MONITORING OF PESTICIDES IN WATER MEDIA OF NORTHEAST PORTUGAL

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Abstract

In this work, an analytical methodology was developed for the monitoring of five emerging pollutants, namely, alachlor, metolachlor, heptachlor, dimethoate, and terbuthylazine. These compounds are among the most used pesticides in the northeast region of Portugal and Tunisia.

A complete experimental methodology was optimized based on the simultaneous extraction and concentration of all five pesticides from aqueous matrices, by means of solid-phase micro-extraction (SPME) followed by detection and quantification using gas chromatography coupled to mass spectrometry (GC-MS).

The optimization of the extraction step was performed using a polydimethylsiloxane–divinylbenzene (PDMS-DVB) coated SPME fiber by direct immersion (DI-SPME) in the aqueous sample. Experimental conditions, such as extraction temperature and time, pH value, salt concentration, and desorption time and temperature in the GC injector port were studied. The optimum value for each one of these parameters was selected based on the maximum total area value obtained in MS detector, using Full Scan Mode, for the mixture of the five pesticides. The extraction optimized conditions were achieved by immersion of a PDMS-DVB fiber in the sample mixture with 10% NaCl, a pH value of 2, at 60ºC for 80 min. Desorption of the compounds from the fiber is done in the GC port at 250ºC during 4 min.

The GC-MS operating conditions were also studied and the main separation and detection parameters were selected. Samples were analyzed using the following oven temperature program: initial temperature of 120°C (held for 2 min), increased by 15°C min^{-1} to 190°C (held for 4 min) and, finally, increased by 10°C min^{-1} to 227°C held for 1min.
The MS instrument operated in the Electron ionization mode (EI) was used for a full scan.
The acquisition was performed in the range of 35–450 (m/z). The ion source temperature was 200 °C and the interface temperature was 270 °C.

The identification and quantification were carried out using calibration curves obtained from the extraction of a standard mixture of the five selected pesticides for at least six concentrations levels, in the same experimental conditions used for the real samples. Detection limits ranged

from 4.2 to 6.6 μg/L. For pesticides with low values of K_{OW}, like dimethoate, the use of a fiber of relatively non-polar nature would be favorable.

The developed experimental methodology was implemented by the analysis of different samples collected from the surface water of three rivers from Bragança, namely, Fervença, Sabor and Onor. All three rivers showed different types and levels of contamination.

Key-Words: Emerging Contaminants, Pesticides, Superficial Waters Micro-Pollutants, Solid Phase Micro-Extraction, Gas Chromatography – Mass spectrometry.

Resumo

Neste trabalho, apresenta-se o desenvolvimento de uma metodologia analítica para a monitorização de cinco poluentes emergentes, nomeadamente, alacloro, metolacloro, heptacloro, dimetoato e terbutilazina. Estes compostos estão entre os pesticidas mais utilizados na região nordeste de Portugal e da Tunísia.

A metodologia experimental é baseada na extração e concentração simultânea de todos os 5 pesticidas presentes em matrizes aquosas, utilizando a micro-extração em fase sólida (SPME) seguida de deteção e quantificação utilizando um sistema de cromatografia gasosa acoplado com espectrometria de massas. (GC-MS).

A otimização da etapa de extração é realizada com uma fibra de SPME com um revestimento de polidimetilsiloxano-divinilbenzeno (PDMS-DVB) e por imersão direta nas amostras aquosas (DI-SPME). As condições experimentais da extração, como sejam, o tempo e a temperatura de extração, o valor de pH, a adição de sal, e o tempo e a temperatura de dessorção na porta do injetor do GC, são estudadas. O valor ótimo para cada um destes parâmetros é selecionado baseado na maximização do valor da área cromatográfica total obtido a partir da mistura dos 5 pesticidas, fornecida pelo detetor de massas. As condições ótimas para a extração são obtidas imergindo a fibra de PDMS-DVB na amostra da mistura, após a adição de 10% (m/m) de NaCl, ajuste do valor de pH para 2, a extração é realizada durante 80 min a 60ºC. A dessorção deve ser realizada na porta do GC a 250ºC durante 4 min.

As condições operatórias do GC-MS foram também estudadas, tendo-se otimizado alguns dos parâmetros de separação e deteção. As amostras são analisadas utilizando o seguinte programa de temperaturas do forno do GC: temperatura inicial de 120ºC (2 min), uma rampa de 15°C.min^{-1} até 190°C (4 min) e, finalmente, nova rampa de 10 °C.min^{-1} até 227°C (1 min).
O detetor de massas foi utilizado em modo de ionização de iões (EI) e em varrimento total de massas (FullScan).

A aquisição de massas foi realizada entre 35-450 m/z. A temperatura da fonte de iões foi de 200ºC e a temperatura de interface foi de 270ºC.

Os limites de deteção e de quantificação estimados através da determinação experimental das curvas de calibração obtidas após a extração de soluções padrão com os 5 pesticidas em estudo, utilizando pelo menos seis níveis de concentração diferentes, nas mesmas condições experimentais utilizadas para as amostras reais. Os limites de deteção obtidos, variam de 4,2 a 6,6 μg/L. Para pesticidas com valores de K_{OW} baixos, como por exemplo, o dimetoato, a utilização de um tipo de fibra de natureza relativamente não-polar pode ser favorável.

O desenvolvimento da metodologia experimental foi seguida da sua implementação através da análise de diferentes amostras, recolhidas das águas superficiais de 3 rios de Bragança, especificamente, rio Fervença, Sabor e Onor. Todas as amostras analisadas revelaram diferentes tipos e graus de contaminação pelos pesticidas em estudo.

Palavras-Chave: Contaminantes emergentes, Pesticidas, Micro-poluentes em águas superficiais, Micro-extração em fase sólida, Cromatografia gasosa – espetrometria de massa.

Résumé

Dans ce travail, une méthodologie analytique est développée pour la surveillance de cinq polluants émergents, à savoir l'alachlor, le métolachlore, l'heptachlore, le diméthoate et la terbuthylazine. Ces composés sont parmi les pesticides les plus utilisés dans la région nord-est du Portugal et de la Tunisie.

Une méthodologie expérimentale complète est optimisée, basée sur l'extraction et la concentration simultanées des cinq pesticides à partir de matrices aqueuses, au moyen d'une micro-extraction en phase solide (SPME) suivie d'une détection et d'une quantification par chromatographie en phase gazeuse avec spectrométrie de masse (GC-MS).

L'optimisation de l'étape d'extraction est réalisée à l'aide d'une fibre SPME revêtue de polydiméthylsiloxane – divinylbenzène (PDMS-DVB) par immersion directe (DI-SMPE) dans l'échantillon aqueux. Les conditions expérimentales, telles que la température et la durée d'extraction, la valeur du pH, l'addition de sel, la durée et la température de désorption dans l'orifice d'injection du CPG ont été étudiées. La valeur optimale pour chacun de ces paramètres a été choisie sur la base de la valeur de la surface totale maximale obtenue dans le détecteur MS, en utilisant le mode de balayage complet, pour le mélange des cinq pesticides. Les conditions d'extraction optimisées ont été obtenues par immersion d'une fibre de PDMS-DVB dans le mélange d'échantillon avec 10% de NaCl, une valeur de pH de 2, à 60°C pendant 80 min. La désorption des composés de la fibre est effectuée dans le port GC à 250ºC pendant 4 min.

Les conditions de fonctionnement de la GC-MS ont également été étudiées et les principaux paramètres de séparation et de détection ont été sélectionnés. Les échantillons ont été analysés en utilisant le programme de température du four suivant : température initiale de 120°C (maintenue pendant 2 min), augmentée de 15°C min^{-1} à 190°C (maintenue pendant 4 min) et enfin augmentée de 10 °C min^{-1} à 227°C maintenu pendant 1 min.
L'instrument MS fonctionnant en mode d'ionisation électronique (EI) a été utilisé pour une analyse complète.
L'acquisition a été réalisée entre 35 et 450 (m / z). La température de la source d'ions était de 200°C et la température d'interface de 270°C.

L'identification et la quantification ont été effectuées à l'aide de courbes d'étalonnage obtenues à partir de l'extraction d'un mélange standard des cinq pesticides sélectionnés pour au moins six concentrations, dans les mêmes conditions expérimentales que celles utilisées pour les échantillons réels. Les limites de détection allaient de 4,2 à 6,6 µg/L. Pour les pesticides à faible K_{OW}, tels que le diméthoate, l'utilisation d'une fibre de nature relativement non polaire serait favorable.

La méthodologie expérimentale développée a été mise en œuvre par l'analyse de différents échantillons prélevés dans les eaux de surface de trois rivières de Bragança, à savoir Fervença, Sabor et Onor. Les trois rivières présentaient différents types et niveaux de contamination.

Mots-clés: contaminants émergents, pesticides, micro-polluants des eaux superficielles, micro-extraction en phase solide, chromatographie en phase gazeuse - spectrométrie de masse.

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List of Abbreviations

AOP	Advanced oxidation processes
AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)-propanoic acid
BTEX	Benzene, Toluene, Ethylbenzene, m-, o-, and p-Xylene
DAD	Diode array detector
DDT	Dichlorodiphenyltrichloroethane
DEHP	Diethylhexyl phthalate
DINP	Diisononyl phthalate
DIDP	Diisodecyl phthalate
EC	Emerging contaminant
ECD	Electron capture detector
EDC	Endocrine disrupting chemicals
EI	Electron ionization
EP	Emerging pollutant
ESA	Acetochlor-ethane sulfonic acid
FID	Flame ionization detector
GAC	Granular activated carbon
GC	Gas chromatography
GC-NPD	Gas chromatography equipped with a nitrogen-phosphorus detector
HDPE	High-density polyethylene
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LLME-SFO	Solidification of floating organic droplet in dispersive liquid-liquid microextraction
MTBE	Methyl tert-butyl ether
MS	Mass spectrometry
MS^n	Tandem mass spectrometry
OXA	Metolachlor-oxanilic acid
PAC	Powdered activated carbon
PBDE	Polybrominated diphenyl ethers
PDMS-DVB	Polydimethylsiloxane/Divinylbenzene
PID	Photoionization detector
PPCP	Pharmaceuticals and Personal Care Products
QA/QC	Quality Assurance/Quality Control
RPLC	Reversed-phase liquid chromatography
SPE	Solid phase extraction
SPME	Solid phase microextraction
UPLC	Ultra performance liquid chromatography
WWTP	Wastewater treatment plant

Chapter 1: Motivation and objectives

1.1 Introduction

In the last few decades, pesticides have been used on an increasingly wider scale throughout the world, though most of them have been banned from use, they are still detected in natural ecosystems. Nowadays, there is a tendency to slow down, or at least a motivation to use less harmful molecules.

The problem of water pollution is a concern for everyone. Nowadays, the question of the effects of "emerging pollutants" is being raised because they generally do not yet have a regulatory status. Furthermore, the identification and the removal of these pollutants is not only time consuming but also extremely expensive. These substances come partly from medical use, but also from industrial production in the watershed, their use in agriculture and the daily use of formulations that use chemistry; anticorrosive, antibacterial, water repellent, flame retardants, cosmetics, etc. The analysis of emerging pollutants in water is fundamental for the protection of health and ecosystems and to assess the effectiveness of water treatment.
Physicochemical and biotechnological analysis have been focused to pesticides, pharmaceutical drugs and toxins.

Due to their large volumes of production and continuous use, some of these compounds have become ‘‘pseudo-persistent’’ substances in the environment. According to Portuguese authorities, about 15×10^6 kg of atrazine, 22×10^6 kg of simazine, 17×10^6 kg of alachlor, and 1×10^7 kg of metolachlor were applied in 1996 in Portugal, mainly in corn, rice and grape plantations [1]. Although most of the non-point source pollution of waters by pesticides has an agricultural origin, in the last years particular attention has been devoted to the non-agricultural uses of pesticides (e.g. highways, railroads and golf courses) [1].

The contamination of the aquatic environment by organic pollutants, such as pesticides is a matter of great concern worldwide. In addition to affecting human health, many pesticides released into the environment can also disrupt the normal endocrine function in a variety of aquatic life and wildlife [2]. Pesticides are a group of compounds in continuous evolution,

characterized by their diversity, different physical and chemical properties as well as their low concentrations in real samples.

The organonitrogen herbicides such as terbuthylazine, alachlor, and metolachlor are among the most commonly used and detected pesticides in water streams around the world. They are among the top ten herbicides used in the United States and Europe. As for insecticides, we can add to our list dimethoate and heptachlor which are the most used insect-killers in the northeast of Portugal [1, 2].

The need for monitoring some pesticides, phenolic compounds, amines, phthalates, alkyl, and aromatic sulfonates in surface waters by state-of-the-art methods is now recognized, being essential for achieving good water-quality objectives followed by the proposal of new and more ecological processes for the treatment of polluted waters.

1.2 Objectives

- **Main objective**

The main objective of this work is to contribute to the development and validation of an experimental methodology that can be applied to the monitoring of pesticides, a specific class of emerging pollutants, in aqueous matrices in the northern of Portugal and Tunisia.

- **Specific objectives**

The analytical methodology development includes both the optimization of solid phase micro-extraction (SPME) technique and optimization of the main operation conditions for gas chromatography coupled with mass spectrometry detector (GC-MS).

The optimization of GC-MS method will be performed by means of the selection of the most promising operating conditions to improve the compounds separation and quantification. Among these, the selection of the GC oven temperature program, the GC injector mode of operation (split/splitless), and the MS FullScan or SIM modes of detection.

For SPME optimization, the main extraction parameters, such as, the type of fiber, the extraction time and temperature, the salt content and pH value of samples, the desorption time and temperature of the fiber in the GC injector port will be studied.

Both GC-MS and SPME optimization will be directed for the maximization of the MS detector signal, of an aqueous sample mixture of different pesticides, in order to optimize the limits of detection and quantification.
The experimental methodology must be validated with the determination of the most relevant statistical parameters, such as, the calibration curves using a confidence level of 95%, the intermediate precision, the repeatability and the limits of quantification and detection.

The developed methodology will be implemented by collecting and analyzing different types of surface water samples in three different rivers at different locations for Bragança region.

1.3 Report organization

This Master thesis report is organized in five chapters. The first one presents a brief introduction to the relevance of the proposed work, the main objectives to be fulfilled and the organization of this report.
In the second chapter it is presented an extensive state of the art, referring some recent published work, in the field of extraction techniques and instrumental methods of analysis for the extraction and quantification of pesticides in aqueous media. Besides, this introductory chapter describes the most relevant equations and statistical parameters used for the experimental methodology validation.
The third chapter is dedicated to list all chemical and materials, the equipment, and the experimental methodology used in this work. In this chapter, it is presented the experimental procedures that can be divided in two main parts. The first one is dedicated to the development of the analytical methodology to extract and to quantify a mixture of five selected pesticides, representative of the most important pesticides currently used in agricultural in the northern of Portugal and Tunisia. The second one is directed to the implementation of the experimental methodology using real aqueous samples collected from three different rivers in Bragança region.
In the fourth chapter, the main experimental results are presented and discussed.
The fifth and final chapter presents the main conclusions obtained in this work and some considerations and suggestions are presented for future works.

Chapter 2: Literature review

2.1 Water

Water has always been inseparable from human activity. On the other hand, the humanity demand of water increased twice as fast as the world population: they were multiplied by more than 7 between 1900 and 2000. The total amount of fresh water is the same since it appeared on Earth about 3 to 4 billion years ago. Today, more than a billion men, women and children around the world do not have 20 liters of water a day to live normally [3].

After, the industrial revolution of the 19th century, water became an essential material for the operation of factories [3]. Parallel to this situation, other phenomena such as the excessive use of pesticides and the uncontrolled waste disposal have contributed to the deterioration of water quality and consequently to the disruption of the entire ecosystem, causing adverse effects on the health of human beings [4].

The classification of water differs from one reference to another, some classify it according to the origin; some authors even speak of rainwater, some others are interested in the use of water.

➢ Natural waters

- **Groundwater**

From a hydrogeological point of view, the aquifers are divided into:

• *Unconfined aquifer:* shallow and fed directly by rainy rainfall or overflowing water.

• *Confined aquifer:* deeper than the first and separated from the surface by an impermeable layer, the feeding of these sheets is ensured by the infiltration on their borders.

The nature of ground is a determinant key for the chemical compositions of water; however, they are also called clean waters because they generally meet the standards of potability.

However, they totally lose their original purity in the case of contamination by pollutants. When groundwater contains a concentration of certain minerals exceeding the standards of potability, but represents therapeutic properties it is distributed in bottles with sometimes a well-defined treatment, these waters are called mineral waters [4].

- **Surface water**

This type of water includes all the waters circulating or stored on the surface of continents (rivers, lakes, ponds, dams). The chemical composition of surface water depends on the nature of the land traversed by these waters during their course in all watersheds.

These waters are the center for the development of a microbial life because of the waste released in it and the important surface of contact with the external environment.
Therefore these waters are rarely drinkable without any treatment [5].

- **Waters of the seas and oceans**

The seas and oceans are enormous reservoirs of water, they represent about 97.4% of the volume of water currently existing on our planet, the rest is the share of the continental waters (groundwater and superficial). The sea waters are characterized by high salinity, they are also called "brackish water", which makes their use difficult [4].

➢ Water consumption

These are waters for domestic consumption experiencing huge increase as a result of demographic development and the improvement of the living conditions of the populations. Even if it is only a small quantity that will be drunk, these waters are only distributed after proper treatment [3].

➢ Wastewater

The use of water creates a new product called effluent or wastewater. The problems related to wastewater are as old as these waters and they worsen due to the enormous growth of population and the development of the industrial activities. Wastewater can be divided into two categories: urban wastewater and industrial wastewater [5].

➢ Chemical composition of water

Water contains, in dissolved or suspended form, mineral and organic substances. If the mineral substances are limited to a hundred compounds, the organic substances are innumerable and their individual identification is very difficult [3].

- **Mineral substances**

Water contains a lot of dissolved ions, the main ones are calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), carbonate (CO_3^{2-}), hydrogen carbonate also called bicarbonate

(HCO_3^-), sulphate (SO_4^{2-}), chloride (Cl^-) and nitrate (NO_3^-). They come mainly from the leaching of soils by rainwater. Also, their content depends directly on the nature of the rocks of the watershed [5].
In less concentration (from microgram to milligram per liter), water contains nutrients, such as nitrogen (contained in ammonia, nitrite and nitrate), phosphorus (contained in phosphates) and silica, but also iron and manganese [6].
Other elements are only present in the trace concentration levels (from 0.1 to 100 micrograms per liter), such as arsenic, copper, manganese, iron, zinc and cobalt, among others. They come from rocks but also sometimes from industrial and domestic activities [5].

- **Organic substances**

Organic substances can be presented in dissolved form (carbohydrates, humic acids, pigments and compounds of artificial origin such as hydrocarbons, chlorinated solvents, or pesticides), or in suspension (vegetable waste, plankton, etc.). They come mainly from the degradation of the organic matter present in the medium or in the soils leached by the rains (decomposition of the plants and the animals), in other hand some compounds resulting from the human activity. Their concentration is very low in deep water but it can reach a few 10 of milligrams per liter in surface water [5].

2.2 Emergent Pollutants

The term "emerging pollutants" includes compounds of chemical or biological nature that may be of industrial, agricultural, domestic or natural origin.
In this report these diverse compounds have been categorized in classes, such as, Pharmaceuticals and Personal Care Products, Hormones and Steroids, Industrial Compounds, Nanomaterials, Flame Retardants and Pesticides.
So, steroids, medicinal products for human or veterinary use, degradation products of nonionic detergents, disinfectants, phthalates, etc., may be classified as emerging pollutants.
The list of pollutants described as emerging is constantly increasing; a non-exhaustive list is also provided on the Norman Network website [2].
Indeed, with the technological advance and progress of analytical methods, many compounds are detected in the different environmental matrices. For many of these compounds, there are

few data on their fate, their behavior in the environment and the likely effects they can have on living things are not yet well defined [2].
Brüsch *et al.* reported [6] an extensive range of pesticide monitoring programs that are being handled, such as, the pesticide leaching assessment efforts in Denmark.
In Sweden, Lindström *et al.* [7], refer the composite sampling for pesticide monitoring in agricultural streams. Two metabolites of dichlordiphenyltrichlorethan 'DDT' and the herbicides bentazone and mecocrop [2], were detected in lakes Tegel and Wannsee in Berlin, Germany.

2.2.1 Pharmaceuticals and Personal Care Products (PPCP)

This group of emergent pollutants include antibiotics, antimicrobial agents, synthetic musks, among other organic groups. Nowadays, more than 3000 known pharmaceutical compounds are produced. Of course, consumption rates vary from country to country according to national legislation and prescription lists. Latest improvements in analytical instrumentation and developments in analytical techniques allowed the detection of these substances at trace levels [8].

The transformation products of these substances can also be persistent in the environment. Advanced technologies such liquid chromatography with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) and gas chromatography with mass spectrometry (GC-MS) or tandem mass spectrometry (GC-MS/MS) can detect pharmaceutical drugs in water at very low concentrations (down to ng/L).

2.2.2 Hormones and Steroids

This category of emerging pollutants includes natural endogenous steroids, such as sex hormones (e.g., testosterone, estrogens and progesterone), faecal indicators and plant sterols, which are excreted from the human body [9].
Synthetic androgens hold oxandrolone, nandrolone and synthetic estrogens (xenoestrogens) such as diethylstilbestrol, which are used as contraceptives.

2.2.3 Industrial Compounds

This group includes substances used in industrial processes and production, especially in the chemical industry. Bisphenol A, the fire retardant (tri(2-chloroethyl) phosphate) and the musk galaxolide are among the most frequently detected substances, especially in groundwaters [9].

However, little is known in the matter of their impact on human health and the environment. MTBE (methyl tertiary-butyl ether) and BTEX compounds (benzene, toluene, ethylbenzene, m-, o-, and p-xylene) are usually detected in groundwater [8].
They are considered as central potential risks for human health and drinking water contamination since MTBE is classified as a potential carcinogen.

2.2.4 Nanomaterials

Nanomaterials are materials with a structure designed at the nanoscale or sub-microscale (1-100 nm) with low permeability, high strength, high conductivity and thermal stability. Their chemical composition differs and consists of nanotubes, metal oxanes, nanosilver, TiO_2 nanoparticles, nanogold and quantum dots. Engineered nanomaterials are used in PPCPs, in cosmetics for sun protection and in hip replacement materials [9].
Although nanotechnology could bear compelling societal benefits, there is a sure risk associated with the release of nanoparticles to the environment and questions arise regarding their potential effects on human health.
These materials have almost all high chemical reactivity, large active surface and biological activity which means that they can enter the body and the cells easier than other larger particles. However, related information about their potential toxicity or the damages they can cause is still limited.

