
	Climbazole	Triphenyl phosphate	0.00096	0.00011	0.0331	0.0063	0.975	0.00496	0.00054	-0.071	0.031	0.977
	Cyanazine	Triphenyl phosphate	0.00201	0.00017	0.0083	0.0092	0.979	0.01258	0.00052	-0.093	0.039	0.993
	Cyfluthrin Isomer 4	Triphenyl phosphate	0.000353	0.000028	0.0118	0.0032	0.988	0.00234	0.00010	0.0019	0.0076	0.993

Compound	I.S	River Water					Standards				
		a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
Cyhalothrin-lambda	Triphenyl phosphate	0.000793	0.000070	0.0080	0.0048	0.977	0.00891	0.00034	-0.053	0.028	0.990
Cypermethrin Isomer 1	Triphenyl phosphate	0.000512	0.000039	0.0019	0.0023	0.989	0.00303	0.00014	-0.001	0.010	0.991
Cypermethrin Isomer 2	Triphenyl phosphate	0.000461	0.000029	0.0016	0.0016	0.988	0.00324	0.00011	-0.0097	0.0081	0.995
Cypermethrin Isomer 3	Triphenyl phosphate	0.000626	0.000049	0.0010	0.0026	0.982	0.003170	0.000081	-0.0148	0.0064	0.997
Cypermethrin Isomer 4	Triphenyl phosphate	0.000549	0.000035	-0.0021	0.0021	0.980	0.00262	0.00013	-0.0099	0.0094	0.991
Cyproconazole	2,4,5,6-Tetrachloro-m-xylene	0.913	0.047	0.2	2.7	0.995	0.0307	0.0017	-0.26	0.12	0.991
Cyprodinil	Triphenyl phosphate	0.00633	0.00030	0.083	0.015	0.991	0.02396	0.00093	-0.119	0.076	0.991
Deltamethrin	Triphenyl phosphate	0.000164	0.000011	-0.0007	0.0011	0.982	0.001408	0.000042	-0.0120	0.0031	0.997
Diazinon	Triphenyl phosphate	0.01947	0.00069	-0.021	0.035	0.995	0.0435	0.0013	-0.13	0.10	0.995
Dichlofenthion	Triphenyl phosphate	0.0000840	0.0000026	0.00095	0.00015	0.998	0.000147	0.000007	0.00065	0.00051	0.991
Ditalimfos	Triphenyl phosphate	0.000469	0.000020	-0.0007	0.0012	0.996	0.000842	0.000050	-0.0023	0.0034	0.990
Esfenvalerate_Fenvalerate Isomer 1	Triphenyl phosphate	0.000931	0.000083	0.0045	0.0048	0.984	0.00655	0.00033	-0.023	0.024	0.990
Esfenvalerate_Fenvalerate Isomer 2	Triphenyl phosphate	0.000438	0.000028	-0.0019	0.0016	0.992	0.002014	0.000073	-0.0095	0.0061	0.991
Ethalfluralin	Triphenyl phosphate	0.00655	0.00041	-0.024	0.024	0.992	0.01328	0.00051	-0.100	0.042	0.991

Compound	I.S	River Water					Standards				
		a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
Ethion	Triphenyl phosphate	0.00997	0.00090	-0.017	0.054	0.961	0.0314	0.0012	-0.22	0.10	0.991
Ethoprophos	Triphenyl phosphate	0.00582	0.00035	-0.023	0.019	0.989	0.01059	0.00044	-0.062	0.034	0.992
Fenamiphos	Triphenyl phosphate	0.00279	0.00018	0.0430	0.0097	0.988	0.0153	0.0014	-0.151	0.081	0.984
Fenitrothion	Triphenyl phosphate	0.01013	0.00072	0.013	0.042	0.990	0.0275	0.0012	-0.258	0.092	0.991
Fenoxycarb	Triphenyl phosphate	0.00369	0.00028	0.043	0.014	0.978	0.01223	0.00042	-0.061	0.035	0.992
Fenthion	Triphenyl phosphate	0.01299	0.00067	0.028	0.068	0.990	0.0471	0.0019	-0.08	0.15	0.992
Iprodione	Triphenyl phosphate	0.000542	0.000043	-0.0071	0.0039	0.958	0.001476	0.000070	-0.0018	0.0055	0.989
Malaoxon	Triphenyl phosphate	0.00678	0.00034	-0.017	0.018	0.992	0.01004	0.00029	-0.067	0.022	0.997
Malathion	Triphenyl phosphate	0.00678	0.00026	-0.032	0.014	0.996	0.02028	0.00064	-0.159	0.052	0.994
Methacrifos	Triphenyl phosphate	0.0165	0.0013	-0.080	0.065	0.976	0.02848	0.00056	0.048	0.047	0.997
Methoprotryne	Triphenyl phosphate	0.00517	0.00028	0.0513	0.014	0.988	0.01968	0.00072	-0.154	0.060	0.991
Methoxychlor (DMTD)	Triphenyl phosphate	0.000292	0.000013	0.0070	0.0013	0.990	0.000766	0.000038	0.0057	0.0029	0.990
Metolachlor	Triphenyl phosphate	0.019	0.001	-0.112	0.059	0.989	0.01860	0.00075	-0.112	0.059	0.992
Myclobutanil	Triphenyl phosphate	0.00470	0.00039	-0.025	0.020	0.973	0.01920	0.00096	-0.154	0.072	0.990

Compound	I.S	River Water					Standards				
		a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
Omethoate	Triphenyl phosphate	0.0090	0.000	-0.050	0.018	0.982	0.00907	0.00022	-0.067	0.018	0.997
Paraoxon Eth	Triphenyl phosphate	0.01587	0.00020	0.002	0.012	1.000	0.0297	0.0015	-0.31	0.11	0.990
Parathion-Ethyl	Triphenyl phosphate	0.0363	0.0019	-0.09	0.11	0.994	0.0672	0.0030	-0.17	0.23	0.992
Parathion-Methyl	Triphenyl phosphate	0.00550	0.00024	0.007	0.013	0.994	0.01567	0.00086	-0.128	0.059	0.991
Penconazole	Triphenyl phosphate	0.00861	0.00027	-0.040	0.016	0.998	0.01273	0.00061	-0.099	0.046	0.991
Permethrin Isomer 1	Triphenyl phosphate	0.00101	0.00011	0.0193	0.0059	0.965	0.00803	0.00025	-0.043	0.020	0.994
Permethrin Isomer 2	Triphenyl phosphate	0.00596	0.00063	0.043	0.037	0.978	0.0327	0.0016	0.09	0.12	0.989
Phosalone	Triphenyl phosphate	0.000347	0.000025	0.0186	0.0014	0.990	0.00411	0.00017	-0.038	0.014	0.990
Phosmet	Triphenyl phosphate	0.000568	0.000029	0.0240	0.0017	0.995	0.00976	0.00047	-0.088	0.035	0.991
Phosphamidon isomer 1	Triphenyl phosphate	0.000526	0.000011	-0.0029	0.0010	0.998	0.001494	0.000070	-0.0366	0.0057	0.993
Phosphamidon isomer 2	Triphenyl phosphate	0.001203	0.000067	0.0023	0.0039	0.994	0.00220	0.00015	-0.0258	0.0086	0.991
Pirimicarb	Triphenyl phosphate	0.01197	0.00072	-0.083	0.039	0.989	0.02189	0.00079	-0.134	0.062	0.994
Pirimiphos ethyl	Triphenyl phosphate	0.0205	0.0011	0.017	0.054	0.989	0.0544	0.0020	0.04	0.15	0.994
Procymidone	Triphenyl phosphate	0.0126	0.0033	0.01	0.11	0.935	0.0368	0.0016	-0.13	0.12	0.991