2.2.5 Flame Retardants

These compounds are used in plastics, textiles and furnishing foam in order to diminish their flammability by intruding with polymer combustion. They can be halogenated or brominated compounds. Polybrominated diphenyl ethers (PBDEs) flame retardants are bio accumulative and are considered as endocrine disrupting chemicals (EDCs) [8].

2.2.6 Pesticides

Chemical pesticides have contributed enormously to the elevated yields in agriculture by controlling pests and diseases and also toward checking the insect-borne diseases (malaria, dengue, encephalitis, filariasis, etc.) in the human health sector [8]. The obligation to increase world food production for the rapidly growing of population is well recognized.

However, the excessive use has been leading to important consequences not only in public health but also in food quality, resulting in an impact load on the environment and hence the development of pest resistance. Through overuse and misuse there is noticeable waste, adding to the cost and contributing to the adverse environmental and health consequences [9].
Inappropriate application of pesticides affects the whole ecosystem by entering the residues in the food chain and polluting the soil, air, ground, and surface water [10].

- **Insecticides**

Insecticides are substances used to kill insects. They include ovicides and larvicides used against insect eggs and larvae, respectively. Insecticides are used in agriculture, medicine, industry and by consumers. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans and/or animals. Some of the most used insecticides are presented in Table 2.1.

- **Herbicides**

Herbicides, also known as weed killers, are chemical substances used to control unwanted plants. Selective herbicides control specific weed species, while leaving the desired crop relatively unharmed, while non-selective herbicides, sometimes called total weed killers in commercial products, can be used to clear waste ground, industrial and construction sites, railways as they kill plant material with which they come into contact. Some of the most used herbicides are presented in Table 2.2.

- **Fungicides**

Fungicides are biocidal chemical compounds or biological organisms used to kill parasitic fungi or their spores. Fungi can cause serious damage in agriculture, resulting in significant losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections in animals. Table 2.3 presents some of the most used fungicides.

2.2.7 Phthalates

Phthalates are diesters of benzenedicarboxylic acids and are the most commonly used plasticizers today. Phthalates with the highest production are diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and bis(2-ethylhexyl) phthalate (DEHP) which is an agricultural spray adjuvant used in all pesticides [8, 11].

Phthalates are generally used as plasticizers to increase stability and flexibility, to prevent brittleness, as a solvent for fragrances, and as inert ingredients. Table 2.3 presents one of the most used phthalates.

Table 2.1. Chemical structure and physicochemical properties of some insecticides.

Insecticides	Chemical structure	Molar Mass (g/mol) [12]	Solubility in water (mg/L) at 25°C	Log (K_{ow})	Boiling point (°C) (760 mmHg)	CAS number [12]
Heptachlor		373.3	0.056 [49]	5.2 [49]	392.3 [50]	76-44-8
Imidacloprid		255.7	610 [51]	0.57 [51]	442.3 [52]	138261-41-3
Dimethoate		229.2	25000 [53]	0.78 [53]	440.6 [53]	60-51-5

Table 2.2. Chemical structure and physicochemical properties of some herbicides.

Herbicides	Chemical structure	Molar Mass (g/mol) [12]	Solubility in water (mg/L) at 25°C	Log (K_{ow})	Boiling point (°C) (760 mmHg)	CAS number [12]
Glyphosate		169.1	12000 [54]	-3.2 [55]	465.8 [56]	1071-83-6
Terbuthylazine		229.7	6.6 [57]	3.4 [57]	373.1 [58]	5915-41-3
Acetochlor		269.8	233 [59]	4.14 [60]	391.5 [61]	34256-82-1
Metolachlor		283.8	530 [62]	3.1 [62]	406.8 [62]	51218-45-2
Alachlor		269.8	240 [63]	3.5 [64]	404.1 [65]	15972-60-8
MCPA		200.6	29390 [66]	2.8 [67]	327.1 [68]	94-74-6

Table 2.3. Chemical structure and some physicochemical properties of some fungicides and one phthalate.

Fungicides	Chemical structure	Molar Mass (g/mol) [12]	Solubility in water (mg/L) at 25°C	Log (K_{ow})	Boiling point (°C) (760 mmHg)	CAS number [12]
Epoxiconazole	N, N–N, O, Cl, F	329.1	7.1 [69]	3.6 [69]	463.1 [70]	135319-73-2
Tebuconazole	N, N–N, HO, Cl	307.1	36 [71]	3.7 [72]	476.9 [73]	107534-96-3
Phthalate	**Chemical structure**	**Molar Mass (g/mol) [12]**	**Solubility in water (mg/L) at 25°C**	**Log (K_{ow})**	**Boiling point (°C) (760 mmHg)**	**CAS number [12]**
Bis(2-ethylhexyl) phthalate (DEHP)	CH_3, CH_3, O, O, CH_3, CH_3	390.6	0.27 [74]	7.6 [75]	-	117-81-7

2.2.8 Sources of emerging pollutants

Most of the convenient data in the literature are related with priority pollutants such as heavy metals, pesticides, and other regulated priority organic pollutants, thus the information available for emerging pollutants (EP) is relatively limited.

The concentration of EP in the environment is very low, as aforementioned (ng/L to µg/L). However, they still can affect ecosystems balance and water quality, and even impact drinking water resources. Therefore, it is decisive that both presently used and prohibited compounds, as well as their metabolites, should be monitored [8].

Since analytical measurements are still time consuming, expensive and require the use of sophisticated advanced equipment, the detection of EP could be challenging in practice. Nevertheless, currently available instrumental methods, such as GC-MS or HPLC-MS allow the detection and study of many EP in the environment.

The passages of entrance for these pollutants into environmental compartments are diverse. They can be classified into point-sources and diffuse pollution sources [11].

- *Originated from point-sources* such as sewage treatment, industrial wastewater, plant effluents, mining activities or landfill leachates.
- *Diffuse pollution sources* are more crucial to be identified since they can be protracted over large geographical areas. This category covers stormwater runoff, terrestrial runoff from roads, urban areas, highways, agricultural land, soil leaching or leachate infiltration.

Compared to point-sources, diffuse sources usually release to the environment lighter loads of pollutants [6]. The use of pesticides in agriculture is contemplated as the major contamination source as well as hormones and veterinary medicines. Besides, treatment plants receive a large range of substances that are not completely eliminated throughout the treatment process stream. After all, that portion mixed with toxic molecules including their metabolites is often reused in many areas as a fertilizer in agricultural land and can be re-introduced this way to the environment.

Living beings are thus exposed to a multitude of compounds that can have harmful effects on them. It is then necessary to be able to identify the potential effects that these compounds may have on humans.

2.3 Factors influencing the detection of pesticides

Data on pesticides and their metabolites properties that determine their fate in the environment are often not available. Therefore, it is important to target the final destination of these molecules or on the other hand, do a risk assessment of pesticides in the environment using information derived from knowledge exchange between researchers [13].

The main results drawn from the laboratory was the need to identify the specific environmental conditions in a specific region and to ascertain how this picture can be harmonized in (regulatory) pesticide fate modeling. Both the weather conditions (e.g., temperature, light, rainfall intensity, snow/frost, altitude-latitude), soil conditions (e.g., soil type, freezing/thawing of soil) vary markedly between different areas.

Furthermore, the agricultural practices in countries differ markedly, being influenced not only by topography and soil and weather conditions, but also by sociocultural conditions and political decisions [13].

There must be agreement regarding both threshold values (surface and groundwater concentrations and toxicity assessment results) and the frequency of surpassing defined thresholds that can be accepted as qualification for more detailed studies.

Besides, it was observed a seasonal alteration of residues in ground water related with several factors namely time of pesticide treatment and irrigation. The steep values were quantified, normally, after pesticide application and during the irrigation period, mainly in summer, and sometimes, in autumn [13].

2.4 Removal of emerging pollutants in treatment plants

Although the concentration of emerging pollutants in the environment is low, continuous exposure to these compounds is a critical concern with unknown long-term impacts. Consequently, the removal of EP gained much attention. Generally, elimination methods of EP fall into three categories: physical, biological and chemical methods. Among chemical methods, advanced oxidation processes (AOP) have been widely investigated [14].

2.4.1 Removal by physical adsorption processes

Adsorption is the most prevalent physical process and is the main processes for removing EP in water. In order to enhance the adsorption capacity for EP, different adsorbents have been researched and developed for adsorption of EP from aqueous solution.

- Activated carbon

Activated carbon has been a widely choice as an adsorbent for the removal of pesticides from wastewater and has a certain application for adsorption of EP from wastewater.
Activated carbon has two structures: powdered activated carbon (PAC) and granular activated carbon (GAC).
But its expensive cost poses an economical problem. Therefore, researchers felt the need for the development of low cost and easily available materials, which can be used more economically on a large scale [11].
It opened the doors of research interests into the production of alternative adsorbents to replace the costly activated carbon that has intensified in recent years. The waste materials and byproducts from the agriculture and other industries are the sources of low-cost adsorbents due to their abundance in nature and because they have processing requirements.

- Agricultural Activated Waste Adsorbents

In recent years, a new class of adsorbents and specifically lignocellulosic materials has been investigated for the same purposes: their attractiveness resulting from their availability, low cost, and biodegradability.
Some previous researches reported their ability to quantitatively accumulate heavy metals and various organic compounds such as dyes and pesticides [14].
Accumulation of these pesticides on agricultural adsorbents is generally achieved through interactions with the hydroxyl and carboxyl groups [15].
Furthermore, the functionalization of this material by the grafting of organic molecules bearing active groups was carried out very successfully. Interestingly, the use of the resulting hybrid materials as an adsorbent lead to significant increases in adsorption capacity (sometimes greater than that of activated carbons) compared to raw materials [15, 16].
Memon *et al.*, [18] reported the adsorption of methyl parathion pesticide from water using chemically and thermally treated watermelon peels as a low-cost adsorbent and it was effective.

Akhtar *et al.,* [20] reported in their work that low-cost agricultural waste (i.e., rice bran and rice husk) can be effectively used to remove triazophos pesticide from water.

- Industrial Waste Adsorbents

Development of low-cost adsorbents for pesticide retention is an important area of research in environmental sciences. Industrial wastes such as sludge, fly ash, and carbon slurry are classified as low-cost materials because of their low cost and local availability and can be used as adsorbents for pesticides removal.

The fly ash, a solid waste from lignite coal-fired thermal power stations, is a low-cost adsorbent and has shown significant adsorption capacity for organic pollutants [21]. Iqbal et al., [19] reported that coal fly ash has significantly high retention capacity for metribuzin, metolachlor, and atrazine.

- Inorganic Natural Adsorbents

Natural clay minerals are well known and familiar to mankind from the earliest days of civilization. Because of their low cost, abundance in most continents of the world, high sorption properties, and potential for ion exchange, clay materials are strong adsorbents. In recent years there has been an increasing interest in utilizing clay minerals such as cloisite, clinoptilolite, eluthrilithe, kerolite, faujasite and montmorillonite for their capacity to adsorb not only inorganic ions but also organic molecules.

More recently, low-cost adsorbents, for example, organoclay complex adsorbents, have been investigated as an alternative to activated carbon. These materials, often used in industrial and technological processes, have been proposed as adsorbents for the immobilization of industrial organic contaminants, for the removal of pesticides from water [22].

According to Darvas, [15] the selectivity of adsorption depends mainly on the polarity, shape, and size of the diffusing molecules relative to the geometry of pores of the zeolites, the presence of exchangeable cations and impurities in the zeolite structure, and the chemical and physical treatments of zeolites.

- Graphene and graphene oxide

Graphene is a kind of new material of single chip structure composed of carbon atoms. The basic structure of graphene is a two-dimensional array of carbon atoms covalently connected via sp^2 hybrid orbitals to form a honeycomb sheet.

Graphene oxide is a precursor of graphene and always prepared via the oxidation of graphite. Recently, graphene and graphene oxide have received increasing attention due to their remarkable properties. Graphene and graphene oxide have higher specific surface area than activated carbon, so it is reasonable to believe that they can be the potentially promising adsorbents to remove the EP. Graphene and graphene oxide can remove the EP. At present, most studies about the adsorption of EP by graphene and its oxide were batch experiments in the laboratory-scale [14].

- Carbon nanotubes

In parallel with graphene, carbon nanotubes have excellent properties, which make them become candidates for many applications such as energy storage and medical devices. Many studies have investigated the removal of EP by carbon nanotubes, such as sulfamethoxazole. These studies indicated that carbon nanotubes have high adsorption capacity to the EP. But the adsorption capacity varied with the surface chemistry and properties of carbon nanotubes.
Also, they had the potential to be used as an effective adsorbent for removal of atrazine from water [15].

2.4.2 Removal by biological degradation processes

Microbial degradation is considered as the most important removal mechanism for organic pollutants in the environment, which has many advantages such as low cost and mild operational conditions. Microorganisms can remove the pollutants by utilizing the pollutants for metabolic functions and in some cases different microorganisms can cooperate together to remove the pollutants.

- Pure cultures

A number of studies have reported that pure cultures isolated from activated sludge, wastewater or sediment can be used to eliminate the frequently detected emerging pollutants.
Some pure cultures isolated from the activated sludge exhibit the capability to degrade a wide range of EP. In addition, for specific EP, many pure cultures can use it as sole carbon and energy source, but with different degradation mechanism [9].

- Mixed cultures

Compared to pure culture, mixed cultures are easier to achieve the goal of degrading the EP because in some cases it is too difficult to get the pure culture. In fact, the most widely used biological treatment process-activated sludge-in the WWTP, depends on the synergy effect of mixed culture to remove the EP [9]. Enhanced removal of EP by adding mixed culture into the activated sludge has been reported.

- Activated sludge process

Activated sludge process was widely used as biological treatment in the conventional WWTP. The removal of EP in the biological treatment is a combined effect of volatilization, adsorption and biodegradation. In general, biodegradation is the main mechanism for the removal of EP by activated sludge. However, biodegradation is not always effective for removing the pollutants in the environment, which is attributed to the low abundance of degraders and the lack of degraders in the environment.

2.4.3 Removal by chemical advanced oxidation processes

As mentioned above, EP are been frequently detected in the effluents of WWTP, although their concentrations are low, suggesting that the conventional wastewater treatment process cannot remove the EP completely. Therefore, advanced chemical processes are needed to deal with the wastewater containing EP. The chemical oxidation processes such as ozonation and other advanced oxidation processes (AOP), involving ozone oxidation, Fenton oxidation, and UV/hydrogen peroxide treatment [14].

- Ozonation

Ozone is the most widely used oxidation method in the removal of EP. This chemical process mainly depends on the strong non-selective oxidizing activity of hydroxyl radicals to eliminate the EP. The mechanism of ozonation is mainly based on the formation of hydroxyl radicals. Ozone has been used as post-treatment process to determine the performance in removing the EP. Results demonstrated that ozone can remove most of EP with removal efficiencies higher than 90%. Thus, concentration of hydroxyl radicals is directly related to the ozonation rate of EP [11].

- Fenton oxidation

Fenton oxidation using iron salts and hydrogen peroxide at acidic conditions is an important oxidation treatment to remove pollutants and used for the treatment of industrial wastewaters. In accordance with ozone oxidation, Fenton oxidation is also dependent on the strong oxidizing capacity of hydroxyl radicals. Fenton oxidation or Fenton-like oxidation has demonstrated its effectiveness in the removal of EP. The core of Fenton oxidation or Fenton-like oxidation is to decompose H_2O_2 to generate hydroxyl radicals using different metal-based catalysts [9].

- UV treatment

Ultraviolet (UV) treatment is a very popular method for disinfecting potable water. UV disinfection is also applied in the wastewater sector, after biological treatment and a sand filtration in case of direct reuse of reclaimed water. The mechanism of UV treatment is to destruct chemical bonds of pollutants by direct UV light, which is called “photolysis”. However, direct UV photolysis is not always effective [8]. In order to increase the capability of UV treatment in removing the EP, was introduced a combination of UV with hydrogen peroxide which is also called “advanced oxidation process”.
Similar to ozone and Fenton oxidation, UV/hydrogen peroxide process is based upon the generated hydrogen radicals resulting from the absorption of UV light by hydrogen peroxide.

2.5 Sampling Quality Assurance/Quality Control (QA/QC)

The collection of a water sample is a delicate operation in which the greatest care must be taken; it conditions the analytical results and the interpretation that will be given.
The sample must be homogeneous, representative, and obtained without modifying the physicochemical characteristics of the water (dissolved gas, suspended solids, etc.). Since in most cases the collection manager is not the analyst, the sampler should have a clear understanding of the sampling conditions and its importance for the quality of the analytical results [2].
Overall, it is therefore necessary to set up a structured organization to have qualified personnel to develop a methodology adapted to each case, to make a wise choice of sampling points and using the proper equipment. In any case, the results of the analysis will be exploitable only if the sample is representative.

In addition, although it is clear that a correct sample is essential to obtain significant analytical results, it is equally important to know the fate of the sample between the sampling and the arrival at the laboratory. The instantaneous sampling is only a reflection of the composition of water which has an evolutionary character. A better appreciation of these variations can result from a multiplication of samples [4].

Also, should be given special attention to sampling equipment. The use of new bottles of borosilicate glass or high-density polyethylene (HDPE) with teflon plugs washed with a hot detergent solution and rinsed with deionized water and dried is recommended. Thus, it is advisable to avoid the reuse of the bottles and especially mixing of the vials used for the analysis of drinking water with those used for industrial water, wastewater, surface water, etc. The maintaining of a moist atmosphere allows, by rinsing the bottle at the time of sampling, to eliminate any contamination [16].

The sampling method will vary depending on the origin of the water. In the case of a river, an open sheet or a tank, the bottle will be plunged at a certain distance from the bottom (50 cm) and also from the surface, far enough from the banks or the edges as well as natural or artificial obstacles.

In the case of a lake or reservoir, several sampling points must be selected at different depths to consider vertical and horizontal heterogeneity.

In the case of groundwater like a well equipped with a pump, the samples will normally be at the end of an interrupted pumping test with a total duration of 30 hours.

In the case of sampling from a tap, if the purpose is the control of the distributed water, it is essential to wait for the stagnant water in the pipes to be eliminated. In practice, it is advisable to open the valve at maximum flow rate for 5 to 10 seconds then to bring it back to an average flow rate for 2 minutes. Then, present the bottle under the tap without having closed it again.

The sampling will inevitably undergo a certain transport time and a possible repose in the laboratory before the analytical start. These times must be reduced to a minimum. In general, transport at 4 °C and in the dark, in isothermal packaging ensures satisfactory preservation [4].

Regarding the water coming from supply networks, it is rarely found in the presence of a significant turbidity and precipitation accessories. The analysis can then be performed directly on the sample.

On the other hand, surface water and some catchments may be sampled with marked turbidity, whether this is pre-existing at the time of sampling or has developed as a result of secondary phenomena [16].

In any case, the presence of a significant turbidity, the analytical results may be distorted by the lack of homogeneity of the sample even after resuspension, by the difficulty of measurements made by molecular absorption spectrophotometry or gravimetry and it will be necessary the separation of the suspended solids [2]. To facilitate the work of the analyst and the exploitation of the results while avoiding errors, the samples should be labeled or numbered very carefully. Each vial must be accompanied by a data sheet to gather useful information in the laboratory and observations made during operations.

2.6 Extraction techniques and analytical methods for pollutants

The physicochemical methods developed for the analysis of organic contaminants in given matrices serve to identify and precisely quantify these contaminants. In general, the steps of physicochemical analysis are extraction, separation of compounds, and finally, the detection and quantification of target pollutants [4].

2.6.1 Extraction technique

The extraction is an analytical step to prepare the sample. It makes it possible to increase the sensitivity of the analysis method and to reach low limits by eliminating the compounds that can interfere with the target compounds and thus allows the pre-concentration of these compounds. This step is necessary for complex matrices such as soils and when the compounds to be analyzed are in the trace concentration levels [16].

The extraction processes are diverse and the selection of the type of extraction depends on the matrix, the objective of the analysis and the physicochemical properties of the targeted compounds. For example, for water, solid phase extraction and solid phase micro-extraction are the most used. For sludge, soil and sediment, liquid-liquid extraction, liquid extraction under pressure, microwave assisted extraction and supercritical fluid extraction are the most commonly used [2]. Separation, detection and quantification are performed by gas or liquid chromatography coupled with specific or universal detectors. These two chromatographic techniques as well as the associated detectors are presented in the next sections.

Traditional methods of analysis for pesticides, involves their extraction by solvent before they are analyzed. This extraction technique is generally, both time and solvent consuming. The need to develop a solvent-free and sensitive technique arises because of the use of organic solvents create pollution-related problems; moreover, organic solvents are costly and require time-consuming procedures.

The solid-phase microextraction is relatively a new approach invented and developed at the University of Waterloo (Ontario, Canada) by Pawliszyn and Associates (Belardi and Pawliszyn) and being sold by Supelco [45]. Introduced as an alternative to traditional sample preparation techniques, because it provides a rapid, simple, effective, solvent-free, and sensitive pretreatment method and can be easily combined with various separation techniques.

The method involves the equilibrium sorption of analytes onto a small microfiber, which is made of a fused-silica optical fiber, coated with a hydrophobic polymer. The fiber is fixed inside a needle of the syringe-like device. It has a small size and cylindrical shape, connected to a stainless-steel tubing that is used to provide additional mechanical strength to the fiber assembly for repeated sampling. Extraction is performed either by immersing the fiber in the gaseous or relatively pure liquid medium or by sampling the analytes from the headspace above the investigated medium. Analytes come into equilibrium with the fiber according to their affinity for the solid phase. The microfiber is incorporated into GC-MS interphase and the analytes are desorbed from the fiber and delivered to the column for separation.

Solid-phase microextraction coupled with chromatographic techniques is gaining wide applicability as an analytical technique. The technique is based on the partition of the analyte between the sample matrix and stationary phase (polyacrylate, polydimethylsiloxane, etc.) coated on fused silica fiber. Depending on the distribution coefficient, the equilibrium is reached between the concentration of the analyte in the sample and the amount of analyte adsorbed on the fiber.

The fiber assembly is reusable and replaceable. Supelco provides seven different types of fibers. Commercially available SPME fibers are expensive and have limited lifetime, since they tend to degrade with increased usage. The difference in length and thickness of SPME fiber coatings may result in variation of analyte enrichment from fiber to fiber [45].

Table 2.4 presents list of the most used fibers for the extraction of pesticides.

Table 2.4. List of some fibers commercially available from Supelco. Properties and recommended use for selected pesticides.

Fiber Core	Thickness [76]	Bond Type [77]	pH range [77]	Operation Temperature (°C) [77]	Application [76]	Recommended Use [17]	Pesticides
Polydimethylsiloxane (PDMS)	100µm 7µm	Non-bonded / Bonded	2-10 2-11	200-280 220-320	Volatile Non-polar / Semivolatile Moderately polar	GC/HPLC GC/HPLC	-
Polydimethylsiloxane/Divinylbenzene (PDMS-DVB)	65µm	Partially crosslinked	2-11	200-270	Polar volatile	GC	Terbuthylazine Alachlor Heptachlor Metolachlor [41]
Carboxen/Polydimethylsiloxane (CAR-PDMS)	75µm	Partially crosslinked	2-11	250-310	Trace-level volatile	GC	Dimethoate [42],[44]
Polyacrylate (PA)	85µm	Partially crosslinked	2-11	220-300	Polar semivolatile	GC/HPLC	Alachlor Heptachlor Metolachlor [36],[41]

2.6.2 Gas chromatography and liquid chromatography

In gas chromatography (GC), the separation of the compounds is based on the distribution of the compounds between the mobile phase, which is a gas called as carrier gas, and the stationary phase, which may be a liquid or a solid adsorbent (alumina, silica...). As column types, the capillary columns are the most used, the most efficient, particularly the narrow-boron columns. Four injection modes can be used: split, splitless, on column and programmed vaporization temperature.
GC is used preferentially for the analysis of thermostable, volatile or semi-volatile, non or medium polar compounds and for easily derivable compounds [17].

In liquid chromatography (LC), the mobile phase is a solvent or a solvent mixture introduced into a constant flow column. Stationary phases of diverse nature are used. These phases determine the type of liquid chromatography.
Thus, adsorption chromatography, ion chromatography, exclusion chromatography, liquid / liquid chromatography and liquid chromatography / graft phase are distinguished. The polarity of the grafted phase can be modified. This modification leads to reverse phase chromatography, which is one of the most used. Indeed, it is suitable for almost all compounds soluble in organic solvents [17]. Liquid chromatography is often preferred over gas chromatography because it does not require derivatization of the compounds.

2.6.3 Detection methods

After separation in the chromatographic column, the compounds are detected. The detection systems differ according to the type of chromatography. In liquid chromatography, the most used detectors are the ultraviolet (UV) detector, the fluorescence detectors and in gas chromatography the flame ionization (FID), electron capture (ECD), photoionization (PID) detectors are most commonly used [4]. However, not all detectors make it possible to precisely identify and characterize the structure of the compounds. As a result, mass spectrometry (MS) and tandem mass spectrometry (MS^n) are increasingly used to detect compounds and determine their structure.

- Mass spectrometry

Mass spectrometry (MS) is used to characterize compounds by measuring the mass-to-charge (m/z) ratio of ionized molecules and their fragmentation products. It can be associated with both liquid and gas chromatography. The GC-MS coupling exists since 1960 and it was much later in 1974 that the LC-MS coupling was done. This coupling has been made possible thanks to the technological evolution of LC-MS interfaces [4].

The different types of existing MS analyzers are the magnetic analyzer, the quadrupole analyzer, the traps (quadrupole ion trap, linear trap, ion trap associated with a Fourier Transform analysis).

- Tandem mass spectrometry

Tandem mass spectrometry (MS^n) allows a better characterization of molecules even in trace. It can be performed by coupling two or more analyzers or be performed with the ion trap. The tandem analysis is carried out initially by selecting an ion characteristic of the substance to be analyzed, the "precursor ion" [17]. This trapped ion will then collapse by collision and give "son ions" which can in turn be selected and give higher generations of ion "small son ions".

2.7 Review on extraction and quantification of various pesticides

In this section, a literature review on extraction and quantification of some pesticides is presented. A brief resume of this literature review is described in Table 2.5.

The detection, identification, quantification and evaluation of the impact of contaminants in the environment involve also physicochemical analysis. These two approaches are complementary.

By physicochemical methods, pollutants can be quantified and characterized structurally. The most used method to extract emerging pollutants from water is solid phase extraction (SPE). However, various analytical techniques have been used for the detection of emerging pollutants such as gas chromatography-mass spectrometry (GC-MS), gas chromatography equipped with a nitrogen-phosphorus detector (GC-NPD), high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS); high-performance liquid chromatography with photodiode array detector (HPLC-DAD).

Table 2.5. Extraction techniques and analytical methods applied for some pesticides.

Pesticides	Extraction Technique	Analytical Method	Reference(s)
Heptachlor	SPE/SPME	GC-ECD or GC-MS	[23],[24],[41]
Imidacloprid	SPE	HPLC-DA RPLC UPLC	[25],[26] [27],[28] [44]
Methyl parathion	SPE XAD-2 SPME	GC-MS UV GC-MS	[29] [30],[31] [32]
Glyphosate	SPE	HPLC-MS LC-MS/MS	[33] [34]
Acetochlor and Alachlor	SPE/SPME	GC-ECD	[35],[36]
Metolachlor	SPE/SPME	LC-MS GC-NPD GC-MS	[36] [37] [43]
Terbuthylazine	SPE/SPME	GC-MS	[41],[43]
Dimethoate	SPE/SPME	GC-MS	[42],[43],[44]
Epoxiconazole	LLME-SFO	GC-MS/MS	[38]
Tebuconazole	SPE	LC-MS/MS HPLC	[39] [40]

For each pesticide referenced in Table 2.5, a detailed description is presented in the following sections 2.12.1 to 2.12.9.

2.7.1 Heptachlor

A review about solid phase extraction (SPE) of heptachlor and other organochlorine pesticides was developed by Zuo *et al.*, [23]. The samples were extracted using hexane-acetone (50/50, v/v). A variety of cleanup steps may be applied to the extract, depending on the nature of the matrix interferences and the target analytes. Suggested cleanups include gel permeation chromatography and the use of Florisil® SPE cartridges. After cleanup, the extract is analyzed by injecting a measured aliquot into a gas chromatograph equipped with either a narrow-bore or wide-bore fused-silica capillary column, and either an electron capture detector (GC-ECD) followed by validation using gas chromatography–mass spectrometry (GC–MS) with negative chemical ionization.

Naghmeh *et al.*, [24] studied the limit of detection and quantification for several organochlorine pesticides, such as heptachlor. In their experiments, a 1000 mL water sample was spiked with 1 mL of 0.080 mg/L standard solution, 5 mL of methanol and passed through a 6 mL capacity C18 cartridge. The cartridge was conditioned with 5 mL of ethyl acetate, 5 mL of dichloromethane, 10 mL of methanol and 10 mL of organic free water before use. Then, it was eluted with 5 mL of ethyl acetate and 5 mL of dichloromethane. The eluted solution was concentrated under a stream of nitrogen to a volume of 1 mL. Then, 1 µL of the concentrated solution was spiked with exactly 1 µL with a 100 mg/L of internal standard before analysis using the GC-ECD.

2.7.2 Imidacloprid

Kookana *et al.*, [25] reported that imidacloprid can be extracted by a C18 column preconditioned with 5 mL of methanol and washed with 5 mL of water. Immediately after the wash, triplicate 10 and 100 mL water samples containing three spike levels of imidacloprid, 10, 1 and 0.5 g/L were flowed through the columns using a flow-rate of 10 mL/min, without allowing the column bed to dry and the eluate was discarded. The columns were then dried by pulling air through for 5 min before the adsorbed compound was eluted with 2 mL of methanol. The methanol eluate was evaporated under N_2 and then re-dissolved in 1 mL of acetonitrile/water (20/80, v/v) for HPLC. The mobile phase was a mixture of 80% of 0.2% phosphoric acid aqueous solution and 20% of acetonitrile. The wavelength of the diode array detector was set at 270 nm for imidacloprid and 224 nm for 6-CNA, with a reference wavelength of 360 nm. The flow rate was 0.8 mL/min, the column temperature was 25°C and the injection volume was 20 µL [26].

Another study, presented by Tao *et al.*, [27] show that an isocratic RPLC method can be used to determine the presence of imidacloprid in surface water. The method used a gradient of 5% to 70% acetonitrile in water with 0.1% formic acid.

A third study developed by Pam *et al.*, [28] studied the separation of 7 neonicotinoids under both acetonitrile and methanol gradient conditions, with a CORTECS phenyl column using UPLC. Two gradient methods were applied. The first was a 5% to 70% acetonitrile in water with 0.1% formic acid and the second was a 5% to 70% methanol in water with 0.1% formic acid. The flow rate was maintained at 0.5 mL/min. The results show a complete separation for the compounds.

2.7.3 Methyl parathion

The determination of methyl parathion was presented by Jiping *et al.*, [29]. A SPE cartridge HLB was first conditioned with 5 mL ethyl acetate to remove air and leach impurities. Then 5 mL of ultrapure water was added to equilibrate the solid phase. A 500 mL water sample was loaded by means of a vacuum pump. Water was then removed, and the vacuum maintained for more 25 min. The analyte was eluted from the sorbent using 2 mL of ethyl acetate. The solution was then transferred to double layer silicon–teflon septum vials placed in the GC auto-sampler and then analyzed by GC-MS.

Furthermore, Paschal *et al.*, [30] extract methyl parathion using a macro reticular resin. A glass column was filled with Amberlite XAD-2 resin up to a height of 10 cm. The column was washed successively with 50 mL of ethanol, diethyl ether and distilled water. Then the water samples were allowed to percolate through the column at an average flow rate of 20 mL/min. The water samples were then, passed through the column, diethyl ether was allowed to flow through the column at 2 to 3 mL/min, after which the ether was removed by passing dry purified nitrogen through the column. The ether was dried by shaking with 2 g of anhydrous sodium sulfate and evaporated to dryness using a rotary evaporator. The residue was dissolved in acetonitrile and the methyl parathion was determined by spectrophotometry by the addition in every tube of 1 mL of MBTH solution and 2 mL of ferric chloride solution and allowed to stand for 15 min and diluted to a final volume with methanol.

Another study about the determination of 23 organophosphorous pesticides in surface water was presented by Xiaojin *et al.*, [32] which reports methyl parathion extraction using SPME followed by GC–MS. The extraction of water samples was carried out by direct immersion of the PDMS/DVB fiber in a 4 mL sample contained in a 5 mL clear glass vial under magnetic stirring for 45 min at 60°C. Sample agitation was done using 1150 rpm. Then the fiber was removed from the sample solution and inserted into the GC-MS injector port for analysis. The SPME fibers were desorbed in the injector using the splitless mode for 5 min and an injector temperature of 250°C was used.

2.7.4 Glyphosate

Delmonico *et al.*, [33] reported the use of solid phase extraction and HPLC to determine glyphosate and AMPA. For SPE extraction, 100 mL of sample containing glyphosate and AMPA was pumped through a cartridge containing 100 mg of anionic resin strongly basic using a flow rate of 5.0 mL/min. Prior to use, the resin was conditioned with 5 mL of HCl 3.0 mol/L and 10 mL of water. After the extraction, the compounds were eluted with 1.0 mL of HCl 0.050 mol/L and the eluate was collected in a 5 mL vial for subsequent analysis. The resin was regenerated to the chloride form by using 10 mL of HCl 0.1 mol/L and 5 mL of ultrapure water. For HPLC determination the analytes were derivatized and analyzed with a C18 column and using a mobile phase consisting of phosphate buffer 0.20 mol/L at pH 3.0 and acetonitrile (85/15, v/v).

Hanke *et al.*, [34] developed a trace level determination of glyphosate in waste water using SPE followed by LC-MS/MS. The SPE cartridges were conditioned with 5 mL of methanol followed by 5 mL of 0.1% formic acid aqueous solution. The samples were extracted using a flow rate of 2.5 mL/min. The analytes were eluted with 9 mL of methanol without using vacuum. The extracts were collected in conical bottom glass vessels. The methanol aliquots were reduced to approximately 50 µL by a gentle flow of nitrogen gas at 50°C. The extracts were transferred to 2 mL amber glass vials with inserts and the volume was reconstituted with 5 mmol/L ammonium acetate solution (pH=9) to approximately 250 µL in order to obtain the initial mobile phase conditions for the injection into the LC-MS/MS. The injection volume for LC-MS/MS analysis was 20 µL. A Waters Xbridge C18 column was used for LC separation. The mobile phase was composed of water buffered with 5 mmol/L ammonium acetate of pH 9 (solvent A) and methanol (solvent B), the flow rate was 0.2 mL/min and the column temperature were 30°C.

2.7.5 Acetochlor and Alachlor

Vuković *et al.*, [35] performed the extraction of two herbicides, acetochlor and alachlor, from drainage water, using solid-phase extraction. Prior to extraction the cartridges were preconditioned with 5 mL of methanol, followed by 5 mL of ultrapure water, using a flow rate of 2 mL/min. Then, the solution was filtered under vacuum using a flow rate of 10 mL/min. Then the cartridges were dried, and the pesticides eluted from the adsorbent with 6 mL of dichloromethane/n-hexane (40/60, v/v) and evaporated to dryness.

The extract was dissolved in 1 mL of methanol, ultrasonically homogenized and analyzed by GC-ECD. The GC injection volume was 3 μL and a splitless injection mode was used. Helium was used as the carrier gas at a flow rate of 1 mL/min.

Another study concerning the extraction and analysis of acetochlor and alachlor was presented by Yoklndey *et al.*, [36] showing that water sample is subjected to purification using a C-18 SPE column. The compound is isolated using 80/20 methanol/water (v/v) for elution. The eluate is reduced to a volume below 1 mL and reconstituted in 10/90 acetonitrile/water (v/v) to the desired final fraction volume. Final analysis is accomplished using LC-MS.

2.7.6 Metolachlor

The extraction and analysis of metolachlor was also studied and was presented by Yokley *et al.*, [36] as referred in section 1.12.5.

Another study was presented by Hassen *et al.*, [37] using a C18 cartridge of 6 mL volume size containing 1000 mg of C18 octadecyl adsorbent mounted on top to an aromatic sulfonic acid cartridge filled with 1000 mg of propylbenzenesulfonyl adsorbent. The cartridges were washed in series with 6 mL of methanol, then with 6 mL of distilled water followed by 2 mL of 0.1% acetic acid using a flow rate of 3 mL/min. A 100 ml aliquot of the sample was centrifuged and filtered through a 0.45 μm nylon filter, to which 0.1 mL of concentrated acetic acid was added, for a final pH value of 4. The sample was later eluted through the two cartridges at a flow rate of 3 mL/min. After the sample had been loaded, the two solid-phase cartridges were rinsed in series with 6 mL of distilled water and then separated. The C18 cartridge was aspirated further for 30 min using vacuum to remove any residual water. The sample extracts emerging from the C18 cartridge were analyzed using a gas chromatograph equipped with a nitrogen-phosphorus detector.
For GC analysis, a DB-5 capillary column was used with helium as the carrier gas at a flow rate of 2 mL/min. The detector was supplied with hydrogen at a flow rate of 3.4 mL/min and with air at a pressure of 35 psi. The injector and detector port temperatures were 250 and 300°C, respectively. The temperature of the column was initially programmed at 50°C. It was increased to 160°C at a rate of 20°C/min, then to 185°C at a rate of 5°C/min and finally to 240°C/min at a rate of 20°C/min. The latter temperature was held for 3 min. The injector was operated in the splitless mode and the injection volume was 1 μL.

2.7.7 Epoxiconazole

Bolzan *et al.*, [38] determined pesticide residues in water by gas chromatography-tandem mass spectrometry. Using LLME-SFO as method of extraction, a 10 mL aqueous solution with 2% w/v NaCl and a pH value of 7.0 was placed in a 15 mL glass tube. A mixed solution of 250 µL of 1-dodecanol, used as extraction solvent, and 1250 µL of methanol, used as the disperser solvent, was added rapidly into the sample solution. After centrifugation for 5 min at 2000 rpm, the organic solvent droplets floated on the surface of the solution. The test tube was then immediately transferred to an ice bath and cooled for 5 min. The floating solvent solidified and then transferred to an Eppendorf tube.

2.7.8 Tebuconazole

Martin *et al.*, [39] used solid-phase extraction to quantify the fungicide tebuconazole. The water sample sizes (0.1–10 mL) were adjusted to pH 6.3 by diluting the sample 1:1 with 100 mM ammonia formate solution (NH_4HCO_2) in the SPE barrel (3 mL) and 1 ng of internal standard (d_6-tebuconazole) was added (50 µL of 20 ng/mL solution). The samples were loaded at a rate varying from 1 to 2 mL/min on mixed-mode anion-exchanger material SPE cartridges (60 mg PAX, Agilent Technologies) mounted on vacuum manifold from Supelco. The two supernatant aliquots were combined, and a 500 µL aliquot was mixed with 500 µL mobile phase A.
The HPLC separation was performed using a reversed phase system consisting of a PEEK frit guard (0.5 µm), a guard column and an analytical C18 column. The mobile phase A consisted of 5% methanol and 95% ultrapure water, and mobile phase B of 95% methanol and 5% ultrapure water. Both mobile phases contained 0.1% formic acid by volume. The analytical system consisted of an Agilent 1260 series HPLC system, equipped with a degasser, autosampler using 50 µL injection volumes. The column flow rate was 0.2 mL/min using isocratic conditions (25% A/75% B, v/v).

Qingxiang *et al.*, [40] studied the determination of fungicides and prometryn in environmental water samples using multiwall carbon nanotubes SPE cartridge conditioned with 10 mL of methanol, then with 10 mL of water before a new pre-concentration procedure started, and then spiked water samples were aspirated through the column at a controlled flow rate. After the sample solution had passed through the SPE column, 10 mL ultrapure water was used to clean the impurity.

Subsequently, the SPE column was dried by negative pressure for 30 min and the target compound retained in the column was eluted with an optimum volume of dichloromethane and the eluent was dried with nitrogen gas in water bath. Then the residue was re-dissolved in 0.5 mL mobile phase. Finally, 20 μL of the final solution was injected for HPLC analysis. The mobile phase was a methanol/water mixture (80%/20%, v/v) at 0.4 mL/min, the injection volume was 20 μL and the detection UV wavelength was set at 220 nm.

2.7.9 Multi-pesticide residues

Ignacio *et al.*, [41], reported in their study the best result obtained to extract some compounds using different polymeric coatings for SPME. For terbuthylazine and methyl parathion the use of polydimethylsiloxane-divinylbenzene (PDMS-DVB) is the most recommended. For Heptachlor using poliacrylate (PA) is most suitable since it has highest fiber-water partition coefficient $K_{fw,}$ although PDMS-DVB fiber showed also good results.

In other research, conducted by Gonçalves *et al.*, [42] to extract by SPME mixed residues of pesticides, a PDMS-DVB fiber was also used to extract terbuthylazine, methyl parathion, heptachlor and dimethoate among others. The SPME extraction procedure adopted for this study consisted on the following: 3 mL-aliquots of the samples were extracted by immersion of a 60 μm PDMS-DVB coated fibre during 60 min; sample agitation was employed at the maximum agitation rate (around 900 rpm) and extraction temperature kept at 60 °C; neither pH adjustment nor ionic strength correction were needed.
Chromatographic analyses were carried out in a Varian 3400 CX gas chromatograph equipped with a CPSil-8 CB low bleed MS capillary column. The split/splitless injection port was maintained in splitless mode for 5 min, the lapse of time for SPME fibre desorption and set at a fixed temperature of 250 ºC. High-purity helium at a flow rate of 1.0 mL/min (150 °C oven temperature) was used as the carrier gas and as the collision gas at the ion trap chamber for MS experiments. Samples were analysed using the following oven temperature programme: initial temperature 80 °C (held for 2 min), increased by 15 °C/min to 190 °C (held for 4 min), then increased by 10 °C/min to 230 °C (held for 5 min) and, finally, increased by 10 °C/min to 290 ºC and held at this temperature for 6 min.
PDMS–DVB and PA are more appropriate for polar, nitrogen-containing herbicides.

An inter-laboratory trial involving the analysis of pesticides demonstrated the validity of SPME using PDMS–DVB fibre in association with added NaCl about 60% saturated sodium chloride solution and the influence of methanol on peak responses (Reasonable extractions may still be carried out at a methanol concentration of 10% vol.) in combination with a GC system.
For equilibrium time experiments, ultrapure water was spiked with standard solutions containing 30 ng/mL of each pesticide in methanolic solution. The linearity of the calibration curve has been studied for all pesticides using SPME at a concentration range of 0.03-30 ng/mL.

A third study developed by Azevedo *et al.*, [43] show that dimethoate, terbuthylazine, methyl parathion and metolachlor can be also extracted by SPE. In that work, OASIS HLB cartridges, 60 mg, were washed sequentially with 6 mL of dichloromethane, 6 mL of acetonitrile and 6 mL of water at a flow rate of 30 mL/min. A 200 mL aliquot of sample was passed though the cartridge at a flow rate of 6 mL/min and then washed with 1 mL of water.
Water residues from cartridges were removed using 30 min of vacuum. Elution was carried out with 2.5 ml of acetonitrile-dichloromethane (1:1) followed by 3.2 mL of dichloromethane at a flow rate of 1 mL/min. Evaporation of the solvent was performed under a stream of nitrogen. The final sample volumes (0.2-0.5 mL) were weighed and corrected by solvent density.
GC–MS analyses were performed using helium as carrier gas and the following conditions: HP-5MS GC column, 60 °C for 1 min, 60–175 °C (4min) at 6 °C/min, 175–240 °C (5 min) at 3 °C/min, 240–300 °C (1min) at 7°C/min. The injector was operated in splitless mode, the temperature of MS interface was 270 °C, the ion source temperature was 200 °C, the temperature of the injector was set at 250 °C. Electron impact ionization at 70 eV was used. All samples were analysed in the SIM mode for quantification purposes of the compounds (major ions corresponding to the typical fragments of the compounds were selected) and the scan mode in the range 70-450 u for confirmation of the spectral data against a real standard and library search.
Ground water samples were tested. The compounds were spiked in 200 mL of water to give a final concentration of 1.0 mg/L and subsequently the water was acidified at a pH value of 4. Immediately after this operation, the water samples were extracted with the ASPEC XL.
The limits of detection were calculated by using a signal-to-noise ratio of 3 (the ratio between the peak intensity under SIM conditions and the intensity of the noise was used).

The limit of detection for most of the compounds were in the range of 0.002-0.08 μg/L by GC-MS being enough for trace levels determination; considering that 200 mL of water were percolated through the cartridge.

Dujakovic *et al.*, [44] studied the determination of imidacloprid using a C18 and (CH_3OH/CH_3CN (1:1)) as elution solvents and dimethoate using HLB and CH_3OH as elution solvents. For this study, 100 mL of deionized water (without pH adjustment; pH~4.5 was spiked with the working standard solution in order to achieve a final concentration of 100 ng/mL for each analyte in the final extract.

The SPE cartridges were preconditioned with 5 mL of selected elution solvent followed by 10 mL of deionized water. Spiked water samples were loaded at a flow rate of 1 mL/min. The cartridges were then dried under vacuum for 10 min and analytes were eluted with 10 mL of selected elution solvent. Extracts were evaporated to dryness and reconstituted with 1 mL of methanol.