Compound	I.S	River Water					Standards				
		a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
Profenophos	Triphenyl phosphate	0.00532	0.00020	-0.010	0.012	0.993	0.01786	0.00073	-0.150	0.060	0.990
Propazine	Triphenyl phosphate	0.01893	0.00023	-0.007	0.013	1.000	0.0302	0.0011	0.018	0.090	0.993
Propham	Triphenyl phosphate	0.00404	0.00030	-0.012	0.016	0.984	0.00703	0.00026	-0.030	0.021	0.992
Prothiophos	Triphenyl phosphate	0.00643	0.00025	-0.009	0.013	0.996	0.0309	0.0010	-0.204	0.082	0.994
Pyrimiphos Me	Triphenyl phosphate	0.010	0.000	0.018	0.000	1.000	0.0361	0.0013	-0.20	0.10	0.994
Quinalphos	Triphenyl phosphate	0.00967	0.00068	-0.037	0.034	0.981	0.0267	0.0011	-0.217	0.086	0.991
Quinoxyfen	Triphenyl phosphate	0.00356	0.00011	-0.0145	0.0059	0.997	0.00878	0.00031	-0.059	0.026	0.992
Quintozene	2,4,5,6-Tetrachloro-m-xylene	0.0270	0.0014	-0.106	0.081	0.995	0.01318	0.00044	-0.051	0.036	0.993
Simazine	Triphenyl phosphate	0.001420	0.000064	0.0053	0.0037	0.996	0.00428	0.00015	-0.015	0.012	0.993
Tebuconazole	Triphenyl phosphate	0.001310	0.000071	0.0215	0.0041	0.994	0.01138	0.00060	-0.088	0.035	0.994
Terbuthylazine	Triphenyl phosphate	0.01893	0.00023	-0.007	0.013	1.000	0.02951	0.00092	0.034	0.069	0.996
Tetrachlorvinphos	Triphenyl phosphate	0.002109	0.000083	0.0118	0.0040	0.992	0.00658	0.00026	-0.065	0.021	0.991
Tetrasul	Triphenyl phosphate	0.001244	0.000066	-0.0037	0.0034	0.989	0.00319	0.00011	-0.0031	0.0087	0.993
Triadimefon	Triphenyl phosphate	0.00774	0.00014	0.0142	0.0084	0.999	0.01957	0.00082	-0.156	0.061	0.993

	Compound	I.S	River Water					Standards				
			a	Sa	b	Sb	R^2	a	Sa	b	Sb	R^2
	Trifluralin	Triphenyl phosphate	0.00746	0.00038	-0.076	0.019	0.990	0.01396	0.00051	-0.086	0.041	0.993
	trans Chlordane	2,4,5,6-Tetrachloro-m-xylene	0.00317	0.00020	0.005	0.044	0.988	0.001826	0.000071	-0.0050	0.0056	0.993
	cis Chlordane	2,4,5,6-Tetrachloro-m-xylene	0.00527	0.00032	0.014	0.019	0.993	0.001962	0.000079	0.0076	0.0059	0.994

4.2 Sensitivity

For the sensitivity Limits of detection (LOD) and limits of Quantification (LOQ) were calculated for the instrument (ILOD, ILOQ) and the method (MLOD, MLOQ). 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. LOQ is the lowest concentration of the analyte that has been validated with acceptablele accuracy by applying the complete analytical method. In this thesis, we estimated the LOD and LOQ for the instrument (ILOD) and method (MLOD). For the estimation we used the below equations:

$$\boldsymbol{LOD = 3.3 \times \frac{S_b}{a}}$$

$$\boldsymbol{LOQ = 10 \times \frac{S_b}{a}}$$

In which,

S_b: Standard deviation of intercept

a: the slope of curve

For ILODs Sb and a were determinated from the calibration curves while MLODS were determinated from standard addition curves. The results of ILODs, ILOQs, MLODs andMLOQs are in **Table 7**. The ranges of ILODs and ILOQs and for MLODs, MLOQs are in **Table 8**.

Table 7: Total results of LODs and LOQs.

Classification	Compound	River Water		Standards	
		MLOD (ng/L)	MLOQ (ng/L)	ILOD (µg/L)	ILOQ (µg/L)
PAHs	Acenapthene	1.4	4.1	6.7	20.4
	Acenapthylene	0.7	2.2	6.1	18.5
	Anthracene	1.1	3.4	5.6	17
	Benzo(a)pyrene	1.0	3.0	9.5	28.9
	Benzo(b)fluoranthene	2.2	6.7	15.6	47.3
	Benz(a)anthracene	2.2	6.6	8.9	26.9
	Benzo(g,h,i)perylene	0.8	2.5	9.8	29.6
	Benzo(k)fluoranthene	1.1	3.3	10.4	31.6
	Chrysene	0.8	2.4	9.1	27.7
	Dibenzo(a,h)anthracene	0.8	2.5	10.0	30.3
	Fluoranthene	2.5	7.5	9.5	28.6
	Fluorene	4.6	14	5.0	15.1
	Indeno(1,2,3-cd)pyrene	1.2	3.8	5.9	18
	Phenanthrene	16.5	50.0	10.7	32.4
	Pyrene	2.2	6.6	6.5	19.8
	Naphthalene	19.8	60	13.1	39.8
PCBs	PCB 101	1.4	4.2	23.8	72.1
	PCB 138	1.7	5.2	12.8	38.8
	PCB 153	22.5	68.2	19.9	60.3
	PCB 180	2.3	7.0	12.4	37.7
	PCB 28	0.9	2.7	16.5	50.1
	PCB 52	1.6	4.8	21.0	63.6
OCPs	2.4-DDT	1.9	5.9	12.8	38.8
	Dichlorobenzophenone	1.9	5.6	10.5	31.8
	4.4-DDD	2.1	6.2	10.0	30.3
	4.4-DDE	1.4	4.2	10.2	30.8
	4.4-DDT	2.7	8.1	8.0	24.2
	Hexachlorobutadiene	3.0	9.0	10.3	31.1
	Dichlorvos	2.3	7.1	8.3	25.2
	a-HCH	2.5	7.6	11	33.4
	b-HCH	1.6	5.0	9.7	29.3
	c-HCH (Lindane)	2.5	7.7	8.8	26.6
	d-HCH	1.7	5.2	10.6	32.0
	Hexachlorobenzene	1.4	4.2	10.1	30.6
	Heptachlor Epoxide	2.0	6.0	9.2	27.9
	Heptachlor	3.4	10.2	10.6	32.0
	Endosulfan alpha	0.6	2	11.3	34.1
	Endosulfan sulphate	1.0	3.0	9.9	30.0
	Aldrin	1.9	5.7	9.0	27.4

	Compound	River Water		Standards	
		MLOD (ng/L)	MLOQ (ng/L)	ILOD (µg/L)	ILOQ (µg/L)
	Endrin	1.2	3.6	11.5	34.8
	Dicofol	3.1	9.5	10.3	31.3
	Dieldrin	0.6	1.8	8.0	24.4
	Isodrin	2.2	6.6	10.8	32.7
	Pentabromoethylbenzene	16.6	50.4	12.4	37.5
	Pentachlorobenzene	0.7	2.0	10.9	32.9
Plant Protection Products (PPPs)	**Acrinathrin**	2.1	6.3	12.1	36.6
	Atrazine	0.2	0.6	8.7	26.3
	Benfluralin	2.1	6.3	8.5	25.9
	Bifenthrin	2.0	6.0	12.0	36.4
	Bromophos Ethyl	0.5	1.6	10.7	32.4
	Bromophos Methyl	3.2	9.6	11.4	34.5
	Chlorfenvinphos mix Z&E isomer 1	1.8	5.5	11.3	34.1
	Chlorfenvinphos mix Z&E isomer 2	1.6	5.0	12.0	36.3
	Chlorpyrifos Ethyl	0.1	0.3	10.3	31.3
	Chlorpyrifos Methyl	2	5.9	11.5	34.8
	Climbazole	4.3	13.1	20.9	63.3
	Cyanazine	3.00	9.1	10.2	31
	Cyfluthrin Isomer 4	6.00	18	10.6	32.3
	Cyhalothrin-lambda major	2.5	7.5	10.5	31.7
	Cypermethrin Isomer 1	2.9	8.9	11.4	34.6
	Cypermethrin Isomer 2	1.7	5.0	8.3	25.2
	Cypermethrin Isomer 3	1.7	5.0	6.7	20.2
	Cypermethrin Isomer 4	2.6	7.8	11.8	35.8
	Cyproconazole	0.1	0.2	12.6	38.3
	Cyprodinil	1.6	4.9	10.4	31.6
	Deltamethrin	4.6	14	7.3	22.1
	Diazinon	1.2	3.6	7.9	24.0
	Dichlofenthion	1.2	3.6	11.5	34.9
	Ditalimfos	1.6	5	13.3	40.4
	Esfenvalerate_Fenvalerate Isomer 1	1.7	5.0	12.3	37.2
	Esfenvalerate_Fenvalerate Isomer 2	2.4	7.4	10.0	30.4
	Ethalfluralin	2.4	7.3	10.4	31.5
	Ethion	3.6	10.9	10.4	31.7
	Ethoprophos	2.1	6.5	10.7	32.5
	Fenamiphos	2.3	6.9	17.4	52.8
	Fenitrothion	2.7	8.2	11.1	33.5
	Fenoxycarb	2.5	7.5	9.5	28.7
	Fenthion	3.4	10.4	10.6	32.0
	Iprodione	4.8	14.5	12.4	37.5

Compound	River Water		Standards	
	MLOD (ng/L)	MLOQ (ng/L)	ILOD (µg/L)	ILOQ (µg/L)
Malaoxon	1.8	5.4	7.2	21.9
Malathion	1.3	4.1	8.5	25.6
Methacrifos	2.6	7.9	5.4	16.4
Methidathion	1.3	4.1	9.8	29.6
Methoprotryne	1.8	5.5	10.1	30.7
Methoxychlor (DMTD)	2.9	8.8	12.3	37.4
Metolachlor	0.2	0.7	10.5	31.9
Monocrotophos	35.1	70.6	9.4	28.5
Myclobutanil	2.8	8.4	12.3	37.4
Paraoxon Eth	0.5	1.5	12.3	37.3
Parathion-Ethyl	2.0	6.2	11.1	33.6
Parathion-Methyl	1.6	4.7	12.4	37.6
Penconazole	0.1	0.4	11.9	36.1
Permethrin Isomer 1	3.9	11.7	8.3	25.3
Permethrin Isomer 2	4.1	12.3	12.4	37.5
Phosalone	2.7	8.3	11.0	33.4
Phosmet	2.0	6.0	11.8	35.8
Phosphamidon isomer 1	1.3	3.9	12.7	38.3
Phosphamidon isomer 2	2.1	6.5	12.9	39.2
Pirimicarb	2.1	6.5	9.3	28.3
Pirimiphos ethyl	1.7	5.3	9.4	28.5
Procymidone	5.9	18	11.2	33.9
Profenophos	1.5	4.6	11.0	33.4
Propazine	0.1	0.3	9.9	29.9
Propham	2.6	7.9	9.9	30.0
Prothiophos	1.4	4.1	8.8	26.6
Pyrimiphos Me	2.2	6.5	9.2	27.8
Quinalphos	2.3	7.1	10.7	32.3
Quinoxyfen	1.1	3.3	9.7	29.3
Quintozene	2.0	6.0	9.1	27.5
Simazine	1.7	5.2	9.4	28.4
Tebuconazole	2.1	6.3	10.1	30.7
Terbuthylazine	0.1	0.3	7.7	23.3
Tetrachlorvinphos	1.2	3.8	10.6	32.2
Tetrasul	1.8	5.4	9.0	27.4
Triadimefon	0.7	2.2	10.4	31.4
Trifluralin	1.7	5.1	9.6	29.1
trans Chlordane	9.2	27.7	10.1	30.5
cis Chlordane	2.3	7.0	9.9	30.1

Table 8: Concentration ranges of ILODs, ILOQs, MLODs and MLOQs.

	MLOD	MLOQ	ILOD	ILOQ
	Concentration Range			
	(ng/L)		(µg/L)	
PAHs	0.7-19.8	2.2-60.0	5.0-15.6	15.1-47.3
PCBs	0.9-22.5	2.7-68.2	12.4-23.8	37.7-72.1
OCPs	0.6-16.6	1.8-50.4	8.0-12.8	24.2-38.8
PPPs	0.1-35.1	0.2-70.6	5.4-20.9	16.4-63.3

5.4.3 Trueness

The accuracy was assesed by measuring the recovery rate for each analyte after the sample preparation of the spiked samples at three concentration levels (6, 20, 60 ng L^{-1}).In this work the recovery is given by the following equation:

$$\%\boldsymbol{R} = \frac{\boldsymbol{Relative\ area_{(spiked\ samples)} - Relative\ area_{(blank\ sample)}}}{\boldsymbol{Relative\ area_{(M.M.\ samples)} - Relative\ area_{(blank\ sample)}}} \times \mathbf{100}$$

The results of % Recoveries for all concentration levels are shown in **Table 9.**

Table 9: % Recoveries at three concentration levels.

Classification	Compound	%Recovery		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
PAHs	**Acenapthene**	170	171	187
	Acenapthylene	60	80	75
	Anthracene	77	67	60
	Benzo(a)pyrene	55	66	61
	Benzo(b)fluoranthene	9	11	16
	Benz(a)anthracene	46	53	48
	Benzo(g,h,i)perylene	51	57	42
	Benzo(k)fluoranthene	44	57	41
	Chrysene	43	61	52
	Dibenzo(a,h)anthracene	40	57	18
	Fluoranthene	130	78	59
	Fluorene	42	56	40
	Indeno(1,2,3-cd)pyrene	37	57	29
	Pyrene	88	72	42

Classification	Compound	%Recovery		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
PCBs	PCB 101	130	168	115
	PCB 138	130	107	110
	PCB 180	92	77	68
	PCB 28	26	35	22
	PCB 52	51	66	46
OCPs	2.4-DDT	53	58	39
	4,4'-Dichlorobenzophenone	82	110	55
	4.4-DDD	43	84	41
	4.4-DDE	47	58	45
	4.4-DDT	46	40	19
	Hexachlorobutadiene	28	8	7
	Dichlorvos	42	37	38
	a-HCH	42	110	18
	b-HCH	52	60	36
	c-HCH (Lindane)	42	108	49
	d-HCH	62	68	41
	Hexachlorobenzene	121	110	105
	Heptachlor Epoxide	62	104	51
	Heptachlor	42	85	49
	Endosulfan alpha	57	103	56
	Endosulfan sulphate	55	61	39
	Aldrin	31	48	35
	Dicofol	61	77	49
	Dieldrin	77	86	65
	Isodrin	45	71	24
	Pentabromoethylbenzene	60	110	87
	Pentachlorobenzene	85	73	82
Plant Protection Products (PPPs)	Acrinathrin	17	28	15
	Atrazine	40	79	38
	Benfluralin	52	89	75
	Bifenthrin	16	35	24
	Bromophos Ethyl	58	87	61
	Bromophos Methyl	81	106	69
	Chlorfenvinphos mix Z&E isomer 1	84	110	65
	Chlorfenvinphos mix Z&E isomer 2	76	107	74
	Chlorpyrifos Ethyl	59	77	69
	Chlorpyrifos Methyl	65	72	59
	Climbazole	42	39	30

	Compound	%Recovery		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
	Cyanazine	52	44	55
	Cyfluthrin Isomer 3	18	38	22
	Cyfluthrin Isomer 4	24	33	37
	Cyhalothrin-lambda major	19	23	16
	Cypermethrin Isomer 1	25	36	19
	Cypermethrin Isomer 2	21	29	15
	Cypermethrin Isomer 3	21	38	19
	Cypermethrin Isomer 4	21	34	17
	Cyprodinil	78	87	65
	Deltamethrin	18	27	19
	Diazinon	61	89	72
	Dichlofenthion	111	121	75
	Ditalimfos	61	98	70
	Esfenvalerate_Fenvalerate Isomer 1	19	29	13
	Esfenvalerate_Fenvalerate Isomer 2	33	30	17
	Ethalfluralin	42	77	41
	Ethion	59	85	76
	Ethoprophos	62	81	65
	Fenamiphos	68	52	42
	Fenitrothion	75	104	81
	Fenoxycarb	72	93	63
	Fenthion	53	89	64
	Iprodione	29	57	26
	Malaoxon	63	83	71
	Malathion	50	114	40
	Methacrifos	52	109	65
	Methidathion	66	87	64
	Methoprotryne	73	81	87
	Methoxychlor (DMTD)	24	32	21
	Myclobutanil	55	41	63
	Paraoxon Eth	73	94	91
	Parathion-Ethyl	110	151	143
	Parathion-Methyl	58	85	67
	Penconazole	6	10	5
	Permethrin Isomer 1	23	35	26
	Permethrin Isomer 2	27	41	30
	Phosalone	52	61	42

	Compound	%Recovery		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
	Phosmet	25	31	23
	Phosphamidon Isomer 1	59	57	46
	Phosphamidon Isomer 2	62	65	42
	Pirimicarb	67	113	54
	Pirimiphos ethyl	75	107	111
	Procymidone	65	121	88
	Profenophos	57	97	73
	Propazine	61	70	84
	Propham	74	64	76
	Prothiophos	39	81	45
	Pyrimiphos Me	67	83	79
	Quinalphos	69	76	72
	Quinoxyfen	51	70	46
	Quintozene	60	57	36
	Simazine	25	32	21
	Tebuconazole	29	18	26
	Terbuthylazine	35	116	52
	Tetrachlorvinphos	84	87	67
	Tetrasul	40	88	29
	Triadimefon	55	73	65
	Trifluralin	41	62	38
	trans Chlordane	110	165	179
	cis Chlordane	121	154	210

Moreover, the average %Recovery of three different concentration levels was calculated and the overall results are shown in **Figure 28**.