After the selection of the SPE sorbent and elution solvent, the effect of sample pH adjustment prior to extraction was evaluated. (pH = 2 was recommended). The deionized water was spiked with working standard solution to produce a concentration of 100 ng/mL for each pesticide. Extraction recoveries of target compounds were determined using both ground water and surface water samples spiked at 40 and 200 ng/L to produce concentrations of 10 and 50 ng/mL in the final extracts, respectively. Prior to analysis, samples used as blanks were proven to be free from the pesticides considered.

Chapter 3: Materials and methods

3.1 Chemicals and materials

All pesticide analytical standards, see Fig. 3.1, were supplied by Sigma-Aldrich (Merck, Darmstadt, Germany). The individual stock standard solutions, for GC analysis without SPME extraction, were prepared by the exact weighting of high-purity substances and dissolving them in methanol HPLC grade (Fisher, Spain). An analytical balance ADA 210/C, ±0.0002 g, Adam Equipment was used for mass measurements. A pH meter from Hanna, model 2020-02, was used to measure the pH value of samples. Sodium chloride with an analytical purity was used without further purification (98%).

Figure 3.1. Sigma Pesticides standards (Dimethoate, Terbuthylazine, Alachlor, Heptachlor and Metolachlor from left to right).

For gas chromatography-mass spectrometry analysis different solutions were prepared in methanol. Five individual stock solutions were prepared with a concentration of 1000 mg/L of each standard and stored in the freeze. All the mixtures and individual standard solutions were prepared daily in the moment of the analysis by dilution with methanol from the stock solution. For SPME analysis the diluted standard solutions were prepared using ultrapure water (resistivity value below 18.2 MΩ.cm - Type I). Sodium chloride, +98%, and HCl solution 1 mol/L were also used in the SPME extraction, both reagents were analytical grade.

3.2 Equipment

Chromatographic analyses were carried out in a Shimadzu GC-MS system, model QP2020, equipped with an AOC-20i autosampler and a Rxi-5ms Low Bleed capillary column (30 m×0.25 mm I.D. and 0.25 µm film thickness) obtained from Restek (Bellefonte, USA), see Fig. 3.2. For automatic injections, the split/splitless injection port was maintained initially in splitless mode for 4 min followed by a split-ratio (1:10).

The injector port was maintained at 250 ºC during all the analysis. The MS acquisition was performed in the range of 35–450 (m/z). The ion source temperature was 200 °C and the interface temperature was 270 °C.

Figure 3.2. GC-MS with autosampler.

All SPME fibers used for extraction were new at the beginning of the study and were conditioned according to the supplier's instructions, in the GC port at 250 °C for 45min. Then the column was cleaned for 2 hours at 300 °C.
Manual operation of the SPME technique was performed using 4 mL amber glass vials, manual SPME holder and an agitation and heating plate with temperature control, as presented in Fig. 3.3.

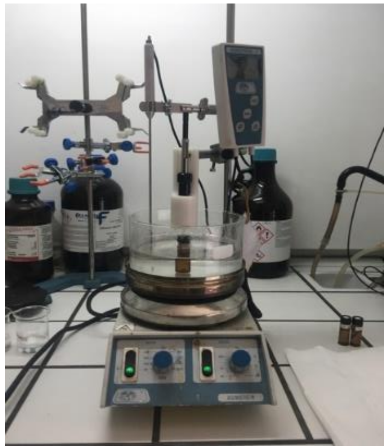


Figure 3.3. SPME extraction using a manual holder, an agitation and heating plate with temperature control and 4 mL amber vials.

Immediately, after the extraction the fiber was retracted, protected inside the needle, inserted in the GC-MS injection port and then exposed for desorption, during a specific time and temperature, as presented in Fig. 3.4.

Figure 3.4. GC-MS with a SPME fiber inserted in the GC-MS injection port.

After the time needed for total desorption, normally 4 or 5 min, the fiber was used again for extraction using a new sample. At the beginning of each day the fiber was pre-conditioned again before use.

3.3 Experimental methodology

The main objective of this study is the development and validation of an extraction and quantification methodology to monitor 5 different of the most used pesticides in northeast of Portugal and Tunisia in aqueous matrices.

The complete development and implementation of a new experimental methodology is a task that requires time. Due to the short time available to perform the experimental work in this double degree diploma program, it was decided to optimize only some of the most important parameters in the extraction technique and to study the proper selected operating conditions of the detection and quantification method [46] [47].

From the set of the pesticides already available at the moment of the experimental work starts, a group of five pesticides was selected based on some published documents that refer some of the most used pesticides in both northeast of Portugal and Tunisia. This group of pesticides includes dimethoate, alachlor, metolachlor, heptachlor and terbuthylazine [46] [47].

Due to the information collected in the literature review presented in previous section, it was decided to develop an experimental methodology based in a first extraction/concentration step using solid phase micro-extraction technique followed by quantification using gas chromatography with mass spectrometry detection.

The experimental work planning includes the following steps:

I. Selection of the most appropriate operating conditions for gas chromatography and mass spectrometry detection. For gas chromatography is intended to select the oven temperature program, the injection temperature and mode (split/splitless) that allows the separation of all the compounds. For mass spectrometry it is important to define the best mode of detection (Full Scan or Single Ion Monitoring) in order to both clearly identify all pesticides and maximize the total detector signal since it can improve de limits of detection and quantification of the method.

II. Optimization of the most important solid phase micro-extraction parameters, such as, the addition of salt to the sample, the pH value of the sample, the extraction time and temperature and the desorption time and temperature in the gas chromatography injector port.

III. Determination of the most relevant statistical parameters mandatory to validate the developed methodology. This task includes the determination for all the five pesticides, the calibration curves and the limits of detection and quantification.

IV. Finally, the implementation of the experimental methodology to monitoring the five pesticides in different surface waters near agricultural lands, such as lagoons or rivers used for irrigation of soils. For this task, different samples will be collected and analyzed near Fervença, Sabor and Onor rivers.

3.4 Optimization of GC-MS operating conditions

Several GC-MS parameters were studied in order to improve the identification and quantification of the 5 pesticides.

After a carefully reading of the literature, three different GC oven temperature programs were selected and tested for the 5 selected pesticides, injected individually or in a mixture using different concentrations.

The main purpose of this first task was to identify the elution order of each compound and compare the resolution and analysis running time need for the three published methods. These methods, used as reference, were obtained from literature and are described from Table 3.1 to Table 3.6.

Table 3.1. GC temperature profile for "Method 1"

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	50	1
25	100	-
5	300	5

Table 3.2. GC temperature profile for "Method 2" [42]

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	80	2
15	190	4
10	290	2

Table 3.3. GC temperature profile for "Method 2 modified"

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	120	2
15	190	4
10	227	1

Table 3.4. GC temperature profile for "Method 3" [43]

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	50	1
25	100	0
5	280	0

Table 3.5. GC temperature profile for "Method 3 modified"

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	80	2
15	180	4
10	280	1

Table 3.6. GC temperature profile for "Method 4" [44]

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
-	45	-
30	130	3
10	240	-
10	280	-

In a second step, the optimization of retention, resolution and running time was performed by small variations in the GC oven temperature program. Then the split/splitless modes of injection were also tested in order to study the effect on the detector signal. Finally, the obtained separation/detection results obtained with single ion monitoring "SIM" and the full scan mode "FullScan", also named "TIC", for mass detection were studied.

After selection of the most favorable GC-MS operating conditions, the optimization of the main SPME parameters was conducted.

3.5 Optimization of SPME main parameters

The read of some published works referring the use of SPME for extraction of pesticides and further analysis using GC or GC-MS, allows the identification of the main parameters for optimization. Due to the lack of time for the experimental part of this thesis, some parameters were fixed, namely, sample agitation (1000 rpm, maximum), desorption time (4 min), desorption temperature (250 ºC), mode of extraction (direct immersion against headspace) and volume of the sample (3 mL in a vial of 4 mL).

The other main parameters, considered as the most important, were selected for optimization at 3 different levels. The extraction temperature (50, 60 and 70 ºC), the extraction time (40, 60, and 80 min), the pH value of the sample (2, 4 and 6) and the addition of salt, i.e., the increase of ionic strength of sample (10, 20 and 30% NaCl).

Using a 4 mL Amber vial, the solutions were prepared in 3 mL volume after dilution with ultrapure water or methanol from 1000 µg/L to 1 µg/L.

For SPME studies, it was noticed that the dilution of the standards in methanol is inappropriate since sodium chloride presents a considerably lower solubility in methanol (14.9 g/L at 25 ºC) when compared to water (359 g/L at 25ºC) [78]. Furthermore, the pH value adjustment with HCl diluted solution requires extremely low volumes of acid, difficult to measure with accuracy. Thus, it was decided to study only the SPME parameters in the same conditions of the samples, i.e., in aqueous media using ultrapure water.

Other experimental observation to be notice is that the SPME extraction of a sample with 30% NaCl is not advised, since the high amount of salt that needs to be added to the sample will damage the PDMS-DVB fiber coating. So, it was decided to study the no addition of salt, 10 and 20% of salt addition.

3.6 Monitoring of pesticides in three different superficial water samples

The experimental implementation of the developed methodology was performed by collecting three samples from three different rivers located in Bragança. The samples were collected in sterile amber flasks and then stored in the freezer, at a temperature of -18°C until the moment of analysis. These three samples were named as “rio Onor” with GPS coordinates (41.803318, -6.697994), “rio Sabor” (41,803139, -6,693960) and “rio Fervença” (41.803705, -6.755356).

The extraction of the samples by SPME and their analysis by GC-MS was performed using the optimized parameters and operating conditions. The limits of detection and quantification were measured, and the experimental values of concentrations were calculated based in the individual calibration curves obtained for each pesticide.

Chapter 4: Results and discussion

4.1 Optimization of GC-MS operating conditions

4.1.1 Identification of the elution order for the selected pesticides

In order to identify all the elution order of the 5 pesticides, individual solutions were prepared by measuring 10 mg each pesticide in 10 mL volumetric flasks using pure methanol as solvent. Each standard solution with a concentration of 1000 mg/L was analyzed in GC-MS using the operating conditions described as "Method 1".

"Method 1" GC-MS operating conditions:

GC	**MS**
Oven initial temperature: 50 °C	Full scan mode; 35-450 (m/z)
Injector: 250 ºC, 1 μL, split 1:50	Ion Trap Temperature: 220 ºC
	Transfer Line Temperature:250 ºC

The obtained results are presented in Figure 4.1, where the five compounds are overlaid.

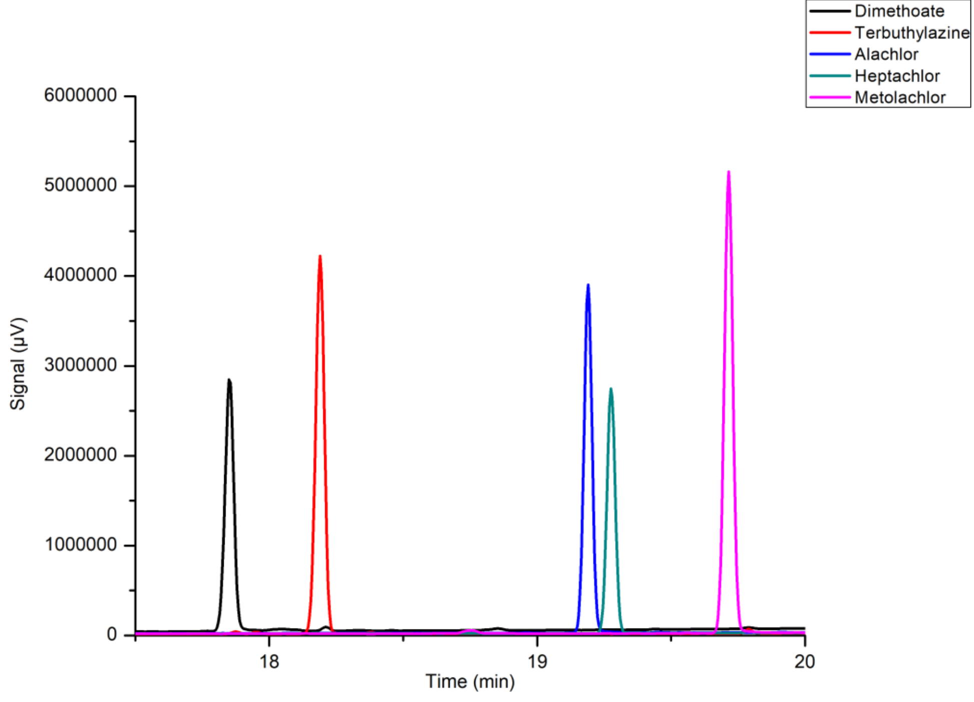


Figure 4.1. GC-MS analysis of the five selected pesticides using a concentration of 1000 mg/L. Overlaid chromatograms and operating conditions referred as "Method 1".

From the analysis of these results the intensity of detector signal (in area) and retention time (in minutes) were collected and are presented in Table 4.1.

Table 4.1. Identification of the elution order and signal intensity for each pesticide using Method 1.

Elution order	Pesticide	Retention time (min)	Area (Counts)
1	Dimethoate	17.850	6526989
2	Terbuthylazine	18.190	9537745
3	Alachlor	19.190	8307548
4	Heptachlor	19.275	5949658
5	Metolachlor	19.715	11692515

The analysis of these results showed that "Method 1" can be used to identify and quantify the 5 pesticides, although, it presents three drawbacks. The first is a gap time of more than 17.5 min to elute the first pesticide (dimethoate). The second is a partial overlay in the baseline between alachlor and heptachlor that will be a disadvantage for quantification of these two compounds. The third one is a final temperature of 300 ºC inside the GC oven that with the increasing number of analysis can damage the packing of the column.

4.1.2 Effect of the GC oven temperature program in the separation

Due to the strong detector signal obtained for an individual concentration of 1000 mg/L, five new individual solutions and a mixture solution were prepared, by dilution with methanol, with an individual concentration of 10 mg/L.

With the diluted mixture solution with a concentration of 10 mg/L, the separation was performed using all the methods collected from literature and presented in section 3.6. The obtained chromatograms are presented in Fig. 4.2.

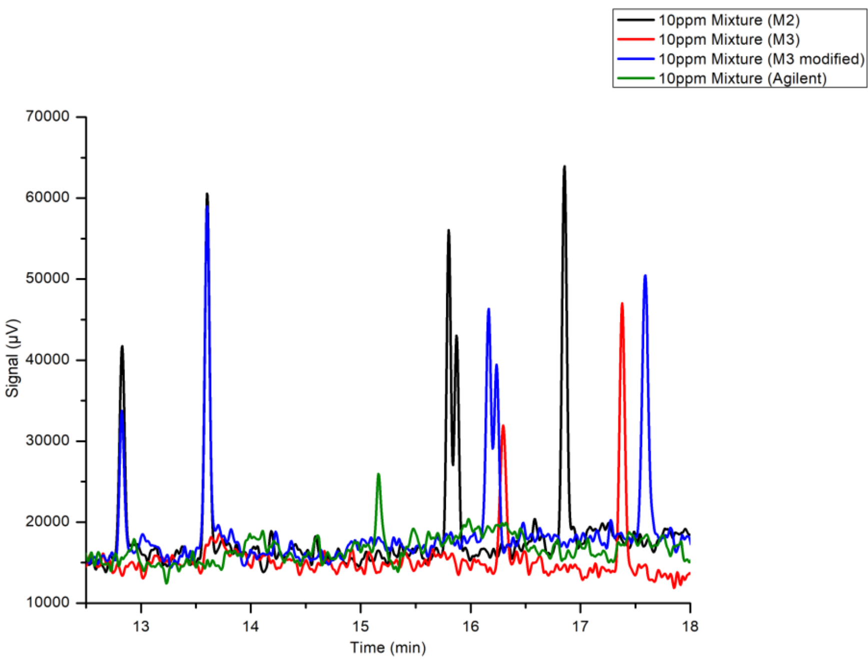


Figure 4.2. GC-MS chromatograms obtained with four different methods for a standard mixture concentration of 10ppm. Experimental conditions presented in Table 4.1.

The analysis of the results presented in Fig. 4.2, clearly shows that the method obtained from Agilent catalogue (2013), "Method 4", is beyond the small detector signal, this method is also time consuming since first compound elutes only after 15 min. "Method 3", obtained from Azevedo *et al.* (2000), presents a very good separation, yet first compound elutes the column only after 16 min. The chromatogram profile obtained with "Method 2", published by Gonçalves *et al.* (2004), represents the better conditions between the four methods despite a considerable overlap between alachlor and heptachlor it has the smaller retention for all the compounds. The lack of resolution between these two compounds could be solved with the SIM mode for clear identification of each compound but surely will result on integration problems. Since results obtained with "Method 3" showed very good resolution but also very high retention times, it was performed several modifications in the GC oven temperature program, in order to decrease the retention and study if the resolution between alachlor and heptachlor were enough for baseline separation. However, the best obtained result obtained with this "modified Method 3" is worse than the one obtained with "Method 2". Since the resolution was slightly better using "Method 2" we decided to select this method for the next tasks of the experimental work.

The retention times obtained with "Method 2" and detector signal for a concentration of 10 mg/L for the 5 pesticides were collected and are presented in Table 4.2.

Table 4.2. Identification of the elution order and signal intensity for each pesticide using Method 2.

Elution order	Pesticide	Retention time (min)	Area (Counts)
1	Dimethoate	12.813	66709
2	Terbuthylazine	13.587	131363
3	Alachlor	15.786	111042
4	Heptachlor	15.857	71759
5	Metolachlor	16.838	136575

As stated above, the selection of "Method 2" will result in integration values between alachlor and heptachlor, however, when leading with very low concentration it is always possible to use the SIM mode to clear identify each pesticide.

4.1.3 Study and selection of the injector operation in split or splitless mode

A careful reading of the GC-MS operating conditions between all studied methods shows that all parameters are very similar. The temperature in the injector is 250 ºC, the oven temperature program starts at 80 ºC, and all the methods use Helium as carrier gas. In the mass detection, they use 200 ºC in ion source temperature and 270 ºC for the transfer line.
The main different is the injector split ratio that can be used for direct injections. A variation is this parameter means a very different detector signal intensity and baseline noise. To study the split ratio parameter, and for a question of time economy, it was decided to work only with the least retained compound, dimethoate.

Several analyses were performed using the following split ratios, maintaining all the other GC-MS parameter constant. The studied split ratios were splitless, split 1:2, 1:10 and 1:50. The obtained chromatograms for dimethoate standard solution with a concentration of 10 mg/L are presented in Fig. 4.3.

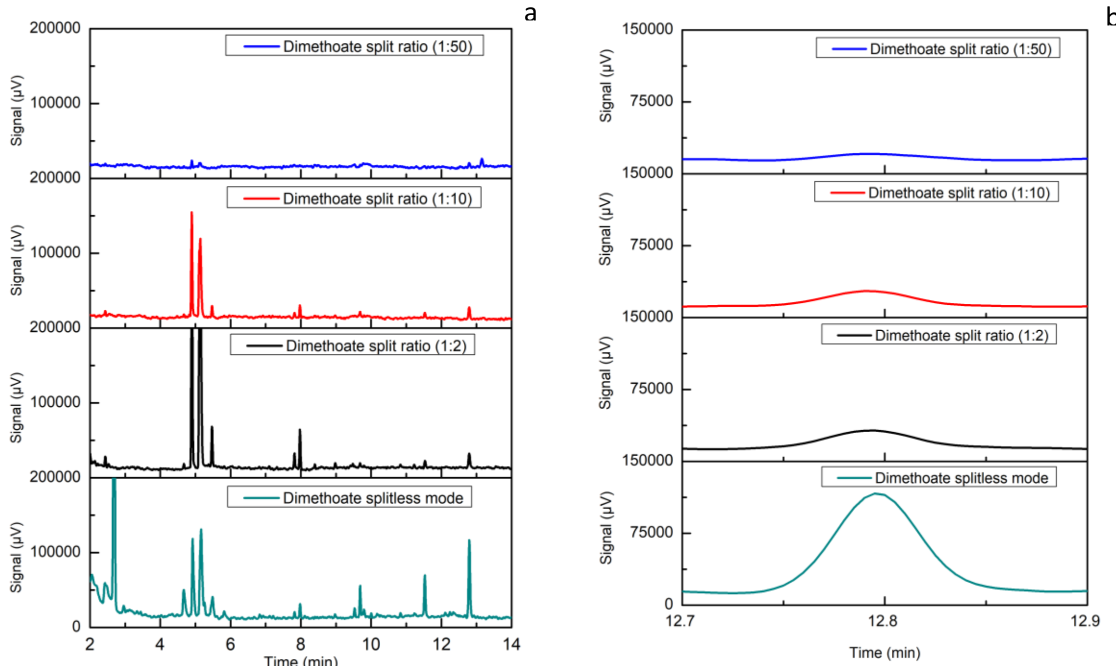


Figure 4.3. Effect of split ratio value in the GC-MS analysis of dimethoate solution 10 mg/L. (a): 10 to 14 min; (b) same results with zoom between 12.7 and 12.9 min.

The retention time and the area of dimethoate are presented in Table 4.3.

Table 4.3. Table of the area of Dimethoate using different Split ratio mode.

Mode	Ret.Time (min)	Area (Counts)
Split (1:50)	12.79	20812
Split (1:10)	12.79	27693
Split (1:2)	12.79	32203
Splitless	12.79	112013

As showed if these results, the splitless mode represents, as expected bigger detector signal, however it represents also a source of contamination, see contaminants between 2 and 4 min. that can affect the identification of compounds in trace concentrations. The split ratio (1:10) and (1:50) enables a strong signal and the contamination is almost absent.

A representation of the zoomed chromatogram obtained for the standard mixture with a concentration of 10 mg/L, for both split 1:10 and 1:50 is presented in Fig. 4.4, for better comparison.

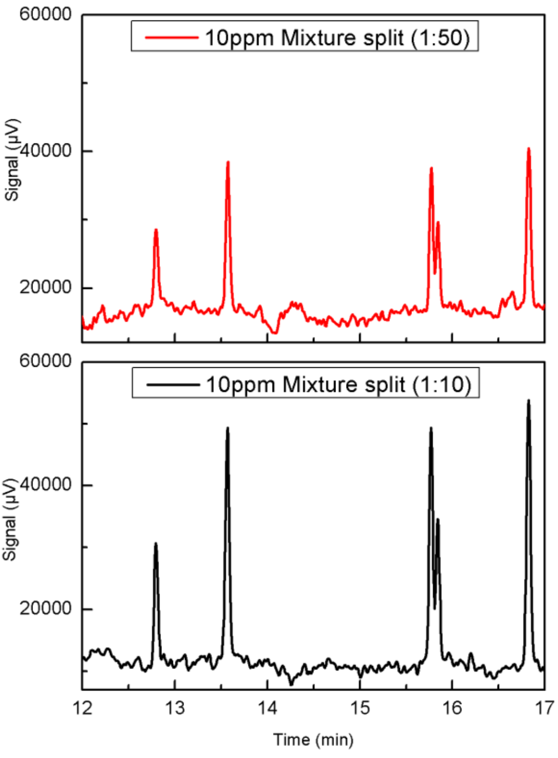


Figure 4.4. GC-MS chromatograms obtained for a standard mixture of pesticides with a concentration of 10 mg/L using 1:10 and 1:50 split ratio.