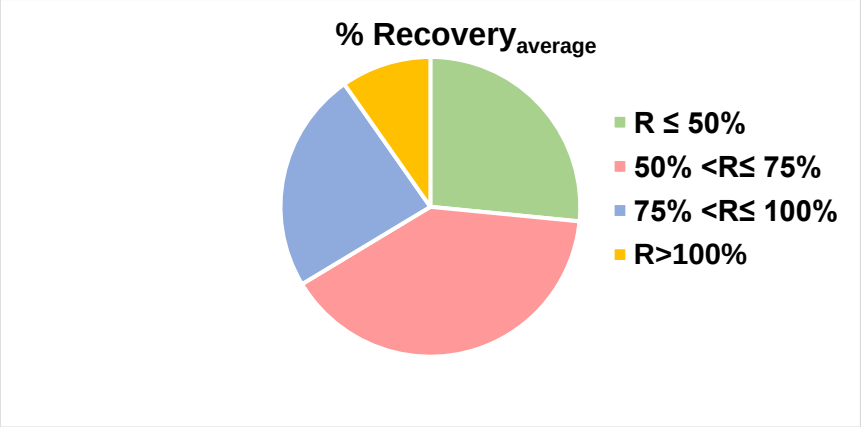

Figure 28: Total results of % Recovery.

For almost 60% of the tested compounds, recoveries were between 50-100%. Apart from the extraction yield, a potential reason for lower recovery rates (<75%) for many compounds, could be attributed to the evaporation until dryness step of the sample preparation. The more volatile analytes (available for GC analysis) are more vulnerable to evaporation which could lead to lower recoveries.

5.4.4 Matrix effect

The determination of ME is an important aspect in order 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 present at the same concentrations in the interface. It is intuitively clear that the efficiency of formation of the desired ions is matrix-dependent due to the competition between the molecule of interest and a number of other undetected but co-eluting molecules present in the system that are capable of reacting with primary ions. This effect may reduce or increase

the intensity of analyte ions and affect the reproducibility and accuracy of the assay.

In order to measure the ME, the matrix factor (MF) is necessary. MF and ME are calculated based on the following equations:

$$\boldsymbol{MF = \frac{Relative\ area_{(matrix\ matched)} - Relative\ area_{(blank\ sample)}}{Relative\ area_{(standard)}}}$$

$$\boldsymbol{ME\ \% = (MF - 1)\ x\ 100}$$

Matrix effect was determined by comparing the response of analyte between matrix matched samples and standard solution of three different concentration levels (6, 20, 60 ng L^{-1}). Results of ME% shown in **Table 10**.

Table 10: % Matrix Effect (ME%) at three concentration levels.

Classification	Compound	ME%		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
PAHs	**Acenapthene**	-52	-40	-40
	Acenapthylene	76	52	61
	Anthracene	30	52	80
	Benzo(a)pyrene	48	-6	37
	Benzo(b)fluoranthene	383	34	-19
	Benz(a)anthracene	39	-6	6
	Benzo(g,h,i)perylene	43	13	39
	Benzo(k)fluoranthene	45	4	79
	Chrysene	28	15	52
	Dibenzo(a,h)anthracene	46	6	17
	Fluoranthene	140	100	130
	Fluorene	-55	-48	-35
	Indeno(1,2,3-cd)pyrene	102	-3	31
	Phenanthrene	-40	-50	-33
	Pyrene	39	4	30
	Naphthalene	92	60	98

Classification	Compound	ME%		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
PCBs	PCB 101	-7	3	-20
	PCB 138	2	-1	-15
	PCB 153	-92	-100	-95
	PCB 180	3	6	0
	PCB 28	-10	-29	-19
	PCB 52	-66	-49	-65
OCPs	2.4-DDT	-53	-68	-53
	4,4'-Dichlorobenzophenone	-65	-64	-56
	4.4-DDD	-32	-48	-42
	4.4-DDE	-42	-45	-48
	4.4-DDT	-89	-91	-87
	Hexachlorobutadiene	87	80	96
	Dichlorvos	-30	-32	-24
	a-HCH	-46	-56	-57
	b-HCH	-65	-65	-73
	c-HCH (Lindane)	-49	-57	-58
	d-HCH	-50	-52	-58
	Hexachlorobenzene	48	30	31
	Heptachlor Epoxide	-54	-57	-60
	Heptachlor	-44	-55	-56
	Endosulfan alpha	-40	-43	-44
	Endosulfan sulphate	-31	-43	-50
	Aldrin	-47	-60	-58
	Endrin	-33	-55	-61
	Dicofol	-60	-62	-54
	Dieldrin	-55	-60	-57
	Isodrin	-51	-54	-48
	Pentabromoethylbenzene	62	44	60
	Pentachlorobenzene	82	70	74
Plant Protection Products	Acrinathrin	-30	-46	-33
	Atrazine	-40	-47	-44
	Benfluralin	-30	-37	-49
	Bifenthrin	-41	-48	-45
	Bromophos Ethyl	-29	-41	-34
	Bromophos Methyl	-45	-47	-52
	Chlorfenvinphos mix Z&E isomer 1	-34	-34	-42
	Chlorfenvinphos mix Z&E isomer 2	-34	-49	-45

Compound	ME%		
	C=6 ng/L	C=20 ng/L	C=60 ng/L
Chlorpyrifos Ethyl	-51	-60	-54
Chlorpyrifos Methyl	-41	-61	-51
Climbazole	-45	-44	-38
Cyanazine	-50	-69	-44
Cyfluthrin Isomer 3	-43	-54	-41
Cyfluthrin Isomer 4	-35	-51	-42
Cyhalothrin-lambda major	-29	-51	-40
Cypermethrin Isomer 1	-39	-45	-30
Cypermethrin Isomer 2	-27	-46	-25
Cypermethrin Isomer 3	-25	-47	-32
Cypermethrin Isomer 4	-24	-49	-20
Cyprodinil	-57	-59	-42
Deltamethrin	-30	-49	-30
Diazinon	-37	-46	-48
Dichlofenthion	-54	-49	-39
Dichlofluanid	-59	-79	-63
Dimethoate	-59	-79	-42
Ditalimfos	-27	-45	-38
Esfenvalerate_Fenvalerate Isomer 1	-21	-49	-26
Esfenvalerate_Fenvalerate Isomer 2	-30	-47	-24
Ethalfluralin	-40	-40	-41
Ethion	-29	-47	-48
Ethoprophos	-29	-49	-41
Fenamiphos	-31	-57	-44
Fenitrothion	-50	-60	-81
Fenoxycarb	-29	-47	-33
Fenthion	-55	-64	-48
Fludioxonil	-48	-46	-51
Folpet	-20	-45	-36
Iprodione	-21	-35	-18
Malaoxon	-15	-22	-37
Malathion	-32	-43	-47
Methacrifos	-30	-46	-47
Methidathion	-40	-47	-27
Methomyl	-35	-42	-48
Methoprotryne	-20	-43	-40
Methoxychlor (DMTD)	-2	-14	41

	Compound	ME%		
		C=6 ng/L	C=20 ng/L	C=60 ng/L
	Metolachlor	-64	-62	-58
	Metribuzin	-61	-69	-46
	Monocrotophos	-64	-38	-35
	Myclobutanil	-28	-57	-44
	Omethoate	-47	-20	-35
	Paraoxon Eth	-40	-41	-42
	Parathion-Ethyl	-50	-54	-58
	Parathion-Methyl	-31	-62	-50
	Penconazole	-85	-100	-74
	Permethrin Isomer 1	-27	-49	-36
	Permethrin Isomer 2	-48	-46	-39
	Phosalone	-12	-64	-30
	Phosmet Fragm 160	-27	-71	-50
	Phosphamidon isomer 1	118	-35	-42
	Phosphamidon isomer 2	49	-17	-36
	Pirimicarb	-38	-51	-48
	Pirimiphos ethyl	-52	-63	-46
	Procymidone	-52	-54	-42
	Profenophos	-46	-66	-52
	Propazine	-134	-98	-74
	Propham	-35	-43	-16
	Prothiophos	-43	-45	-47
	Pyrimiphos Me	-52	-49	-48
	Quinalphos	-38	-68	-48
	Quinoxyfen	-44	-57	-59
	Quintozene	36	15	9
	Simazine	-104	-89	-62
	Spiroxamine Isomer 1	-47	-51	-48
	Spiroxamine Isomer 2	-45	-47	-49
	Tebuconazole	-23	-45	-42
	Terbuthylazine	-100	-98	-74
	Tetrachlorvinphos	-16	-51	-47
	Tetrasul	-41	-48	-43
	Triadimefon	-45	-47	-44
	Trifluralin	-24	-39	-45
	trans Chlordane	28	18	15
	cis Chlordane	17	20	37

The results of ME% are summarized in the **Figure 29** for 6, 20 and 60 ng L^{-1} concentration levels.