As it was already expected and can be confirmed for the detector signal in Table 4.4, there is no changes in the retention times of all the 5 pesticides, and the higher detector signal is obtained with the split 1:10.

Table 4.4. Comparison of detector signal for GC-MS analysis of a mixture of 5 pesticides with a concentration of 10 mg/L using split 1:10 and split 1:50.

Peak#	Name	Area_1:10 (Counts)	Area_1:50 (Counts)
1	Dimethoate	56954	48828
2	Terbuthylazine	113476	73030
3	Alachlor	113509	59784
4	Heptachlor	113509	38531
5	Metolachlor	137161	80521
Total Area		534609	300694

4.1.4 Study of MS analysis using SIM and FullScan modes

In order to improve the safety when performing the SPME experiments, in which the solvent is ultrapure water, it was decided to increase the initial oven temperature from 80 to 120 ºC to avoid possible solvent condensation inside column that will damage the stationary phase. This modification, decided only at this moment, implies changes in the retention times of all the compounds. This new modified method was called "Method 2 modified".

To start study the use of single ion monitoring (SIM) mode, we collected from the literature and from the NIST MS library present inside the Shimadzu software sold with the equipment (see appendix for MS data of each pesticide). The selected m/z values (target ions) were:

- Dimethoate: 87 and 125
- Alachlor: 45, 160 and 188
- Heptachlor: 100, 272 and 65
- Terbuthylazine: 214, 43 and 173
- Metolachlor: 162, and 238

"Method 2 modified" GC-MS operating conditions:

GC	**MS**
Oven initial temperature: 120 °C	Full scan mode; 35-450 (m/z)
Injector: 250 ºC, 1 □L, split 1:10	Ion Trap Temperature: 200 ºC
	Transfer Line Temperature:270 ºC

The chromatograms obtained for a standard mixture of the 5 compounds with a concentration of 50 mg/L and a split ratio of 1:10 are presented, overlapping in Fig. 4.5.

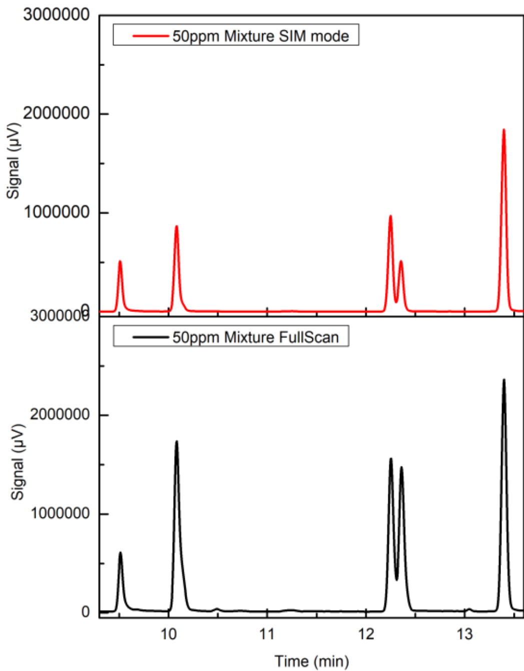


Figure 4.5. GC-MS chromatograms obtained for a 50 mg/L, a split ratio 1:10 for a standard mixture of 5 pesticides and using SIM and FullSan modes.

The obtained retention times and areas for SIM and FullScan mode are presented in Table 4.5.

Table 4.5. Retention times and obtained areas for all the 5 pesticides using the "Method 2 modified" and the SIM and FullScan mode.

Elution order	Pesticide	Retention time (min)	Area (Counts) SIM	Area (Counts) FullScan
1	Dimethoate	9.511	1565788	2019000
2	Terbuthylazine	10.083	2941875	7144227
3	Alachlor	12.249	3142328	5759764
4	Heptachlor	12.355	1683284	5385840
5	Metolachlor	13.396	6459946	8742523

As it can be observed in Figure 4.5, the SIM mode will produce a baseline without contaminants since the detector is only considering the target selected ions for each pesticide. This is a huge advantage if we consider only that the main goal is to clear identify each compound present in the obtained chromatogram.

However, and as it can be observed in Fig. 4.5 and in the area values of Table 4.5, the SIM mode represents a small detector signal since we are considering the mass to charge ration (m/z) of two or three fragments of the overall mass spectrum of each compound. So, it was decided to follow the experimental work using the FullScan mode for quantification and the SIM mode if we would live to confirm the detection of trace concentration, especially in the case of alachlor and heptachlor that are not baseline resolved.
Another observation is that the modification in the initial oven temperature helped, since increased, lightly, the resolution between alachlor and heptachlor.

4.1.5 Repeatability studies for GC-MS analysis with optimum conditions

To quantify the precision of developed GC-MS "Method 2 modified" it was analyzed the 5 individual standards and the mixture standard with a concentration of 50 mg/L. A batch GC-MS automatic file method was built to inject 6 times each solution, corresponding to 36 analyses. The obtained results are presented in Table 4.6.

Table 4.6. Precision studies for the GC-MS analysis of individual and mixture standards with a concentration of 50 mg/L using the developed GC-MS method.

Individual standards				
Pesticide	**Retention Time (min)**	**Average Area (Counts)**	**Standard Deviation**	**Coefficient of Variation (%)**
Dimethoate	12.780	375046	34809	9.33
Terbuthylazine	13.560	379871	33111	8.24
Alachlor	15.765	506773	43559	8.25
Heptachlor	15.831	54126	42497	8.71
Metolachlor	16.806	838595	78269	9.63
Standard mixture				
Pesticide	**Retention Time (min)**	**Average Area (Counts)**	**Standard Deviation**	**Coefficient of Variation (%)**
Dimethoate	12.780	372775	28021	7.47
Terbuthylazine	13.546	401503	26832	7.06
Alachlor	15.749	527687	25953	5.12
Heptachlor	15.821	487499	18536	3.42
Metolachlor	16.806	812630	20671	2.46

The individual average chromatograms obtained for the analysis of each pesticide are presented in Fig. 4.6a, as overlay, and the average chromatogram obtained from the 6 analyses of the standard mixture is presented in Fig. 4.6b.

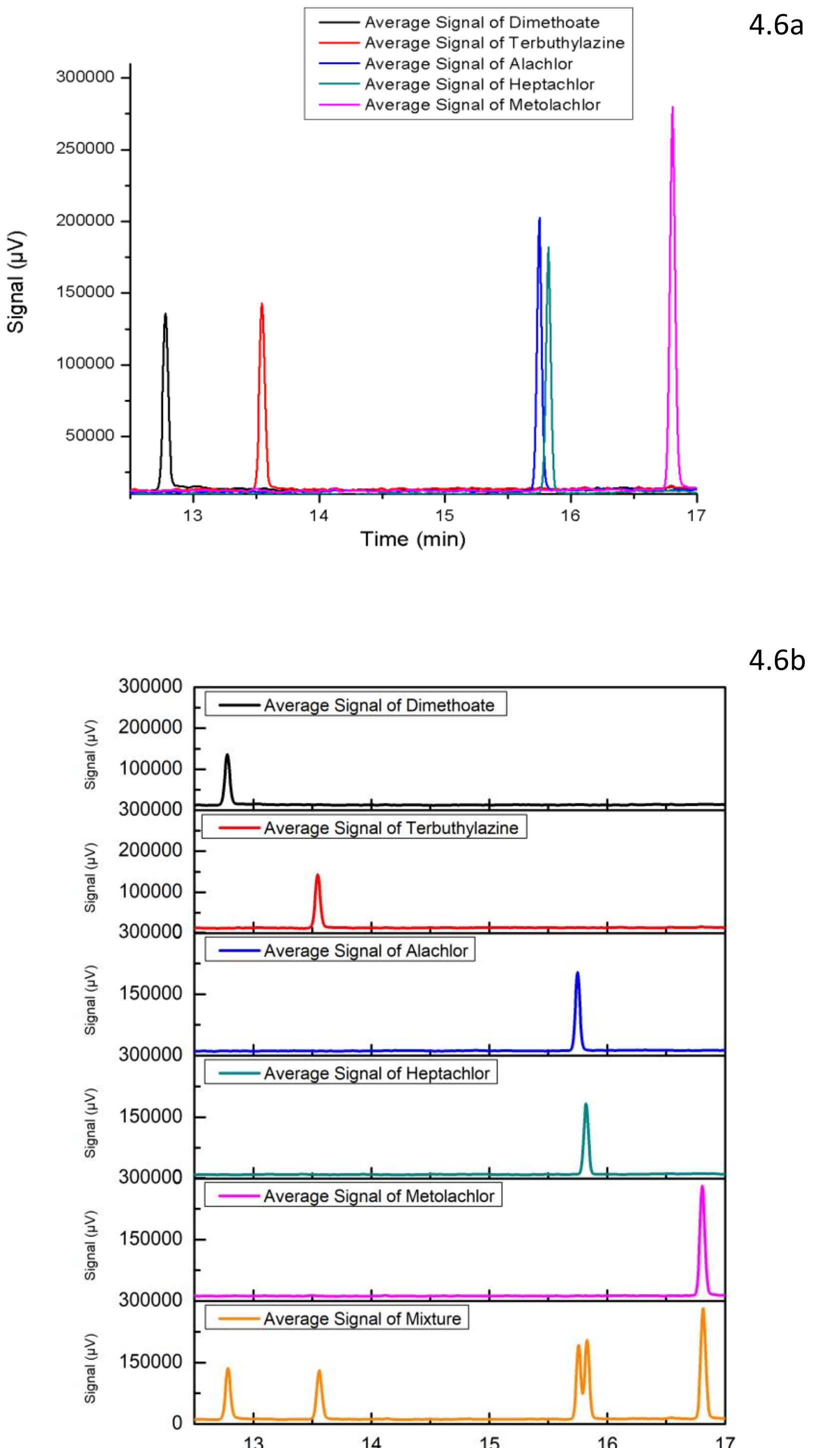


Figure 4.6. Average GC-MS chromatograms obtained for each of the 5 pesticides, overlaid (Fig.4.6a) and the average GC-MS chromatogram obtained for the standard mixture of the 5 pesticides (Fig.4.6b), all solutions with a concentration of 50 mg/L.

As it can be observed in Table 4.6, using a standard mixture would be more accurate than extracting the samples individually. The coefficient of variation (CV%) is between 2.5% and 7.6% as the highest value related to Dimethoate. But when extracting the standards individually, the coefficient of variation (CV%) increase to 8.2 – 9.6%
Therefore, extracting our standards as a mixture is the suitable method.

4.2 SPME preliminary studies

4.2.1 Study of ionic force and acidity effect in the extraction efficiency

The ionic force increases and decrease of the pH value of samples is referred in some published references that can have a strong effect in the extraction efficiency of some compounds. In principle, non-volatiles compounds or semi-volatiles compounds will be favorable extracted if the ionic strength of sample is increase. The effect of pH value it will affect by the polarity of sample. For clear conclusions, experimental measurements are always advised for each individual compound.
To study these two parameters, standard conditions SPME conditions were collected from references. It was decided to perform 4 different extractions with or without adjustment of salt, 10% NaCl, i.e., 10 mg/mL and a pH value of 2. For the other parameters, the standard mixture of the 5 pesticides was prepared in a concentration of 1 mg/L with ultrapure water, the extraction was carried under 1000 rpm agitation, using a temperature of 60 ºC and during 40 min. The obtained results are presented in Fig. 4.7.

"Method 2" GC-MS operating conditions:

GC
Oven initial temperature: 80 °C
Injector: 250 ºC, 1 μL, split 1:10

MS
Full scan mode; 35-450 (m/z)
Ion Trap Temperature: 200 ºC
Transfer Line Temperature:270 ºC

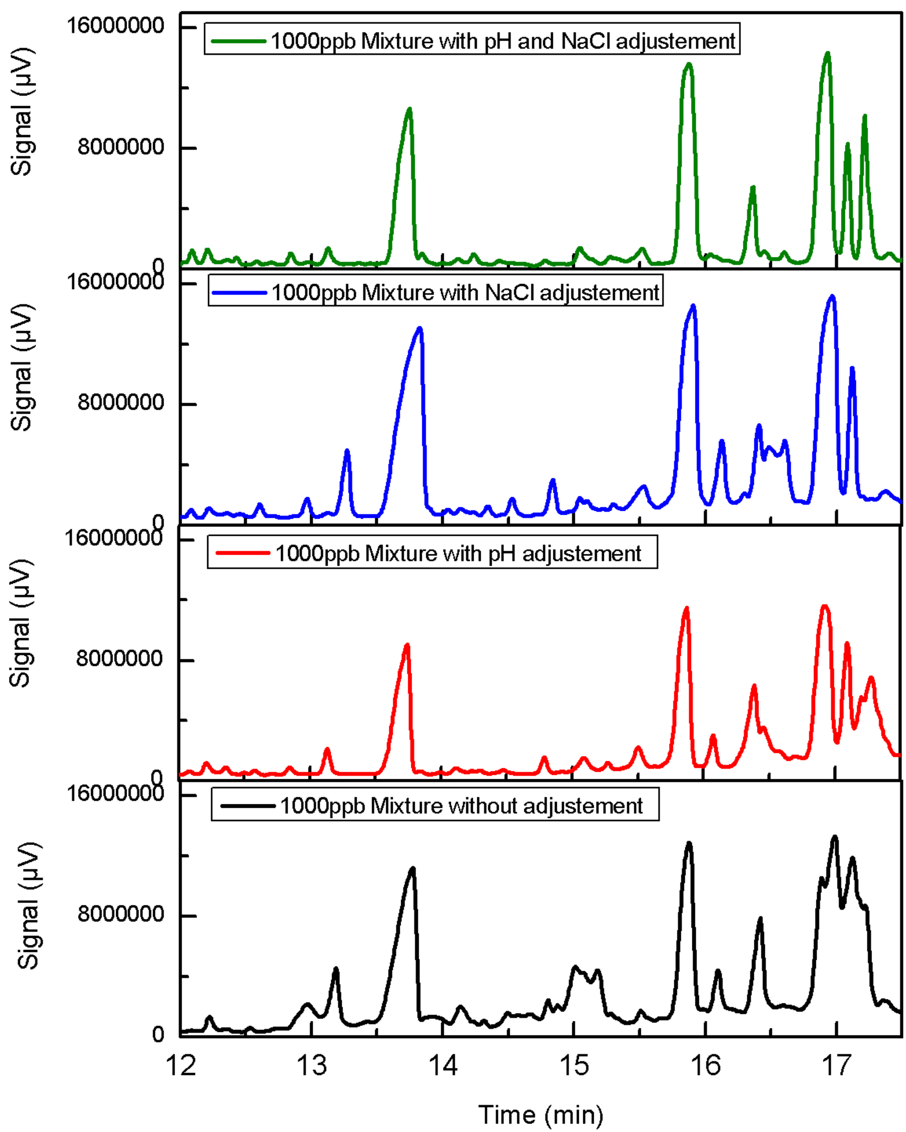


Figure 4.7. 4 SPME/GC-MS analysis obtained for the studies of salt addition and pH value of sample for the extraction of a 1 mg/L mixture of pesticides.

Using ''Method 2'' to study the effect of ionic force and pH on our mixture, we obtained these chromatograms of 4 different parameters with a concentration of 1000 µg/L and a split ratio of 1:10 are presented, stacked, in Fig. 4.7.

It was clear that when applying both NaCl and pH adjustment we received the best resolution for almost all compounds. Alachlor and Heptachlor they were still tangled together at a retention time of 15.97 min.

As for Metolachlor without adjustment it was impossible to identify or even integrate its peak, but we observed a gaussian peak for Metolachlor alone when using an adjusted mixture at a retention time of 17.01 min.
We also observed the same behavior for Dimethoate at 12.79 min. Finally for Terbuthylazine, even without adjustment we detected a high intensity signal and a good resolution.

Although the use of so high value of sample concentration, it can be observed that the addition of salt to the sample is favorable, but the identification of all compounds is possible only when both addition of 10% of salt and decreasing the pH value to 2 is done.

These conclusions may be easy to confirm if the same study is done using samples with a small value of concentration. The contamination present in these samples could be justified with a lower purity of the ultrapure water available from our laboratory. To test this hypothesis, different ultrapure water was taken from another laboratory (CIMO). These experiments are presented in section 4.2.2.

4.2.2 SPME/GC-MS experiments using different ultrapure water and different standard solutions concentrations

Four different SPME/GC-MS experiments were conducted in order to study if the ultrapure water used in our laboratory has poor quality and if the concentration of sample can affect the separation/identification of each compound. Additionally, in these experiments it was used the "Method 2 modified", that considers and initial oven temperature of 120 ºC using splitless for the 5 first minutes and the split ratio of 1:50 until the end of the GC run. For SPME extraction the conditions were, samples with 10% salt and pH of 2, extraction during 60 min and during 60 min, under 1000 rpm agitation.
First analysis was done using a concentration of 500 µg/L of each pesticide, second analysis was done with a 250 µg/L concentration, third analysis was done with a 100 µg/L concentration and finally the fourth analysis was done for a 100 µg/L concentration and different ultrapure water (CIMO).
The results obtained for these experiments are presented in Fig. 4.8.

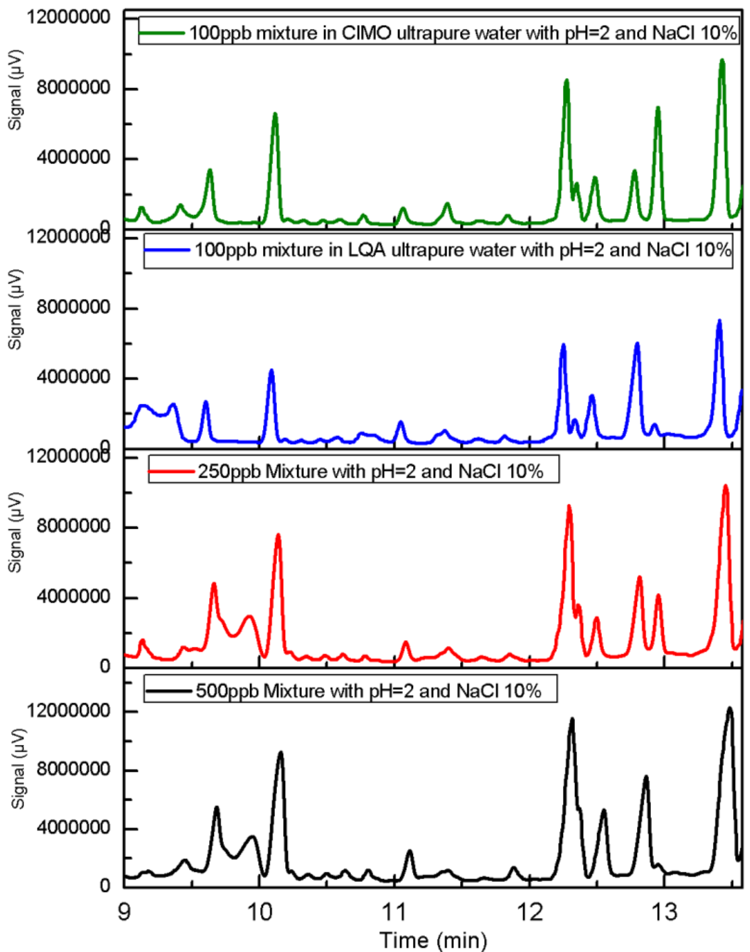


Figure 4.8. 4 SPME/GC-MS analysis obtained with same adjustment but different concentrations.

It seems that the decrease in concentration in this range improves the resolution of compounds.

To discuss furthermore, using a 500 µg/L adjusted mixture it was impossible to integrate the peak of Heptachlor nut decreasing the concentration to 100 µg/L we observed a better separation between Alachlor and Heptachlor with a retention time of 12.25 min and 12.35 min respectively.

As, for Terbuthylazine in both 500 µg/L and 250 µg/L detected at 10.08 min we noticed a contaminant peak next to it but when decreasing the concentration to 100 µg/L the contaminant peak did vanishes.

Comparing the results between two different ultrapure water, we can say that the two chromatograms were very similar. In CIMO we don't have contamination from 9 to 9.5 min, but we have more contamination near 13 min. However, both contaminations do not affect the identification/quantification of the 5 selected pesticides.

4.2.3 Study the effect of FullScan and SIM modes in the detector signal

After, samples with 50 ppb, 10 ppb and 1 ppb were prepared and adjusted to pH=2 and NaCl 10%. Analyzing these samples using (M2) optimized (initial temperature 120 °C, 5min splitless then split ratio mode (1:50) with two scan modes (FullScan / SIM mode).

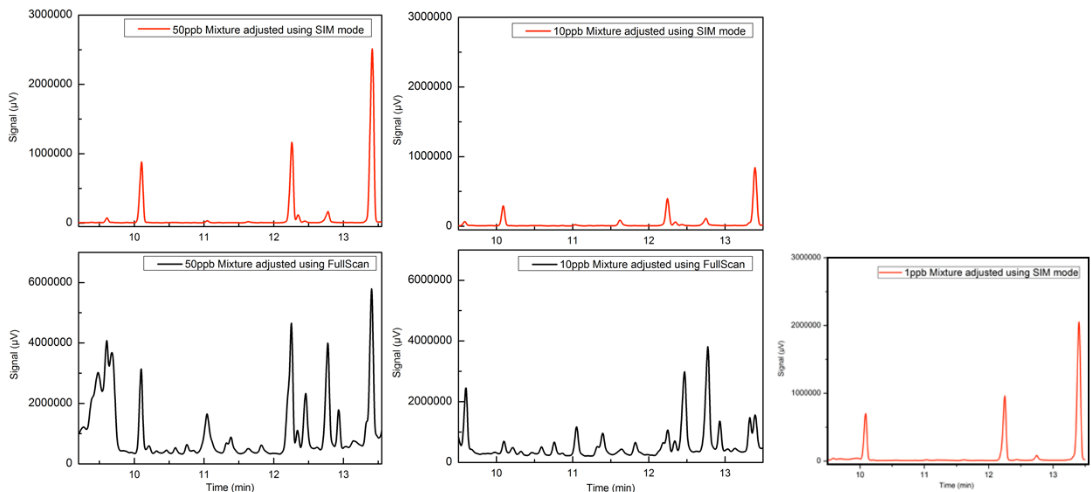


Figure 4.9. Comparison between the obtained chromatogram obtained using the SIM and FullScan MS modes. SPME/GC-MS analysis of a standard mixture solution with 50, 10 and 1 µg/L concentration, from left to right.