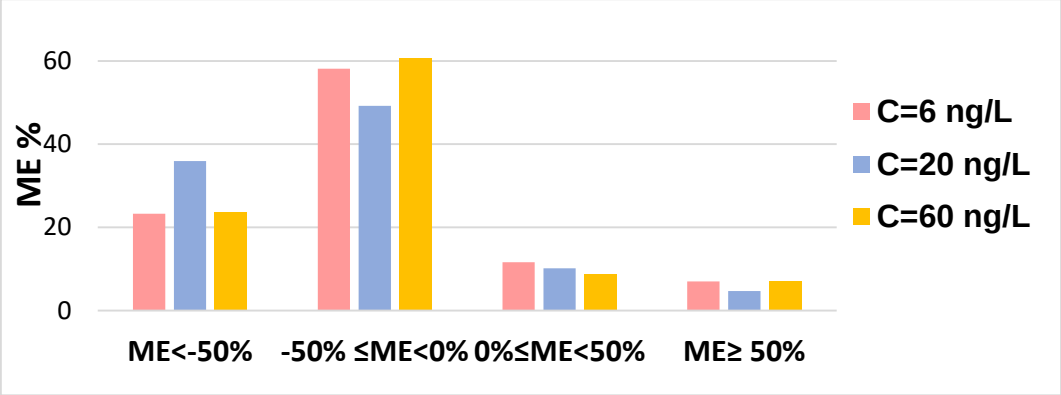

Figure 29: %ME at three concentration levels.

Ideally, the ME % is equal to zero meaning that neither ion suppression nor enhancement takes place. It has to be noted that negative values of ME% indicate ion suppression while positive ion enhancement. Total results of matrix effect for three different concentration levels shown that ME % had negative values (Ion suppression) for most of the tested compounds.

5.4.5 Precission

Precision was assessed by the evaluation of repeatability (intra-day precision) and reproducibility (inter-day precision).Total results for the estimation of precission presented in **Table11**.

Table 11: Total results for Precission.

Classification	Compound	RSDr %				RSDwR%	InRSDr%
		C1=6 ng/L	C2=6 ng/L	C=20 ng/L	C=60 ng/L	C=6 ng/L	C=100 µg/L
PAHs	Acenapthene	37	3	17	3	24	2
	Acenapthylene	40	4	12	17	27	5
	Anthracene	10	17	18	11	18	6
	Benzo(a)pyrene	74	24	10	25	49	6
	Benzo(b)fluoranthene	84	10	11	38	49	5
	Benz(a)anthracene	76	18	20	37	47	6
	Benzo(g,h,i)perylene	45	17	32	12	48	4
	Benzo(k)fluoranthene	30	21	21	29	25	3
	Chrysene	10	8	29	24	15	2
	Dibenzo(a,h)anthracene	80	13	22	9	54	5

	Compound	RSDr %				RSDwR%	lnRSDr%
		C1=6 ng/L	C2=6 ng/L	C=20 ng/L	C=60 ng/L	C=6 ng/L	C=100 µg/L
	Fluoranthene	23	7	43	22	15	4
	Fluorene	21	13	10	18	17	3
	Indeno(1,2,3-cd)pyrene	73	8	16	17	49	1
	Pyrene	44	19	38	24	35	5
PCBs	**PCB 101**	50	23	9	16	49	9
	PCB 138	64	11	3	40	61	7
	PCB 180	71	24	9	22	44	13
	PCB 28	20	34	13	22	30	9
	PCB 52	67	49	4	14	44	5
OCPs	**2.4-DDT**	51	20	9	19	37	5
	4,4'-Dichlorobenzophenone	41	33	16	27	34	3
	4.4-DDD	45	24	19	29	36	6
	4.4-DDE	83	11	21	25	49	5
	4.4-DDT	36	33	35	43	34	4
	Hexachlorobutadiene	81	11	110	79	86	8
	Dichlorvos	54	40	36	15	49	7
	a-HCH	47	28	8	21	38	2
	b-HCH	87	25	13	45	59	3
	c-HCH (Lindane)	69	24	15	43	41	4
	d-HCH	26	36	29	22	49	17
	Hexachlorobenzene	6	18	33	10	19	5
	Heptachlor Epoxide	36	35	24	24	34	2
	Heptachlor	48	22	24	20	33	6
	Endosulfan alpha	63	49	21	34	48	34
	Endosulfan sulphate	22	34	41	39	45	6
	Aldrin	86	64	11	17	68	3
	Endrin	38	30	14	30	31	8
	Dicofol	37	35	16	26	34	3
	Dieldrin	24	22	7	29	31	2
	Isodrin	71	48	7	12	54	5
	Pentachlorobenzene	42	8	22	12	28	4
Plant Protection Products (PPPs)	**Acrinathrin**	87	30	35	35	58	16
	Atrazine	48	41	43	45	47	4
	Benfluralin	28	25	12	29	54	7
	Bifenthrin	70	19	14	54	45	5
	Bromophos Ethyl	37	19	21	14	31	19
	Bromophos Methyl	30	38	18	21	34	15

Compound	RSDr %				RSDwR%	InRSDr%
	C1=6 ng/L	C2=6 ng/L	C=20 ng/L	C=60 ng/L	C=6 ng/L	C=100 µg/L
Chlorfenvinphos mix Z&E isomer 1	28	37	21	25	35	14
Chlorfenvinphos mix Z&E isomer 2	14	32	28	16	30	5
Chlorpyrifos Ethyl	63	31	16	26	46	9
Chlorpyrifos Methyl	55	29	13	9	41	6
Climbazole	58	95	93	31	70	20
Cyanazine	47	73	72	20	56	6
Cyfluthrin Isomer 3	10	32	37	38	19	11
Cyfluthrin Isomer 4	7	27	39	34	18	9
Cyhalothrin-lambda major	10	40	14	31	50	4
Cypermethrin Isomer 1	61	33	30	30	49	7
Cypermethrin Isomer 2	33	27	13	32	32	8
Cypermethrin Isomer 3	13	30	50	29	40	8
Cypermethrin Isomer 4	27	25	30	36	34	4
Cyproconazole	96	57	80	10	76	9
Cyprodinil	17	53	38	19	43	5
Deltamethrin	34	25	30	22	38	10
Diazinon	39	32	15	14	42	5
Dichlofenthion	28	18	19	18	19	14
Ditalimfos	32	42	30	23	47	18
Esfenvalerate_Fenvalerate Isomer 1	11	37	24	28	28	6
Esfenvalerate_Fenvalerate Isomer 2	12	11	34	19	14	4
Ethalfluralin	31	34	9	22	31	3
Ethion	52	18	33	0	44	5
Ethoprophos	36	30	11	17	39	7
Fenamiphos	47	87	95	11	93	5
Fenitrothion	42	46	20	32	44	8
Fenoxycarb	12	25	29	24	18	4
Fenthion	53	47	28	34	56	5
Iprodione	2	8	21	34	16	7
Malaoxon	20	36	42	14	35	18
Malathion	63	60	20	18	62	9
Methacrifos	27	40	9	8	36	2
Methidathion	44	23	28	32	35	15
Methoprotryne	9	37	51	21	28	3
Methoxychlor (DMTD)	92	87	87	11	90	7

Compound	RSDr %				RSDwR%	InRSDr%
	C1=6 ng/L	C2=6 ng/L	C=20 ng/L	C=60 ng/L	C=6 ng/L	C=100 µg/L
Myclobutanil	72	74	86	26	74	9
Paraoxon Eth	7	42	35	19	27	4
Parathion-Ethyl	22	43	17	24	49	8
Parathion-Methyl	16	41	19	15	40	7
Penconazole	110	125	142	60	110	4
Permethrin Isomer 1	91	28	28	25	63	5
Permethrin Isomer 2	58	22	18	34	49	5
Phosalone	26	45	37	6	43	7
Phosmet	26	46	25	22	35	5
Phosphamidon isomer 1	18	35	44	23	36	12
Phosphamidon isomer 2	23	54	52	13	47	5
Pirimicarb	36	32	15	17	43	8
Pirimiphos ethyl	30	34	22	24	34	7
Procymidone	11	41	28	22	33	5
Profenophos	32	9	9	16	19	4
Propazine	100	133	62	85	120	8
Propham	59	40	59	29	45	4
Prothiophos	59	16	17	26	39	6
Pyrimiphos Me	30	50	15	13	49	9
Quinalphos	16	33	26	9	30	8
Quinoxyfen	41	21	24	20	44	4
Quintozene	9	8	41	45	17	5
Simazine	110	128	95	49	115	3
Tebuconazole	97	47	91	7	72	9
Terbuthylazine	90	91	62	70	145	8
Tetrachlorvinphos	5	31	30	18	23	12
Tetrasul	30	10	18	25	48	4
Triadimefon	20	47	38	22	41	11
Trifluralin	42	22	15	34	69	7
trans Chlordane	38	42	44	28	41	21
cis Chlordane	32	26	55	21	27	9

5.4.5.1 Repeatability

Repeatability (InRSDr %) of the instrument was obtained by performing six injections of the same spiked sample (100 µg/L) in the same day.

Repeatability for the instrument was ≤ 10% for 96% of the tested compounds (**Figure 30**).

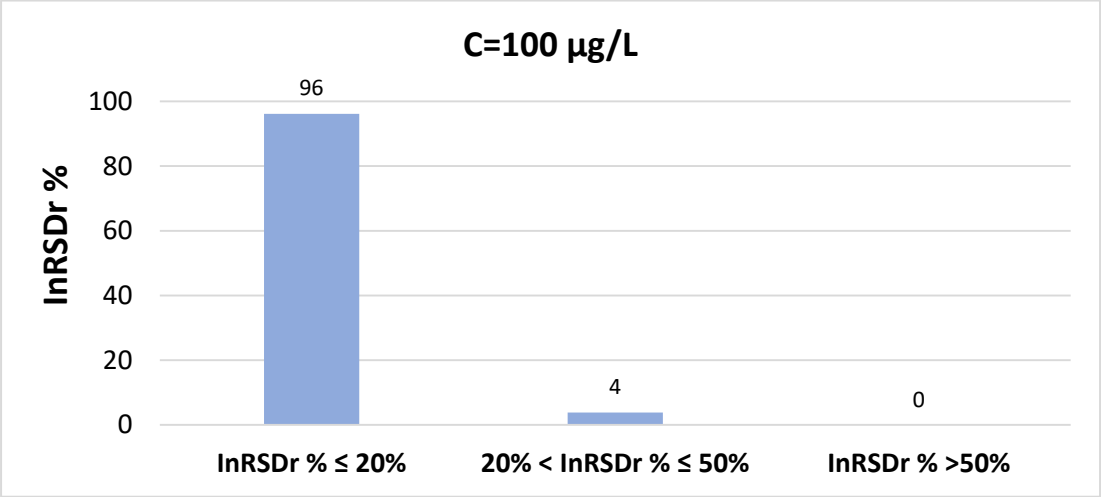

Figure 30: Repeatability (InRSDr %) for the instrument.

Also, repeatability (RSDr %) for the method was tested by performing three different spiked samples at three different concentration levels (20, 40, 60 ng/L) in the same day. The repeatability for the method for the most of the tested compounds was between 20% and 50% (**Figure 31**).

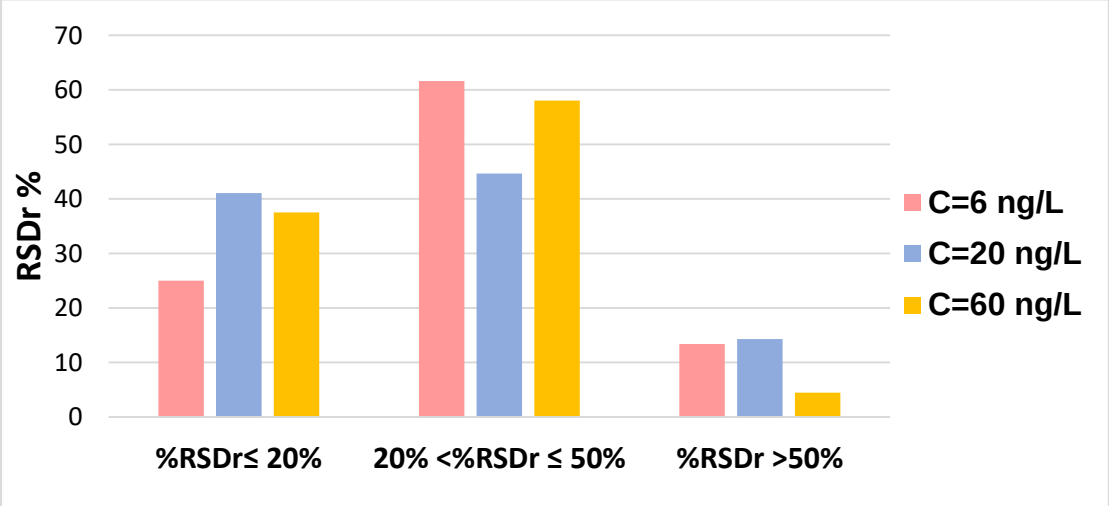

Figure 31: Repeatability (RSDr %) for the method.

This happening because repeatability was calculated in low concentration levels and little differences in low concentrations leads to bigger errors.

5.4.5.2 Reproducibility

Reproducibility was tested over two different days by performing three injections per day of three different spiked samples (C=6 ng L^{-1}). Therefore, a total of six spiked samples was used for this evaluation. The values of reproducibility (RSDwR%) have been summarized in **Figure 32**.

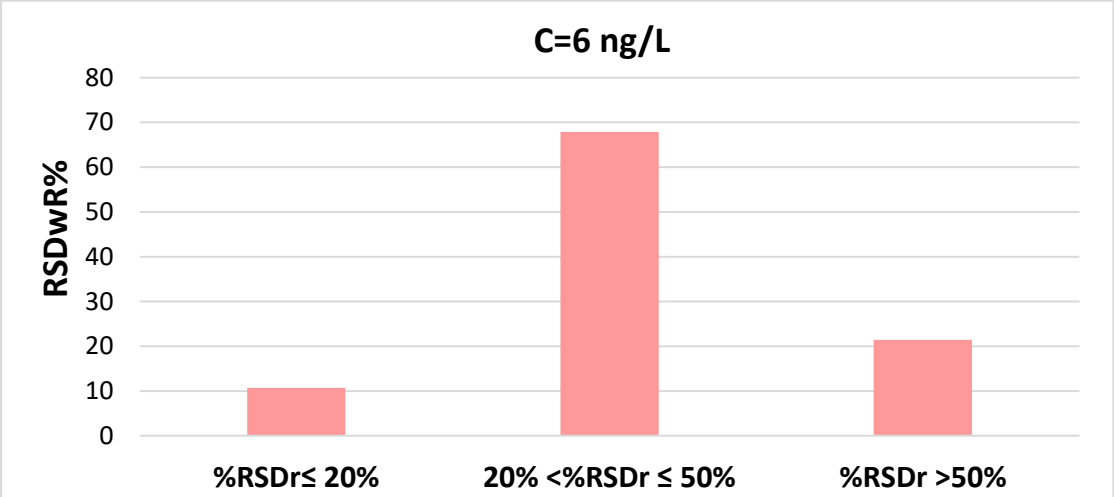

Figure 32: Reproducibility of the method (RSDwR%).

The reproducibility for the most of the tested compounds was between 20% and 50%. As it was mentioned before, precission was calculated in low concentration levels. Little differences in low concentrations leads to bigger errors and so it was expected to had these results for the precission,for the most of the tested compounds.

5.5 Application of target screening in real river water samples

River water 24h samples from Asopos river basin were collected consecutively from 20.11.2018 to 26.11.2018 using two portable autosamplers (one at each sampling point). River water samples were collected from two sampling points, the first one was close to the estuaries of Asopos river (R1) and the second one was close to industrial and agricultural activities (R2).These samples have been analyzed using GC-APCI-QTOFMS in order to assess the contamination of the river from organic contaminants.

For this purpose, all the samples were screened using the software TASQ CLIENT 2.1 (Bruker Daltonic). A method was composed using the the already

mentioned in-house database and the screening of samples was based on the identification criteria which mentioned before (Chapter 4.1).

For the quantification of the samples the following procedure was followed. Firstly, the ideal internal standard was selected for each analyte (for PAHs deuterium labeled standards were used). 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. Finally, the MLOD and MLOQ as well as the concentration levels of the detected analytes were compared with the environmental quality standards(EQs),(Directive 2013/39/EU)

Table 12: Total results of the determination of organic compounds in river water samples.