The analysis of these results shows that, as expected, the SIM mode will be considerable better that FullScan mode to eliminate the noise of the baseline and thus improve resolution. However, the obtained detector signal is considerable smaller that the obtained using FullScan. If the target ions, previously referred, for each pesticide, the detector will only seek for these fragments and, so, everything else will be discarded. This is an advantage for the identification and, we agree that it could also be an advantage for integration.

The typical results of SIM mode are presented in Fig. 4.9, were the screenshots for the three concentrations analysis using the SIM mode are presented.

Figure 4.10. Comparison between the obtained chromatograms using SIM MS mode for SPME/GC-MS analysis of a standard mixture solution with 50, 10 and 1 μg/L concentration, from top to bottom.

It can be observed that at least a 1 μg/L concentration is possible to integrate the 3 compounds and for at least 10 μg/L, using SIM mode it is possible to integrate all the five compounds.

Despite of these observations it was decided to work with some eventual problems for the integration but also with the strong advantage to work with higher signals (areas), since the idea is to achieve lower values for limit of detection and quantification.

4.2.4 Study of GC Split ratio value for SPME/GC-MS analysis

Before starting the optimization of SPME parameters, one mode tuning was performed for the GC injector split ratio. It was studied the difference between split 1:10 and 1:10 and the MS was used in SIM mode.

The previously detailed parameters and operation conditions were settled as, extraction from a standard solution with 100 g/L concentration, pH=2, 10% NaCl, 60 ºC and 60 min, and for GC-MS the "M2 method modified" was used. The obtained results are presented in Fig. 4.11.

Between split 1:10 and 1:50 there is a slight change in the area and carefully analysis it is possible to notice that the separation using 1:10 is slightly better. So, it was concluded that the use of the split 1:10 will be an advantage to use for SPME optimization.

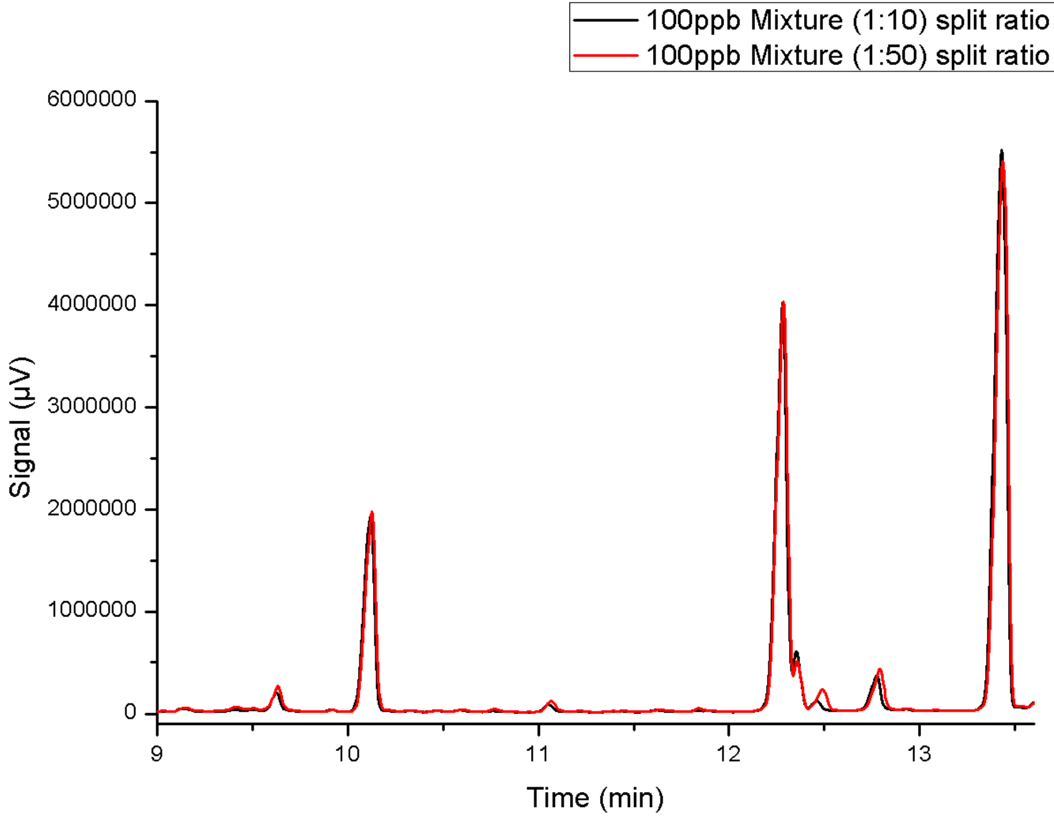


Figure 4.11. Comparison between the obtained chromatograms using SIM mode for SPME/GC-MS analysis of standard mixture solution with 100 µg/L concentration, using split ratio (1:10) and (1:50).

4.2.5 Study of MS mode for SPME/GC-MS analysis for a 100 g/L concentration

To confirm if the same results are confirmed for the main differences between the SIM and FullScan modes after SPME extractions, an additional experiment was done using a 100 g/L concentration and a split of 1:50 for better comparison results already presented in Fig. 4.11. This new comparison, now for a concentration of 100 µg/L is presented in Fig. 4.12.

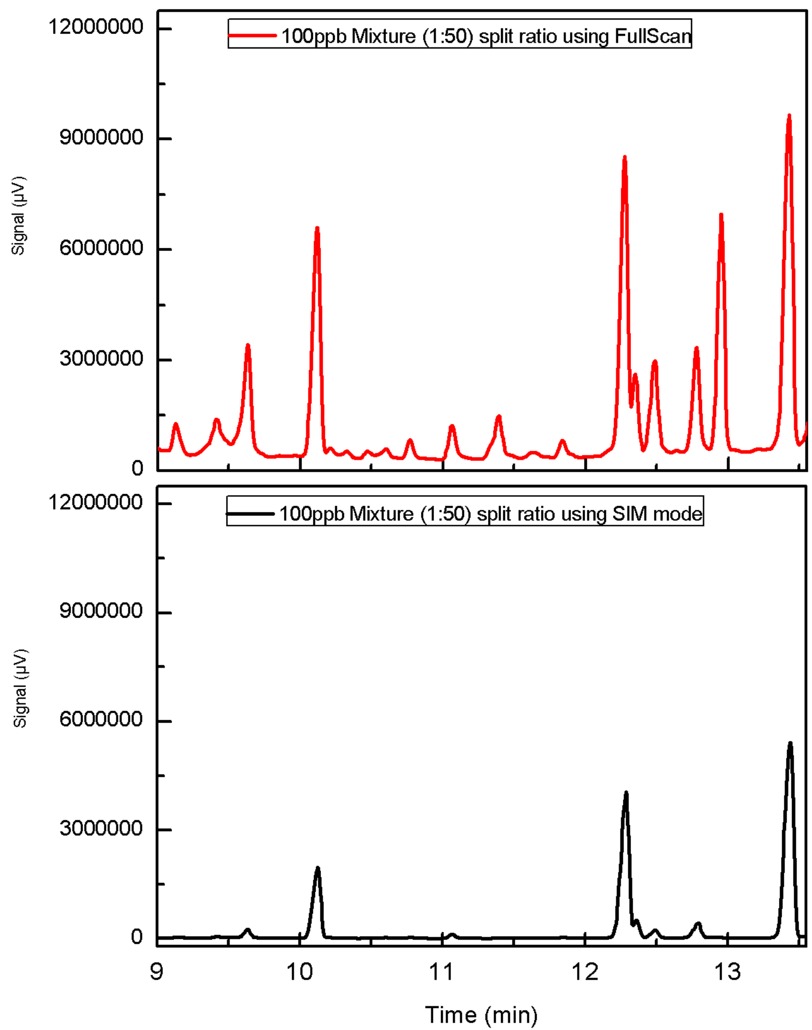


Figure 4.12. Comparison between the obtained chromatogram obtained using the SIM and FullScan MS modes. SPME/GC-MS analysis of a standard mixture solution with 50 µg/L concentration.

The comparison can also be done in terms of area, as presented in Table 4.12.

Table 4.7. Retention times and obtained areas for all the 5 pesticides in 100 µg/L mixture using the "Method 2 modified" and the SIM and FullScan mode.

Peak#	Name	Ret.Time (min)	Area (SIM)	Area (FullScan)
1	Dimethoate	9.633	567557	4298467
2	Terbuthylazine	10.125	7295267	23667814
3	Alachlor	12.286	15669004	24487817
4	Heptachlor	12.358	1171149	2668459
5	Metolachlor	13.437	23264750	37004321

These results presented in Fig. 4.12 and Table 4.12, confirm, once more, the previously presented results in section 4.2.3, also for a 100 g/L concentration. The baseline it is almost clear using the SIM mode, but the SIM results are poor in terms of detector signal as the area values for FullScan mode in Table 4.12 shows. Since the main goal is to optimize the limits of detection and quantification, we decided to use the FullScan mode.

4.3 Optimization of the SPME main parameters

4.3.1 Studying of pH, NaCl % and extraction time effect

After preparing 4 samples in 100 ppb using ultrapure water as solvent with different adjustments, we got these results;

- S1: pH = 2 and NaCl 10%
- S2: pH = 2 and NaCl 20%
- S3: pH = 4 and NaCl 10%
- S4: pH = 4 and NaCl 20%

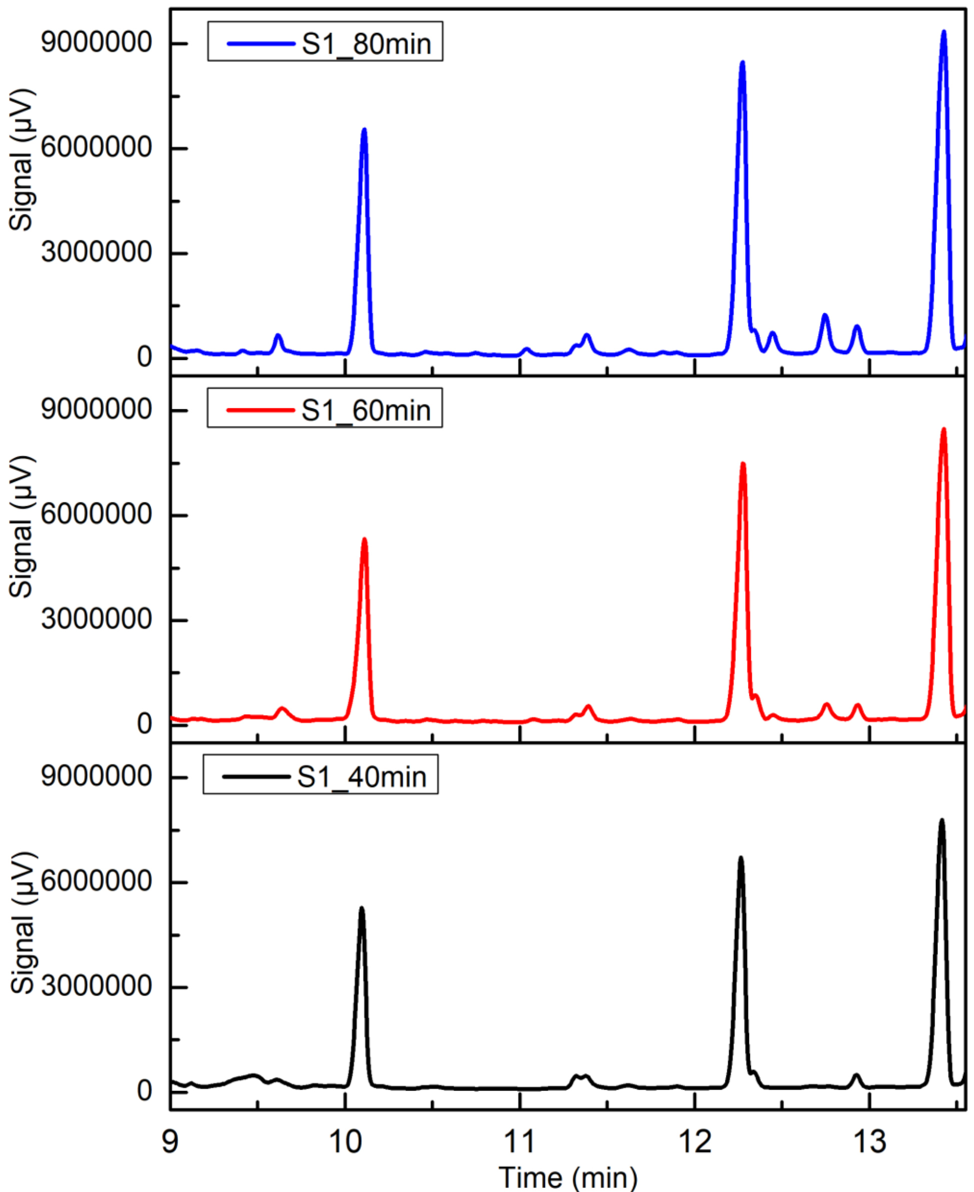


Figure 4.13. Comparison of 3 SPME/GC-MS analysis of 100 µg/L mixture S1 using different extraction time.

For the first sample S1 we detected all the 5 compounds when we used 80 min as an extraction time, also using 60 min we observed the 5 compounds but the peak of Dimethoate wasn't gaussian but when we applied 40 min extraction time, we couldn't identify Dimethoate.

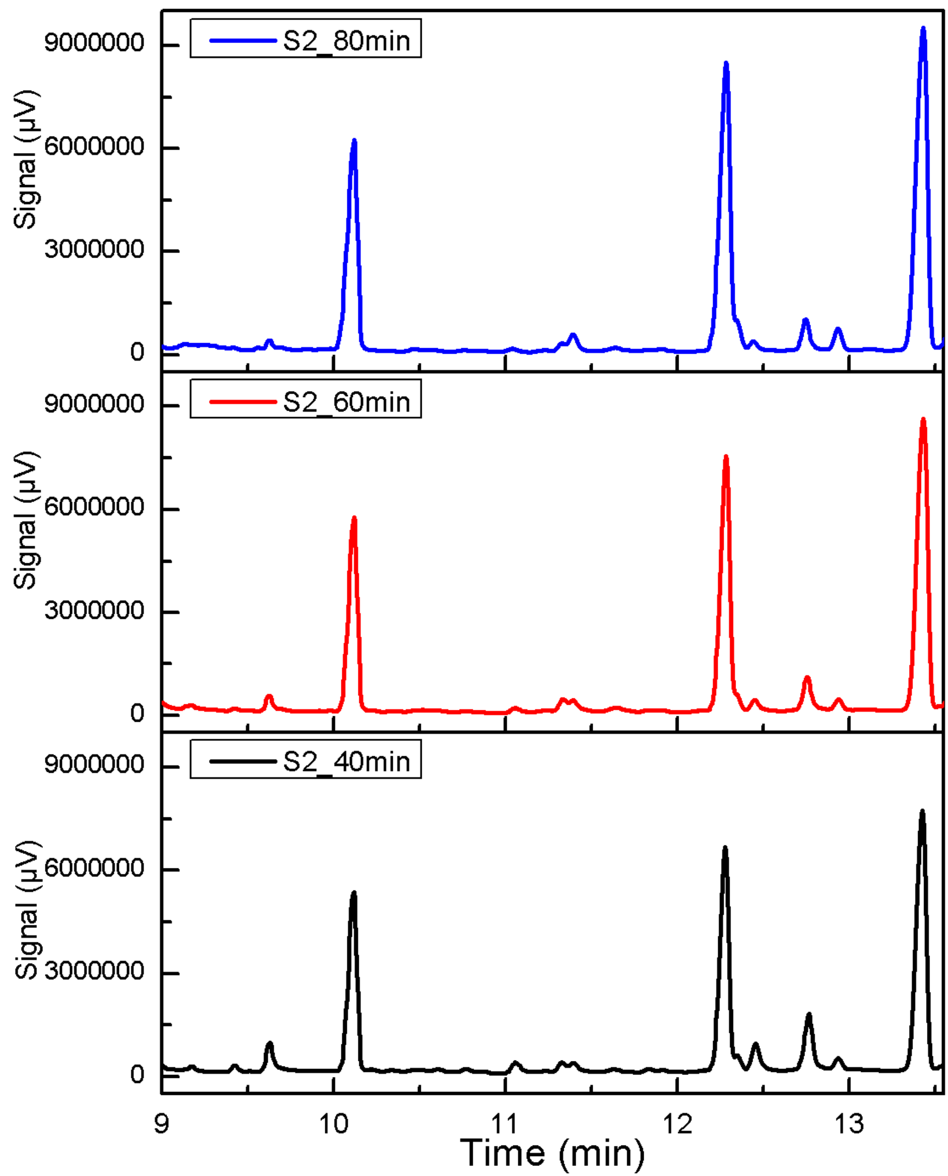


Figure 4.14. Comparison of 3 SPME/GC-MS analysis of 100 µg/L mixture S2 using different extraction time.

For the second sample S2 we detected all the 5 compounds when we used 40 min as an extraction time, but using 60 min or 80 min Alachlor and Heptachlor peaks were tangled. Also, for the use of 80 min extraction time we observed a very small peak of Dimethoate.

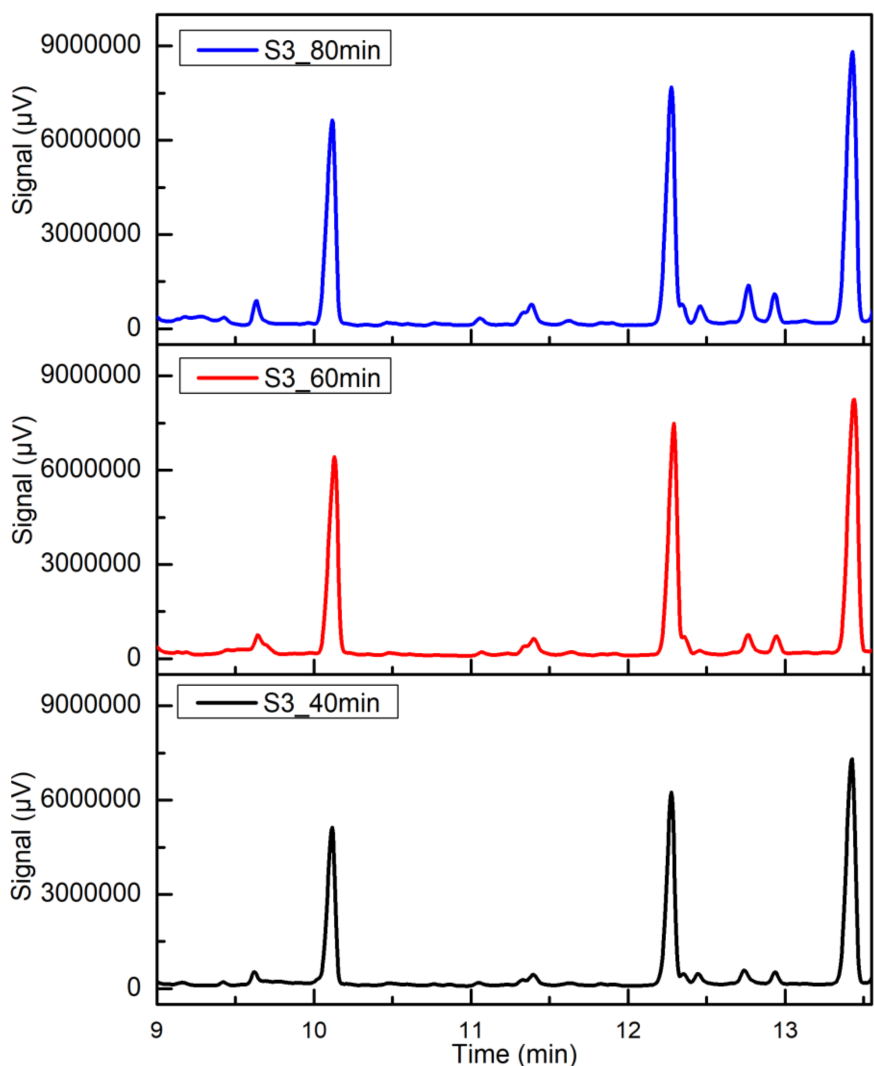


Figure 4.15. Comparison of 3 SPME/GC-MS analysis of 100 µg/L mixture S3 using different extraction time.

For the third sample S3 we detected all the 5 compounds when we used 80min as an extraction time, also using 60 min we observed the 5 compounds but the peak of Dimethoate wasn't gaussian but when we applied 40 min extraction time, we couldn't identify Heptachlor.

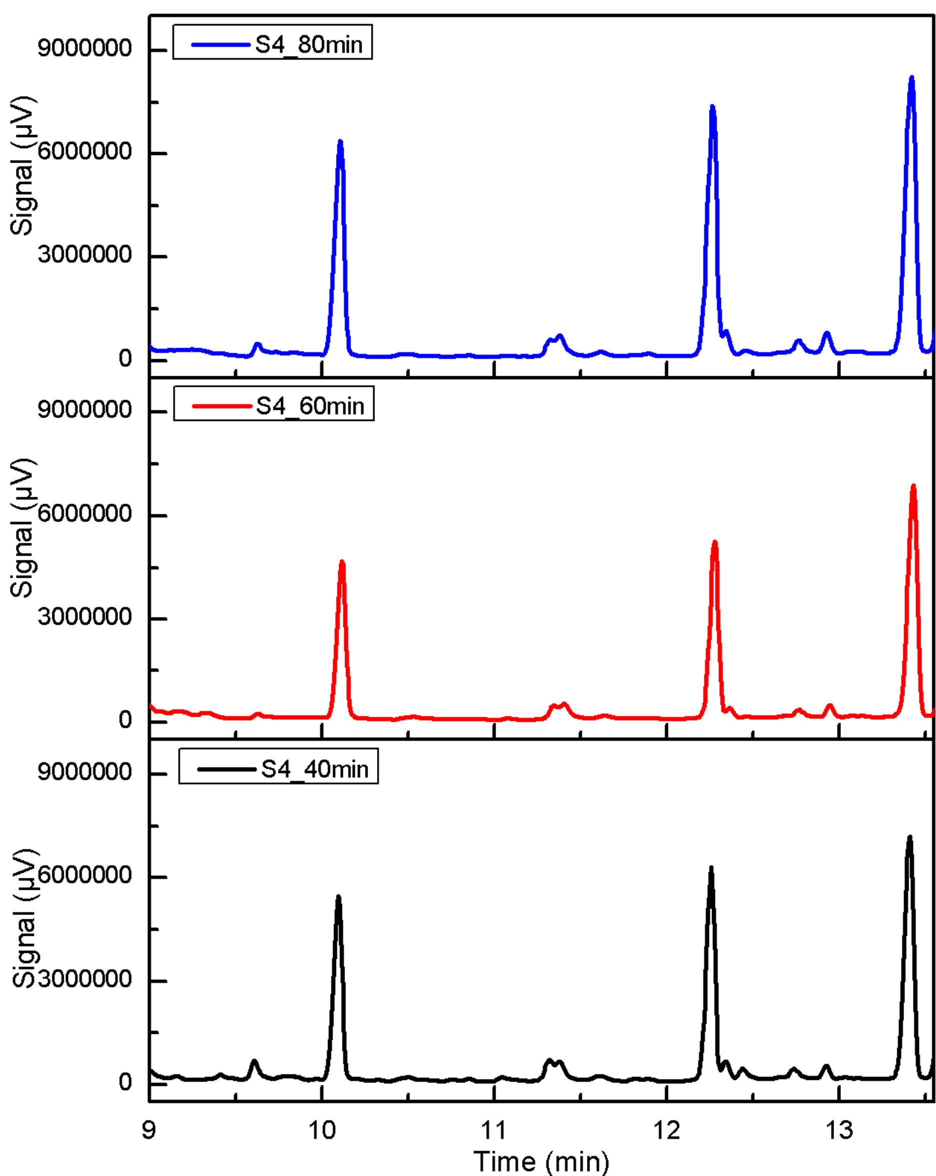


Figure 4.16. Comparison of 3 SPME/GC-MS analysis of 100 µg/L mixture S4 using different extraction time.

For the fourth sample S4 we detected all the 5 compounds when we used 80min as an extraction time, also using 40 min we observed the 5 compounds and we received a great separation of Alachlor and Heptachlor but when we applied 60 min extraction time we couldn't identify Dimethoate.

The best results in each sample from the same adjustment were selected and they were compared in the Fig 4.17.

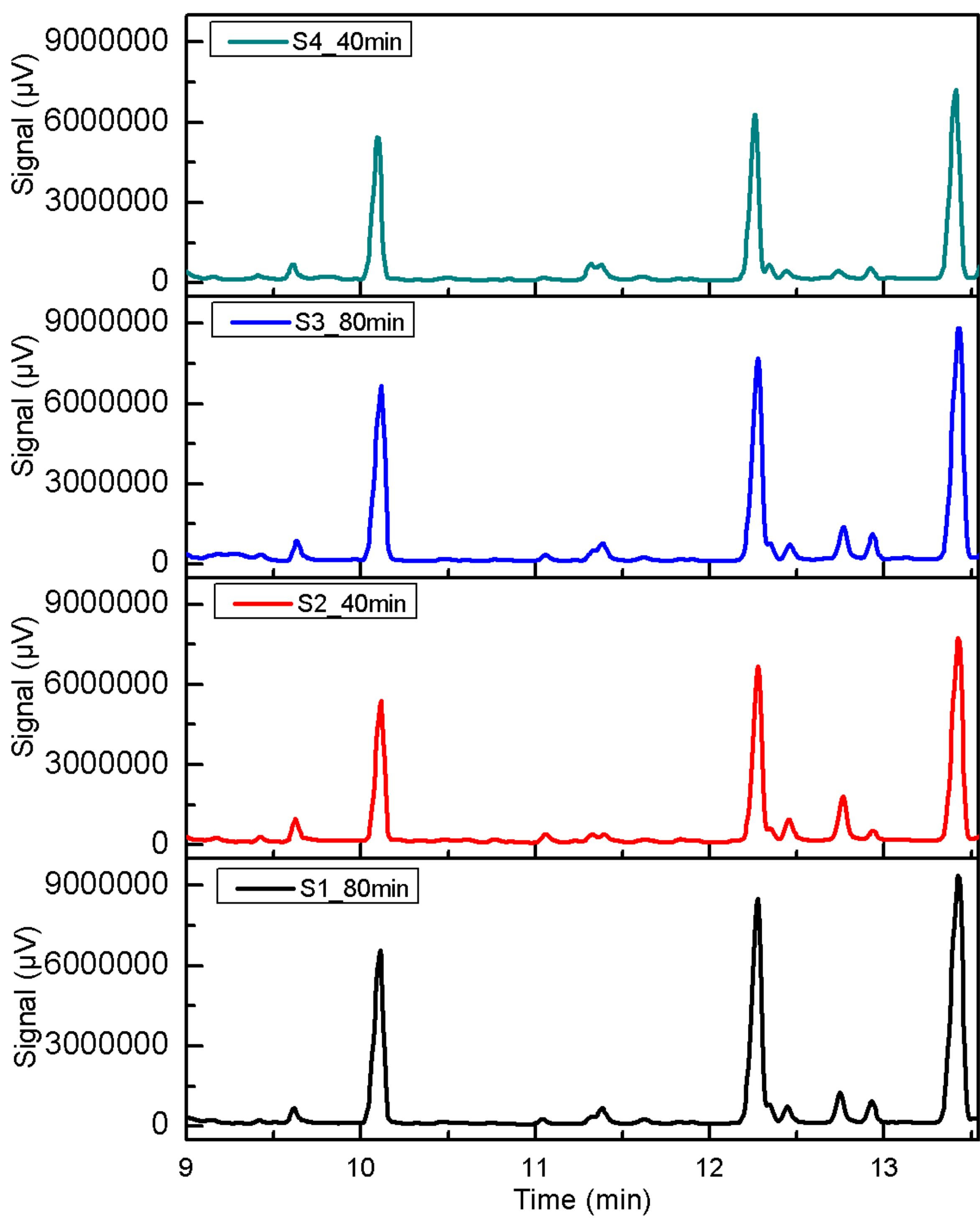


Figure 4.17. Comparison of 4 SPME/GC-MS analysis of 100 µg/L mixture with different adjustment and extraction time.

Table 4.8. Retention times and obtained areas for all the 5 pesticides in 100 µg/L mixture using the "Method 2 modified" with different adjustment and extraction time.

Peak#	Name	Area_S1_80min (Counts)	Area_S2_40min (Counts)	Area_S3_80min (Counts)	Area_S4_40min (Counts)
1	Dimethoate	1552305	2753980	*2470525*	1759052
2	Terbuthylazine	24471915	18661639	26153127	18611907
3	Alachlor	32742023	23703841	29674002	21541039
4	Heptachlor	1370832	960005	*1453363*	1275514
5	Metolachlor	41156128	30636802	37739462	27010484
Total Area		**101293203**	76716267	97490479	70197996

We got a different results while searching for the best extraction parameters, if we want to extract the maximum amount of Dimethoate and Heptachlor combined we should choose S3_80min but if we want the maximum amount of all the compounds we clearly should use S1_80min.

Since our main criteria in this work is to obtain the best extraction for all the 5 pesticides we continued our study using S1_80min because it's related to the highest value of Total Area.

4.3.2 Studying of Extraction temperature effect

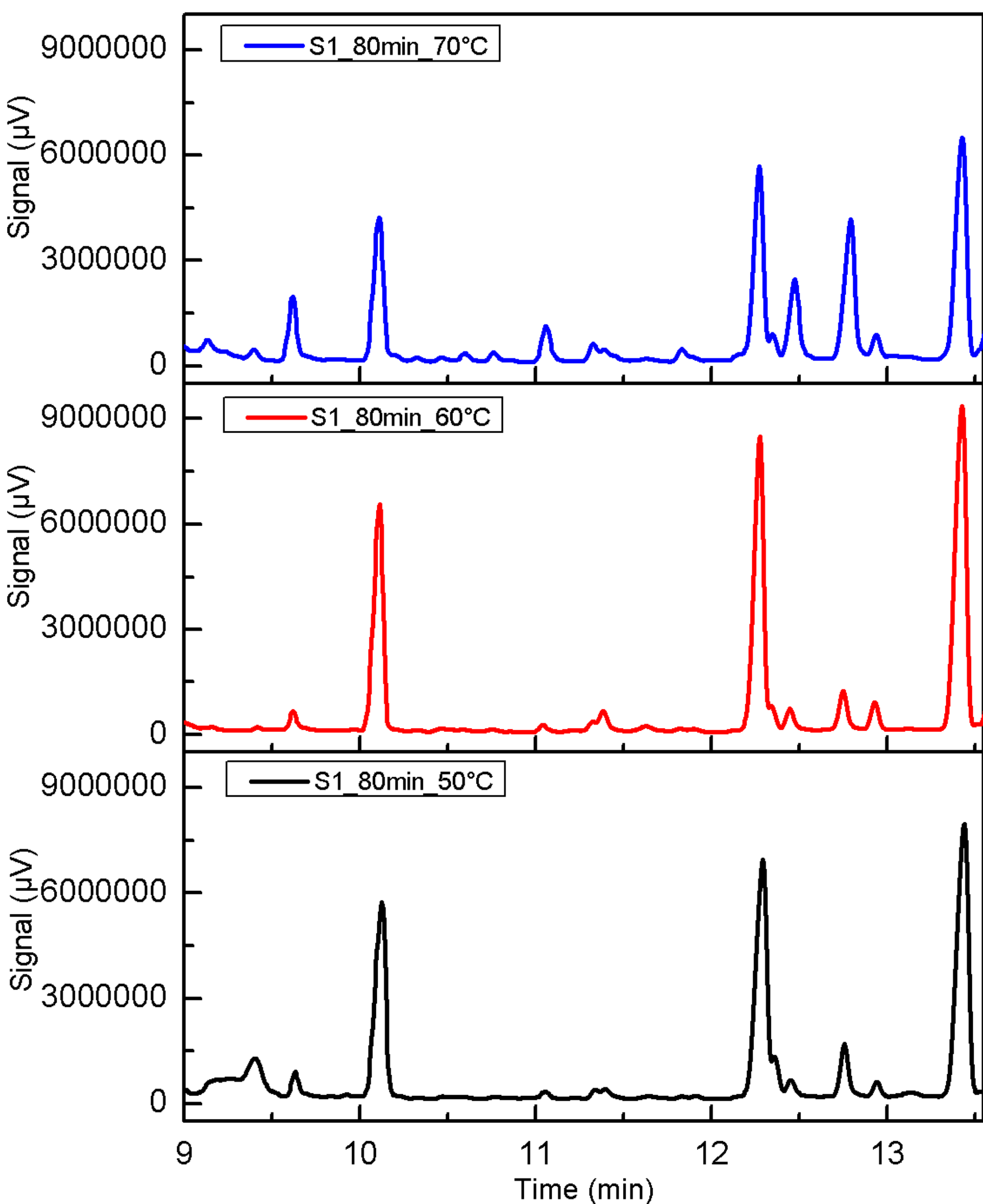


Figure 4.18. Comparison of 3 SPME/GC-MS analysis of 100 µg/L mixture with same adjustment but different extraction temperature.

Table 4.9. Retention times and obtained areas for all the 5 pesticides in 100 µg/L mixture using the "Method 2 modified" with different extraction temperature.

Peak#	Name	Area_70°C (Counts)	Area_60°C (Counts)	Area_50°C (Counts)
1	Dimethoate	6409654	1552305	1991795
2	Terbuthylazine	16027011	24471915	23432833
3	Alachlor	19801346	32742023	29876866
4	Heptachlor	1555868	1370832	2201447
5	Metolachlor	27860449	41156128	36095453
Total Area		71654328	101293203	93598394

The last parameter was the extraction temperature and the highest total area registered was related to the 60 °C parameters.

To conclude, the extraction procedure adopted for this study consisted of the following: 3ml-aliquots of the samples were extracted by direct immersion of a PDMS-DVB (65 µm of Thickness) fiber into the sample containing 10% of NaCl to adjust the ionic force and 120 µL of HCl to maintain a pH=2. The mixture was stirred for 80 min at 60 °C and desorption of the pesticides was carried out at 250 °C in the GC-MS injector for 4 min.

4.4 Statistical validation of the experimental SPME/GC-MS methodology

The validation of the developed experimental methodology is carried out by the determination of the statistic parameters of precision, accuracy, calibration curves and limits of detection and quantification.

The statistic equations used in this work were obtained from Miller and Miller (2010) [48], and the statistic parameters of calibration curves were obtained for a 95% level of confidence and using a t Student distribution. The calibration curves of all the 5 pesticides were determined by dilution with ultrapure water a mixture standard solution for at least 6 different levels of concentration between 10 and 100 g/L.

The optimized parameters for SPME extraction and the optimize operation conditions for GC-MS were used for the analysis of each sample. All experimental data and data treatment, with the equations used for the determination of calibration curves is presented in the appendix.

The determined parameters, for the 5 calibration curves, are presented in Table 4.15, and the it´s graphical representation is presented in Fig. 4.19.

Table 4.10. Calibration curve [y=a+bx] for the selected 5 pesticides obtained using the SPME/GC-MS experimental methodology.

Pesticides	**Linear Range (µg/L)**	$a \pm t.S_a$	$b \pm t.S_b$	R^2	**LOD (µg/L)**	**LOQ (µg/L)**
Dimethoate	25 - 75	1458830 ± 139412	15620 ± 2639	0.9916	6.1	20.2
Terbuthylazine	10 - 60	130279 ± 1580494	342397 ± 38503	0.9963	4.2	14.1
Alachlor	10 - 60	1090704 ± 2362839	408635 ± 57562	0.9942	5.3	17.6
Heptachlor	25 - 75	-543942 ± 297762	30679 ± 5637	0.9901	6.6	22.0
Metolachlor	10 - 60	2659877 ± 3521017	533439 ± 85776	0.9924	6.0	20.1

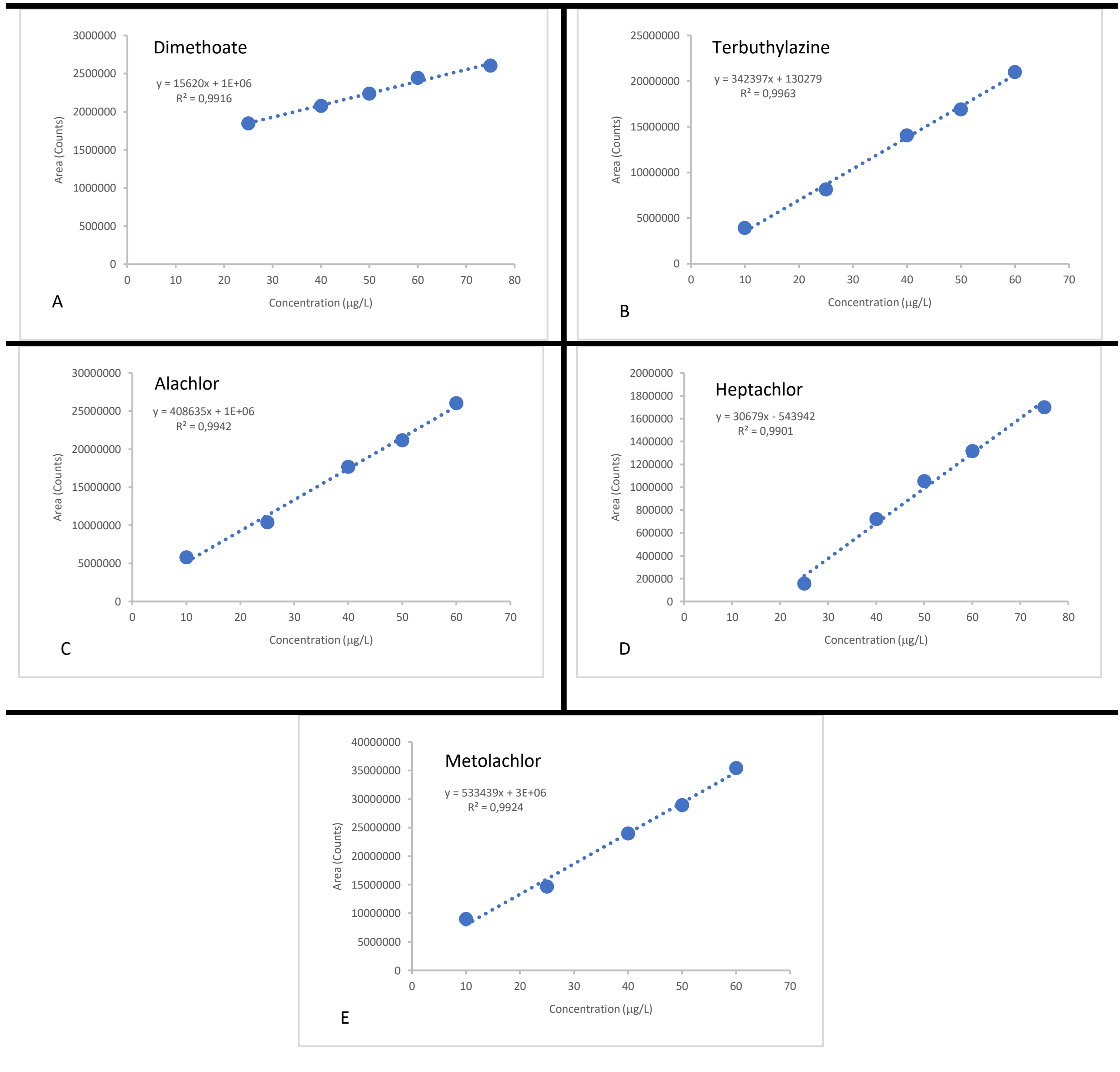


Figure 4.19. Calibration curves obtained using the developed SPME/GC-MS methodology for the selected 5 pesticides. (A : Dimethoate, B : Terbuthylazine, C : Alachlor, D : Heptachlor, E : Metolachlor).

- **Equations**

Where x = concentration / y = area / a = intercept / b = slope.

$$y = a + b.x \quad (1)$$

$$a = \bar{y} - b.\bar{x} \quad (2)$$

$$b = \frac{\sum_i\{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2} \quad (3)$$

$$r = \frac{\sum_i\{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2} \quad (4)$$

$$S_{y/x} = \left\{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}\right\}^{1/2} \quad (5)$$

$$S_b = \frac{S_{y/x}}{\{\sum_i (x_i - x)^2\}^{1/2}} \quad (6)$$

$$S_a = S_{y/x} \left\{\frac{\sum_i x_i^2}{n \sum_i (x_i - \bar{x})^2}\right\}^{1/2} \quad (7)$$

4.5 Validation of the developed methodology using real samples

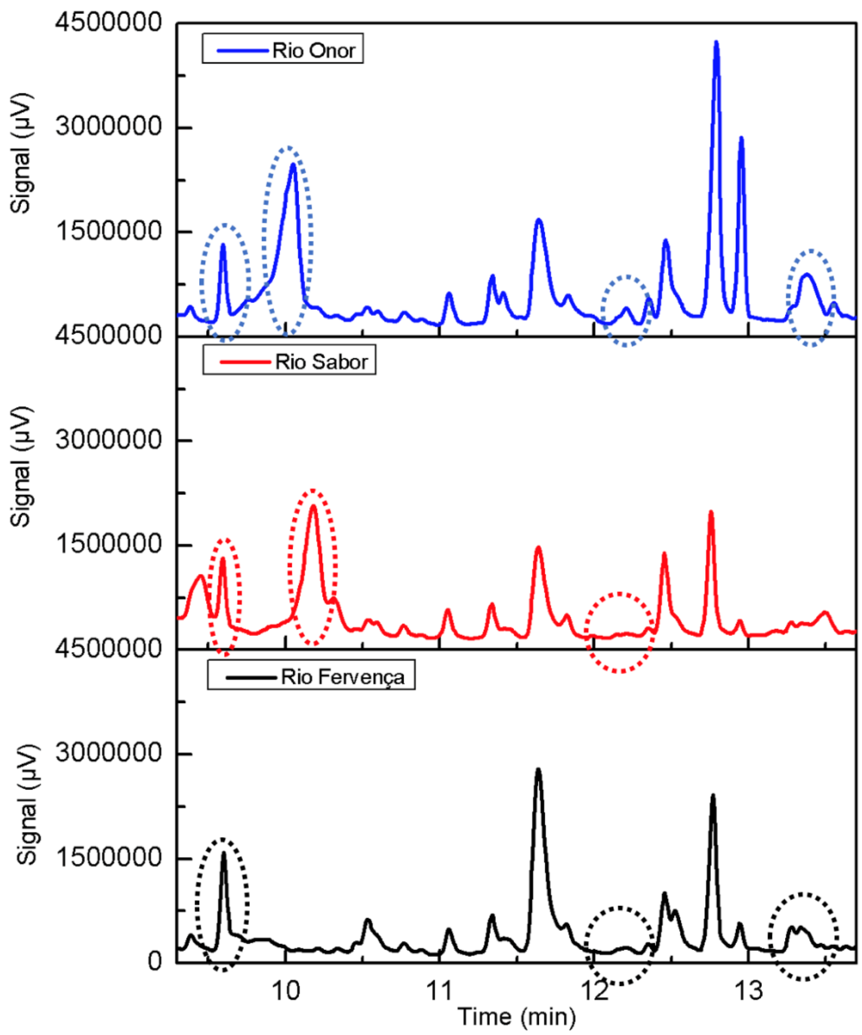


Figure 4.20. Comparison of three SPME/GC-MS analysis of three real samples.

In summary, the water of the rivers of Bragança contains the 5 selected pesticides, only in rio Fervença we didn't detect or quantify Terbuthylazine since its LOD and LOQ where below the ones calculated. As for Metolachlor we couldn't integrate the peak in the rio Sabor because it was very overlaid with another contaminant.

Table 4.11. Concentration (ppb) for the 5 pesticides in 3 real samples using the "Method 2 modified".

Concentration (ppb)	Dimethoate	Terbuthylazine	Alachlor	Heptachlor	Metolachlor
Rio Onor	133.7	26.6	-	22.4	3.7
Rio Sabor	58.7	44.8	-	-	-
Rio Fervença	200.9	-	-	-	-

Using the calibration curves of each compound, we did quantify Dimethoate in the three rivers, in the rio Fervença we collected the highest concentration 200 µg/L, this value was 10 times bigger than the limit of quantification (LOQ).
Also, for Terbuthylazine we successfully quantify it in both rio Sabor and rio Onor with 44 and 26 µg/L respectively both were above the LOD and LOQ calculated from calibration curves.
As for Alachlor we couldn't quantify it because it's concentration in all the rivers were below the limit of quantification (LOQ).
Finally, in rio Onor only we quantified Heptachlor with a 22 µg/L equal to (LOQ) and Metolachlor with 3 µg/L below the (LOD).

All these interpretations and the values obtained are related to the linear range of each pesticide. Furthermore, there is no values referred in legislation about the maximum concentration of these pesticides in drinking water.

Chapter 5: Conclusions

Multi residue analysis is the commonest way of determining pesticides. We have carried out experiments on mass spectrometric determination of pesticide residues in water samples after solid-phase microextraction and successful method was established. The PDMS-DVB coating proved to be efficient on the extraction of the 5 pesticides and, thus, suitable for multi residue analysis. Subsequently, the GC–MS technique was selected due to its high selectivity i.e. high discriminating power among analytes and between these and matrix interferences.