Compound	C (ng/L)													
	R1							R2						
	20_11	21_11	22_11	23_11	24_11	25_11	26_11	20_11	21_11	22_11	23_11	24_11	25_11	26_11
Acenapthene	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Acenapthylene	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ
Anthracene	6.29	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	3.53	8.76	LOD-LOQ	LOD-LOQ	LOD-LOQ	3.76	6.41	8.52
Benzo(a)pyrene	LOD-LOQ	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ	LOD-LOQ	<LOD	<LOD
Benzo(b)fluoranthene	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD
Benz(a)anthracene	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Benzo(g,h,i)perylene	3.42	LOD-LOQ	3.28	<LOD	<LOD	<LOD	2.80	3.44	4.54	<LOD	4.59	LOD-LOQ	<LOD	<LOD
Benzo(k)fluoranthene	4.78	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	4.69	<LOD	<LOD	<LOD
Chrysene	7.99	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	2.62	LOD-LOQ	LOD-LOQ	5.39	<LOD	<LOD	<LOD
Dibenzo(a,h)anthracene	2.85	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	2.98	<LOD	<LOD	3.11	<LOD	<LOD	<LOD
Fluorene	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Indeno(1,2,3-cd)pyrene	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5.08	5.36	LOD-LOQ	<LOD	<LOD	<LOD	<LOD
Phenanthrene	LOD-LOQ	<LOD	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ
Pyrene	LOD-LOQ	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	10.42	LOD-LOQ	<LOD	<LOD	<LOD
Naphthalene	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	LOD-LOQ	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
PCB 101	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.32	<LOD	<LOD	11.05	<LOD	<LOD
PCB 138	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10.35	<LOD	<LOD
PCB 153	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD
PCB 180	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
PCB 28	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	LOD-LOQ
PCB 52	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
2.4-DDT	5.88	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	8.01	<LOD	8.74	<LOD	<LOD	<LOD
4.4-DDD	6.59	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	6.31	<LOD	<LOD	<LOD

Compound	C (ng/L)													
	R1							R2						
	20_11	21_11	22_11	23_11	24_11	25_11	26_11	20_11	21_11	22_11	23_11	24_11	25_11	26_11
4.4-DDE	4.39	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	6.37	LOD-LOQ	5.42	<LOD	<LOD	<LOD
4.4-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	53.63	<LOD	<LOD	<LOD
Hexachlorobutadiene	<LOD	11.23	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	12.79	<LOD	LOD-LOQ	<LOD	LOD-LOQ	<LOD
Hexachlorobenzene	7.65	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.38	<LOD	<LOD	LOD-LOQ	<LOD	<LOD
Heptachlor Epoxide	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Heptachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Endosulfan alpha	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Endosulfan sulphate	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Endrin	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dieldrin	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Isodrin	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pentachlorobenzene	4.64	3.37	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	4.78	<LOD	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ
Atrazine	0.87	1.77	0.81	0.84	0.96	0.81	0.61	LOD-LOQ	LOD-LOQ	0.86	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ
Bifenthrin	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	7.89	<LOD	<LOD	<LOD
Bromophos Ethyl	4.49	1.66	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	1.98	<LOD	2.85	<LOD	<LOD	<LOD
Bromophos Methyl	1.25	0.53	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Chlorpyrifos Ethyl	1.45	0.41	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	0.40	<LOD	0.55	LOD-LOQ	<LOD	<LOD
Chlorpyrifos Methyl	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cyhalothrin-lambda major	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Cypermethrin Isomer 1	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Cypermethrin Isomer 2	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Cypermethrin Isomer 3	LOD-LOQ	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Cypermethrin Isomer 4	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Cyproconazole	0.52	0.36	0.33	<LOD	<LOD	<LOD	<LOD	0.33	0.24	LOD-LOQ	0.20	1.46	0.75	0.33

Compound	C (ng/L)													
	R1							R2						
	20_11	21_11	22_11	23_11	24_11	25_11	26_11	20_11	21_11	22_11	23_11	24_11	25_11	26_11
Cyprodinil	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
Esfenvalerate_Fen Iso 1	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Esfenvalerate_Fen Iso 2	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Metolachlor	2.38	3.93	1.08	1.10	LOD-LOQ	0.67	LOD-LOQ	1.24	6.27	5.58	4.68	2.81	2.99	2.05
Penconazole	2.09	1.20	2.57	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	1.67	3.81	2.11	2.78	4.34	3.68	3.35
Permethrin Isomer 1	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	14.04	<LOD	<LOD	<LOD
Permethrin Isomer 2	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	LOD-LOQ	13.65	<LOD	<LOD	<LOD
Prothiophos	4.89	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Quinoxyfen	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	<LOD	<LOD	<LOD
Simazine	<LOD	5.57	<LOD	<LOD	<LOD	<LOD	<LOD	10.21	27.95	<LOD	<LOD	<LOD	<LOD	<LOD
Terbuthylazine	0.50	0.56	1.64	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	1.10	2.97	1.38	1.04	1.77	1.97	1.59
Tetrasul	LOD-LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	LOD-LOQ	LOD-LOQ	<LOD	LOD-LOQ	LOD-LOQ	<LOD	<LOD
Trifluralin	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ	LOD-LOQ
Fluometuron	3.30	4.15	7.86	1.51	0.84	1.52	0.60	1.73	14.46	14.47	12.96	17.95	17.65	6.28
Fluopicolide	<LOD	<LOD	0.26	<LOD	<LOD	<LOD	<LOD	0.49	0.52	<LOD	2.16	0.82	0.88	1.12

In total, 62 compounds were detected in river water samples, from different groups, however the vast majority were plant protection products (PPPs). The overall results indicate that the tested river water samples were contaminated. This points the need for efficient workflows for the estimation of the aquatic environment contamination. As it is shown in **Table 12** most of the compounds were below of the LOD of the method. There are also many compounds that were detected in low levels which were not able to be quantified (between MLOD and MLOQ). Furthermore, the presence of these organic compounds into the river water samples shouldn't concern us, as the concentrations in the samples are lower compared to the environmental quality standards (EQS). This was not the case for benzo(a)pyrene, heptachlor and cypermethrin, since their concentrations were above the established EQS.

Moreover, the overall results confirmed that river water samples which were collected near industrial and agricultural activities (R2) were more contaminated in comparison with the samples which were collected near the the estuaries of the river (R1). The number of the detected compounds and the total concentration of all analytes were higher in samples near the sampling point R2. In addition, PPPs seem to have higher contribution in the contamination of sampling point R2, where agricultural activities take place.

Furthermore, some analytes were detected only in samples from sampling point R2 . Most of those compounds are PPPs, such as the fungicide Cyprodinyl (PPPs), and PCBs. PCBs are used as coolants, plasticizers in paints and cements, flame retadants, or sealants for coating of electrical cables and electronic components. Consequently, their detection in river water samples could be related with the industrial activities that take place near the sampling point R2.

It is also important to mention that some organic compounds exhibited high detection frequency.These compounds were detected in all the samples during the week which were collected from both sampling points. Maybe these PAHs (Acenapthylene, Anthracene) and PPPs (Atrazine, Penconazole, Fluometuron, Metolachlor, Terbuthylazine) which demonstrated high detection frequency can be prioritized for routine analysis of river waters in the future.

Some of the detected PPPs, may be related with the agricultural activities near Asopos river. For example, the herbicide Metolachlor (**Figure 33**) was detected at both sampling points during the week. Metolachlor is an organic compound that is widely used as a herbicide. It is used for grass and broadleaf weed control in corn and cotton which. It is also used in combination with other herbicides.

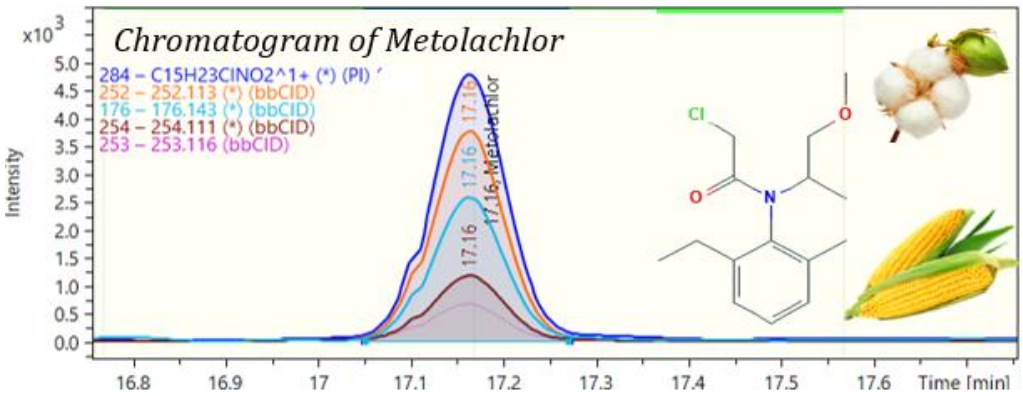

Figure 33: Chromatogram of herbicide Metolachlor.