Used for monitoring or screening purposes a single MS method in the FullScan mode showed adequate sensitivity, selectivity and precision for pesticide analysis. However, its improved sensitivity was accomplished on the expense of qualitative data and thus for confirmation of positive results a second analysis in SIM mode should be conducted. Additionally, in certain circumstances only concentrations well above the LOQ could be confirmed due to the presence of high background in the spectra.

For a set of pesticides currently found as contaminants in groundwater samples from Rio Sabor, Rio Onor and Rio Fervença, a validation with real samples from Tunisia couldn't be achieved by reason of difficulty to collect the samples, also an SPME/GC–MS method was developed. This approach allowed important improvements in selectivity and sensitivity and thus in identification and quantification capabilities for low traces of pesticides in water samples. Background noise and interferences were almost completely eliminated and clean secondary spectra permitted identifications with high certainty.

We have optimized and applied a SPME/GC-MS method for extracting and detecting 5 pesticides in 3 river water samples from Portugal during 6 months whereas for the target polar compounds was used.

The insecticide dimethoate was found in the 3 river samples. Other herbicides such as heptachlor, alachlor and metolachlor also appeared in very small quantity. We also detected other triazines as terbuthylazine in relevant concentration levels. These results are not surprising as these sites have agricultural activities. This is the first pilot study undertaken in Portugal that monitors 5 priority pollutants in different surface waters. By performing the present combined methodology used here we provide the analytical tools to carry out advanced monitoring in environmental analysis.

This investigation is a new start line in IPB and there is a lot of work to be improved like re-do all the optimized method of GC-MS using a SIM mode scan or maybe use a different type of GC column, last but not least we can study different types of fibers such as CAR-PDMS or PA and finally extend the list of pesticides for better monitoring of the water quality in the northeast of Portugal.

References

[1] Food and Agriculture Organization of the United Nations 'FAO', "The future of food and agriculture Trends and challenges", *Food and Agriculture Organization of the United Nations*, pp. 9-70, 2017.

[2] The International Food Policy Research Institute 'IFPRI', Global Food Policy Report, *International Food Policy Research Institut*, pp. 6-24, 2017.

[3] W. W. Development, "Population and Water", *Population Institute,* pp. 209–210, 2010.

[4] V. Mboula, "Devenir de polluants émergents lors d'un traitement photochimique ou photocatylitique sous irradiation solaire", pp. 230, 2013.

[5] T. Ahmad, M. Rafatullah, A. Ghazali, O. Sulaiman, R. Hashim, "Removal of Pesticides from Water and Wastewater by Different Adsorbents", *Journal of Environmental Science and Health*, vol. 28, pp. 231–271, 2010.

[6] W. Brüsch, A. Rosenbom, N. Badawi, L. Gudmondsson, von Platten-Hallermund F, Nielsen CB, Plauborg F, Laier T, Olsen P; The Danish Pesticide Leaching Assessment Programme. GEUS, København, *J. of Environ Sci Pollut Res,* vol. 14, pp. 82–94, 2015.

[7] B. Lindström, M. Larsson, J. Kreuger, Från miljöövervakningen av bekämpningsmedel (växtskyddsmedel). Årssammanställning, *Report, Swedish University of Agricultural Sciences, Institute of Water and Environment,* Uppsala, vol. 3, pp. 21–43, 2013.

[8] A. Stefanakis and J. Becker, "A Review of Emerging Contaminants in Water", *Environmental Science & Technology*, vol. 49, no. 11 pp. 55–80, 2014.

[9] DJ. Lapworth, N. Baran, M. Stuart, "Emerging organic contaminants in groundwater: A review of sources, fate and occurrence", *J. of Environmental Pollution*, vol. 163, pp. 287–303, 2012.

[10] A. Jurado, J. Carrera E. Pujades, D. Barceló, "Science of the Total Environment Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context", *Sci. Total Environ.*, vol. 440, pp. 82–94, 2012.

[11] Y. Wang, J. Liu, D. Kang, C. Wu, and Y. Wu, "Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: a review", *Rev. Environ. Sci. Biotechnol.*, vol. 16, no. 4, pp. 717–735, 2017.

[12] J.W. Munch, ''Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry", *Environmental Protection Agency*, Method 525.2, Revision 2.0, pp. 2-3, 1995.

[13] J. Wang and S. Wang, "Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review", *J. Environ. Manage.*, vol. 182, pp. 620–640, 2016.

[14] V. Gupta, B. Gupta, A. Rastogi, S. Agarwal, and A. Nayak, “Pesticides removal from waste water by activated carbon prepared from waste rubber tire”, *Water Res.*, vol. 45, no. 13, pp. 4047–4055, 2011.

[15] A. Székács, M. Mörtl, and B. Darvas, “Monitoring pesticide residues in surface and ground water in Hungary: Surveys in 1990-2015”, *J. Chem*, vol. 39, no. 12, pp. 82–94, 2015.

[16] M. Stenrød, M. Almvik, M. Eklo, R. Holten, M. Larsbo, L. Putelis, K. Siimes, I. Turka, “Pesticide regulatory risk assessment, monitoring, and fate studies in the northern zone: recommendations from a Nordic-Baltic workshop”, *Environ. Sci. Pollut. Res.*, vol. 23, no. 15, pp. 15779–15788, 2016.

[17] J. Rodier, B. Legube, N. Merlet et Coll “L’analyse de l’eau”, 9th edition, 7th Chapter, pp. 1510, 2009.

[18] M. Bhanger, M. Akhtar, F. Talpur,” Adsorption of methyl parathion pesticide from water using watermelon peels as a low-cost adsorbent”, *Sci. Total Environ,* vol. 92, pp. 616–621, 2008.

[19] M. Akhtar, S. Iqbal, M. Bhanger, M. Moazzam. “Utilization of organic by-products for the removal of organophosphorous pesticide from aqueous media”, *J. Hazard Mater*, vol. 162, pp. 703–707, 2009.

[20] MJ. Sanchez-Martin, MS. Rodriguez-Cruz, MS. Andrades, “Efficiency of different clay minerals modified with a cationic surfactant in the adsorption of pesticides”, *Appl Clay Sci.* vol. 31, pp. 216–228, 2006.

[21] NA. Suciua, E. Capri, “Adsorption of chlorpyrifos, penconazole and metalaxyl from aqueous solution by modified clays”, *J. Environ Sci Health B*. vol. 44, pp.525–532, 2009.

[22] M. Cruz-Guzman, R. Celis, MC. Hermosin, J. Cornejo, “Adsorption of pesticides from water by functionalized organobentonites”, *J. Agric Food Chem.* vol. 53, pp. 7502–7511, 2008.

[23] H. Zuo, J. Zhu, and C. Zhan, “A method developed for determination of heptachlor and its metabolites from pork”, *Environ Monit Assess*, vol. 186, pp. 2399–2412, 2014.

[24] N. Saadati, P. Abdullah, Z. Zakaria, S. Belin, T. Sany, and M. Rezayi, “Limit of detection and limit of quantification development procedures for organochlorine pesticides analysis in water and sediment matrices”, *Chemistry Central Journal*, vol. 7, pp. 1–10, 2013.

[25] R. Kookana, “Determination of the insecticide imidacloprid in water and soil using high-performance liquid chromatography” *Journal of chromatography*, vol. 787, pp. 271–275, 1997.

[26] Z. Papp and L. Bjelica, “A rapid spectrophotometric determination of imidacloprid in selected commercial formulations in the presence of 6-chloronicotinic acid”, *J. Serb. Chem. Soc*, vol. 74, no. 12, pp. 1455–1465, 2009.

[27] T. Ding and B. K. Lavine, “Separation of imidacloprid and its degradation products using reversed phase liquid chromatography with water rich mobile phases”, *J. Chromatogr*. A, vol. 1218, no. 51, pp. 9221–9226, 2011.

[28] P. Longenbach, “Separation of Neonicotinoids, Under Both Acetonitrile and Methanol Gradient Conditions, Using High Efficiency CORTECS Phenyl Columns”, *Water corporation Journal*, 2015.

[29] J. Ma, R. Xiao, J. Li, X. Zhao, B. Shi, and S. Li, “Determination of Organophosphorus Pesticides in Underground Water by SPE-GC – MS”, *Journal of Chromatographic Science*, vol. 47, pp. 110–115, 2009.

[30] D. C. Paschal, R. Bicknell, and D. Dresbach, “Determination of Ethyl and Methyl Parathion in Runoff Water with High Performance Liquid Chromatography”, *Analytical Chemistry*, vol. 49, no. 11, pp. 1551–1554, 1977.

[31] M. Sastry, “Determination of Malathion and Methyl Parathion in Water samples by Spectrophotometry”, *Talanta*, vol. 34, pp. 372-374, 1987.

[32] X. Li, P. Gan, R. Peng, C. Huang, and H. Yu, “Determination of 23 Organophosphorous Pesticides in Surface Water Using SPME Followed by GC – MS”, *Journal of Chromatographic Science*, vol. 48, no. March, pp. 183–187, 2010.

[33] E. L. Delmonico, J. Bertozzi, N. E. De Souza, and C. Celestino, “Acta Scientiarum Determination of glyphosate and aminomethylphosphonic acid for assessing the quality tap water using SPE and HPLC”, *Acta Scientiarum Technology*, vol. 36, pp. 513–519, 2014.

[34] I. Hanke, H. Singer, and J. Hollender, “Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry: performance enrichment and detection”, *Anal Bioanal Chem*, vol. 391, pp. 2265–2276, 2008.

[35] S. Vuković, “Determination of some pesticides in drainage water”, *Plant Protection Society of Slovenia,* vol. 11, pp. 454-458, 2013.

[36] R. A. Yokley, C. Mayer, SB. Huang, D. Vargo, “Analytical Method for the determination of Metolachlor, Acetochlor, Alachlor, Dimethenamid and their corresponding Ethanesulfonic and Oxanillic Acid degradates in Water Using SPE and LC/ ESI-MS/MS”, *Anal. Chem*, vol. 74, no. 15, pp. 3754–3759, 2002.

[37] H. Sabik, S. Cooper, P. Lafrance, “Determination of Atrazine, its degradation products and Metolachlor in runoff water and sediments using Solid-phase extraction”, *Talanta*, vol. 42, no. 5, pp. 717–724, 1995.

[38] C. Bolzan, S. Sergiane, S. Bruno, G. Ednei, "Dispersive liquid–liquid microextraction based on solidification of floating organic droplet for the determination of triazine and triazoles in mineral water samples", *Journal of Separation Science*, vol. 42, pp. 1-22, 2012.

[39] M. Hansen, R. Poulsen, X. Luong, D. L. Sedlak, and T. Hayes, "Liquid chromatography tandem mass spectrometry method using solid-phase extraction and bead-beating-assisted matrix solid-phase dispersion to quantify the fungicide tebuconazole in controlled frog exposure study: analysis of water and animal tissue", *Anal Bioanal Chem*, vol. 406, pp. 7677–7685, 2014.

[40] Q. Zhou, J. Xiao, and Y. Ding, "Sensitive determination of fungicides and prometryn in environmental water samples using multiwalled carbon nanotubes solid-phase extraction cartridge", *Analytica chimica acta,* vol. 2, pp. 223–228, 2007.

[41] I. Valor, M. Pérez, C. Cortada, D. Apraiz, J. C. Moltó, and G. Font, "SPME of 52 pesticides and polychlorinated biphenyls: Extraction efficiencies of the SPME coatings poly(dimethylsiloxane), polyacrylate, poly(dimethylsiloxane)-divinylbenzene, Carboxen-poly(dimethylsiloxane), and Carbowax-divinylbenzene," *J. Sep. Sci.*, vol. 24, pp. 39–48, 2001.

[42] C. Gonçalves and M. F. Alpendurada, "Solid-phase micro-extraction-gas chromatography-(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities," *J. Chromatogr. A*, vol. 1026, pp. 239–250, 2004.

[43] N. Dujaković, S. Grujić, M. Radišić, T. Vasiljević, and M. Laušević, "Determination of pesticides in surface and ground waters by liquid chromatography-electrospray-tandem mass spectrometry," *Anal. Chim. Acta*, vol. 678, pp. 63–72, 2010.

[44] J. S. Aulakh, A. K. Malik, V. Kaur, and P. Schmitt-Kopplin, "A review on solid phase micro extraction - High performance liquid chromatography (SPME-HPLC) analysis of pesticides," *Crit. Rev. Anal. Chem.*, vol. 35, no. 1, pp. 71–85, 2005.

[45] P. L. Chang, M. M. Hsieh, and T. C. Chiu, "Recent advances in the determination of pesticides in environmental samples by capillary electrophoresis," *International Journal of Environmental Research and Public Health*, vol. 13, no. 4. 2016.

[46] Lista de pesticidas a pesquisar em água destinada ao consume humano, Triénio 2019-2021", Ao abrigo do disposto no Decreto-Lei nº 306/2007, de 27 de agosto, alterado pelo Decreto-Lei nº 152/2017, de 7 de dezembro. Direção Geral de alimentação e veterinária. Repúplica Portuguesa. Lisboa 2018.

[47] Projet d´intensification de l´agriculture irriguée en Tunisie (PIAIT)". Ministère de l'Agriculture, des Ressources Hydrauliques et de la Pêche (MARHP). Direction Générale du Génie Rural et de l'Exploitation des Eaux (DGGREE). République de Tunisie. Version définitive, Mars 2018.

[48] J. N. Miller and J. C. Miller, "Statistics and Chemometrics for Analytical Chemistry", Sixth Edition, Prentice Hall, England, 2010. ISBN: 978-0-273-73042-2.

[49] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/heptachlor#:~:text=Heptachlor%20is%20soluble%20in%20organic,solubility%20at%2025%20%C2%B0C).

[50] https://pubchem.ncbi.nlm.nih.gov/compound/Heptachlor

[51] http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/imidacloprid-ext.html

[52] https://www.chemicalbook.com/ChemicalProductProperty_US_CB9730575.aspx

[53] https://pubchem.ncbi.nlm.nih.gov/compound/Dimethoate

[54] https://soils.wisc.edu/facstaff/barak/virtual_museum/glyphosate

[55] https://substances.ineris.fr/fr/substance/getDocument/2984

[56] http://www.chemspider.com/Chemical-Structure.3376.html

[57] https://pubchem.ncbi.nlm.nih.gov/compound/Terbuthylazine#section=Environmental-Water-Concentrations

[58] https://www.chemsrc.com/en/cas/5915-41-3_1091285.html

[59] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/acetochlor#:~:text=Acetochlor%20has%20a%20boiling%20point,%2C%20toluene%2C%20and%20carbon%20tetrachloride.

[60] https://pubchem.ncbi.nlm.nih.gov/compound/Acetochlor

[61] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/acetochlor#:~:text=Acetochlor%20has%20a%20boiling%20point,%2C%20toluene%2C%20and%20carbon%20tetrachloride.

[62] https://pubchem.ncbi.nlm.nih.gov/compound/Metolachlor#section=Color-Form

[63] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/alachlor

[64] https://substances.ineris.fr/fr/substance/pdf/335

[65] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/alachlor#:~:text=Alachlor%20is%20a%20white%20to,137%20%C2%B0C%20(cc).

[66] https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/427.htm

[67] https://substances.ineris.fr/fr/substance/getDocument/3212

[68] https://pubchem.ncbi.nlm.nih.gov/compound/4-Chloro-2-methylphenoxy_acetic-acid

[69] http://www.etongchem.com/products_detail_es/id/4.html

[70] https://sitem.herts.ac.uk/aeru/iupac/Reports/267.htm

[71] https://pubchem.ncbi.nlm.nih.gov/compound/Tebuconazole

[72] https://substances.ineris.fr/fr/substance/getDocument/3059

[73] https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/610.htm

[74] https://www.ncbi.nlm.nih.gov/books/NBK373186/

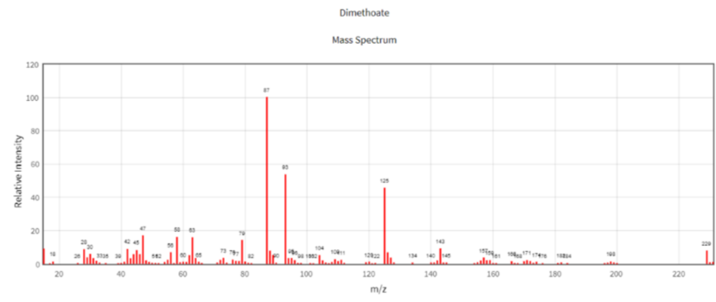
[75] https://pubchem.ncbi.nlm.nih.gov/compound/Bis_2-ethylhexyl_-phthalate

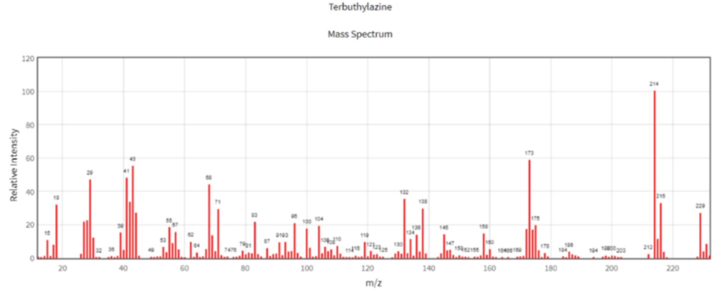
[76] https://www.researchgate.net/figure/Properties-of-commercially-available-SPME-fibers-adapted-from-31-CW-TPR_fig2_263432842

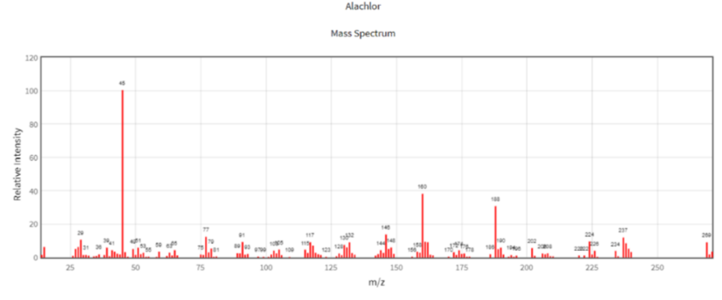
[77] https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/General_Information/1/spme-gc-brochure.pdf

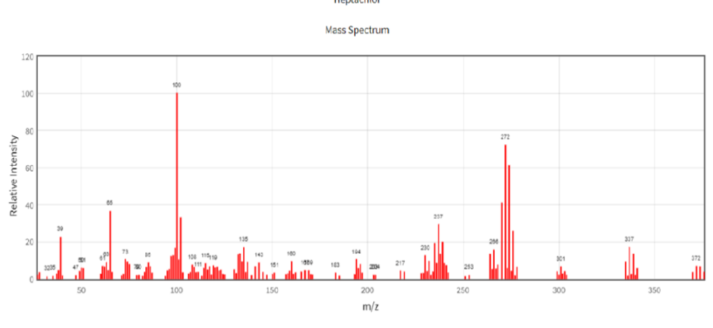
[78] https://www.researchgate.net/publication/231536036

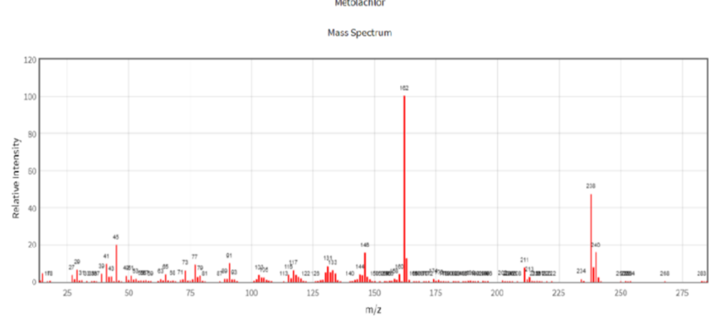
Appendix











MS Data for 5 pesticides

Table of different area related to different concentration of Dimethoate.

Concentration (ppb)	Area (Counts)
25	1845062
40	2073507
50	2234832
60	2442173
75	2603550

Table of different area values related to the confidence interval of Dimethoate.

Concentration (ppb)	Area (Counts)	Lower limit	Upper limit
25	1845062.0000	2054723.2832	1643931.1444
40	2073507.0000	2328612.2128	1838639.3183
50	2234832.0000	2511204.8325	1968444.7675
60	2442173.0000	2693797.4522	2098250.2168
75	2603550.0000	2967686.3817	2292958.3907

Table of different area related to different concentration of Terbuthylazine.

Concentration (ppb)	Area (Counts)
10	3914947
25	8150079
40	14046654
50	16896024
60	20987094

Table of different area values related to the confidence interval of Terbuthylazine.

Concentration (ppb)	Area (Counts)	Lower limit	Upper limit
10	3914947.0000	5519769.0436	1588723.8108
25	8150079.0000	11233263.6135	6147132.7663
40	14046654.0000	16946758.1833	10705541.7218
50	16896024.0000	20755754.5632	13744481.0254
60	20987094.0000	24564750.9431	16783420.3291

Table of different area related to different concentration of Alachlor.

Concentration (ppb)	Area (Counts)
10	5791834
25	10399828
40	17662304
50	21169887
60	26027213

Table of different area values related to the confidence interval of Alachlor.

Concentration (ppb)	Area (Counts)	Lower limit	Upper limit
10	5791834.0000	8115514.4108	2238601.8930
25	10399828.0000	15108471.4744	7504705.9940
40	17662304.0000	22101428.5379	12770810.0950
50	21169887.0000	26763399.9136	16281546.1623
60	26027213.0000	31425371.2893	19792282.2297

Table of different area related to different concentration of Heptachlor.

Concentration (ppb)	Area (Counts)
25	157584
40	722460
50	1053490
60	1317458
75	1698942

Table of different area values related to the confidence interval of Heptachlor.

Concentration (ppb)	Area (Counts)	Lower limit	Upper limit
25	157584.0000	661716.3829	-215671.4036
40	722460.0000	1206453.8267	159948.3250
50	1053490.0000	1569612.1226	410361.4774
60	1317458.0000	1932770.4184	660774.6298
75	1698942.0000	2477507.8622	1036394.3584

Table of different area related to different concentration of Metolachlor.

Concentration (ppb)	Area (Counts)
10	8998493
25	14682485
40	23994829
50	28912561
60	35397139

Table of different area values related to the confidence interval of Metolachlor.

Concentration (ppb)	Area (Counts)	Lower limit	Upper limit
10	8998493.0000	12373043.6822	3615479.5710
25	14682485.0000	21661268.4880	10330410.0689
40	23994829.0000	30949493.2939	17045340.5669
50	28912561.0000	37141643.1644	21521960.8989
60	35397139.0000	43333793.0350	25998581.2309

Table of 5 compounds with their area found in 3 different location.

Area (Counts)	Dimethoate	Terbuthylazine	Alachlor	Heptachlor	Metolachlor
Rio Onor	3547446	9244790	1067098	1231128	4652466
Rio Sabor	2376466	15493316	475326	328872	-
Rio Fervença	4598154	-	511256	395508	920836