The concentration range was 1.20 - 6.3 ng/L and 0.66 - 4 ng/L for samples collected from sampling point R2 and R1 respectively.

Furthermore, the fungicide Cyproconazole was detected in the river water samples with higher detection frequency at sampling point R2 (**Figure 34**). Cyproconazole is both a prevention and treatment agricultural fungicide. It used to control a wide rage of fungi on cereal and other crops.

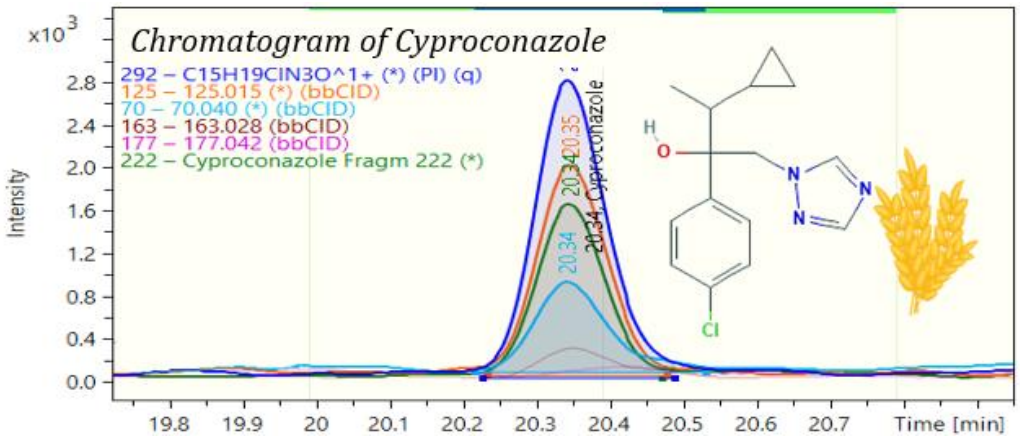

Figure 34: Chromatogram of fungicide Cyproconazole.

Farmers from Asopos river basin confirmed that near the river there are corn, cotton and cereal crops. So the results may be related with these agricaltural activities.

As it is already mentioned, the weather was recorded for each day during the sampling period in order to check if the weather can affect the daily detection trend of the organic compounds in river water samples. As an example, the herbicide Terbuthylazine was detected in river water samples during the week (20-26.11.18) at both sampling points.

Terbuthylazine is a herbicide which is used for corn crops and may be used at agricultural activities near the sampling point R2. During this week, Tuesday was a rainy day and with the water's runoff the analyte transfered from the soils into the river. The next day (Wednesday) the analyte was detected in higher concentration in samples from the sampling point R2 (**Figure 35**). Due to the water flow, the analyte was detected on Thursday in higher concentration at sampling point R1, near the estuaries of Asopos river (**Figure 35**).

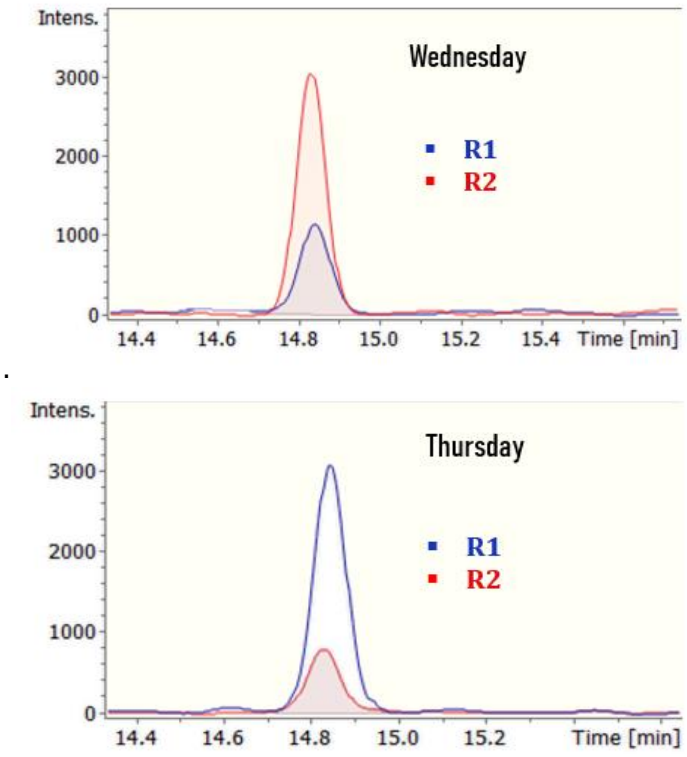

Figure 35: Detection of Terbuthylazine in two different days.

CHAPTER 6
Conclusions

In conlcusion, 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 PPs 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 PPs and ECs has been developed. SPE with C18 EC cartridges was used for the the pre-concentration of the analytes and the clean-up of the river samples. The developed method was validated for 130 analytes concerning linearity, sensitivity, trueness, matrix effect and precission.

River water samples were collected from two sampling points and they were screened for the determination of more than 300 organic compounds. More than 60 organic compounds from different groups (PAHs, PCBs, PPPs, OCPs) were detected in river water samples from Asopos.

Target analysis in water samples highlighted that the industrial zone and agricultural activities near the river (sampling point R2) may affect the detection of the analytes. Overall results show that the number and the total concentration of the detected analytes was higher in river water samples from sampling point R2. Moreover the weather contitions can potentially affect the concentration and the transport of the detected analytes.

CHAPTER 7
Future perspectives

This master thesis is a part of the programme “Monitoring of Asopos river basin waters - Assessment of pollution/ contamination and qualitative and quantitative investigation of contamination levels and possible sources of pollution” which financed by the Region of Attica. Therefore, the developed method will be used in the future for the determination of organic compounds in more river water samples. So far, 102 river water samples have been collected. Samples were collected for 30 concecutive days during winter and for 7 concecutive days during each other season (spring, summer and autumn).

Moreover, the developed method wil be used in underground and drinking water samples from Asopos river basin. The total results will indicate the overall contamination of Asopos river basin due to the occurrence of GC-amenable PPs & ECs and the detection trend of the detected pollutants on a monthly and seasonal basis.

Furthermore, all these samples will be analyzed with LC-ESI-QTOFMS. Results from both methods and instuments will can compered and through this way to provide more reviable results

Finally, suspect and non-target screening analysis of the samples shoyld be done, for the detection of more organic compounds

ABBREVIATIONS AND ACRONYMS

Table 13: Abbreviations and acronyms.

EPA	Environmental Protection Agency
PPs	Priority Pollutants
ECs	Emerging Contaminants
VOCs	Volatile organic compounds
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorobiphenyls
PBDs	Polybromodiphenylethers
OCPs	Organochlorine pesticides
WFD	Water Framework Directive
EC	European Comission
EU	European Union
COMMPS	combined monitoring-based and modeling-based priority setting
EQs	Environmental Quality Standards
WWTPs	Wastewater treatment plants
PPPs	Plant protection products
PCNs	Polychlorinated naphthalene
PFRs	Phosphorus flame retardants
LLE	Liquid-liquid extraction
SPE	Solid phase extraction
GSC	Gas-solid chromatography
GLC	Gas-liquid chromatography
HRMS	High Resolution Mass Spectrometry
LRMS	Low Resolution Mass Spectrometry
EI	Electron Impact Ionization
CI	Chemical Ionization
ESI	Electrospray Ionization
APCI	Atmospheric Pressure Chemical Ionization
MS	Mass Spectrometry
QqQ	Triple Quadrupole
IT	Ion trap
TOF	Time of Flight
DDA	Data Depentent Acquisition
DIA	Data Independent Acquisition
SRM	Single Reaction Monitoring
IPs	Identification points
HLB	Hydrophilic-Liophilic-Balanced
GC-ECD	GC with Electron Capture Detector
FC43	Perfluorotributylamine
RC	Regenerated cellulose

CRM	Certified Reference Material
LOQ	Limit of Detection
LOQ	Limit of Quantification
ME	Matrix Effect
MF	Matrix factor
R	Recovery
RSDr	Repeatability
RSDrw	Reproductivity
GC	Gas Chromatography
LC	Liquid Chromatography
QTOF	Quadrupole time of flight mass spectrometer
EtoAC	Ethyl Acetate
ACN	Acetonitrile
MeOH	Methanol

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