Andrés Castaño García

Analysis of organic contaminants in water intended for human consumption – analysis by GC-MS with different sample preparation techniques (LLE and SPE)

Universidade do Algarve/Empresa Portuguesa das Águas Livres

2015-2016

Andrés Castaño García

Analysis of organic contaminants in water intended for human consumption – analysis by GC-MS with different sample preparation techniques (LLE and SPE)

Thesis supervised by: Dr Vitor Vale Cardoso

Erasmus Mundus Master in Quality Analytical Laboratories

Universidade do Algarve/Empresa Portuguesa das Águas Livres

2015-2016

Analysis of organic contaminants in water intended for human consumption – analysis by GC-MS with different sample preparation techniques (LLE and SPE)

Declaro ser o(a) autor(a) deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Acknowledgements

This research project has been carried out at the Central Laboratory of the Empresa Portuguesa das Águas Livres (EPAL), in collaboration with Universidade do Algarve. The Master thesis is the second year of the European Master, Erasmus Mundus in Quality Analytical Laboratories (EMQAL).

I have been fortunate to study the first year of the Master in Universitetet i Bergen, at which I am very grateful for all the knowledge acquired and for letting me meet that fantastic country. I chose to come to Lisbon for doing the research project and I can say that I did not regret the decision, it was a fantastic year, professionally and personally.

I would like to thank Prof. Isabel Cavaco and all the EMQAL team for making possible this project. I also want to show my gratitude to Eng. María João Benoliel, EPAL's laboratory director, for allowing me to carry out my research project at EPAL.

I want to say thank you – a big thank you - to Dr. Vitor Vale Cardoso, who guided and supported me throughout this year. Thanks also Dr. Vitor, for giving me enough work independence, because that made me think by myself, helping my professional development.

This year doing my thesis at EPAL would not be the same without the people that embraced and shared every moment with me, so I want to thank everyone in the laboratory. I want to give another big thanks to the people that make up the organic chemistry department, specially to Dr Alexandre Rodrigues, Dra Cristina Correia, Dra Ana Penetra, Dra Marta Loureiro, Sara Anacleto, João and Nelson, because they are the people that I would definitely choose as work colleagues and friends.

On a personal level, I want to say thanks to all my apartment colleagues, Edoardo, Michele, Beppe, Aldo and Vincenzo, because we shared happiness and pains, but I will never forget that year, and they are one of the main reasons.

I also wish to thank my family warmly for all the love and encouragement received. The last one, buy maybe the most important and heartfelt thanks is to Yara, my girlfriend, for sharing the good and the bad moments and withstand all the difficulties of the distance.

Andrés Castaño García

Abstract

Water is a fundamental resource for human life, as well as for other organisms. Pesticides are one of the most critical group of components that can contaminate rivers and lakes, affecting the quality of water. EPAL, as the public water company of Lisbon, is responsible for monitoring the water quality, analyzing microbiological, inorganic and organic parameters in water, according to the established water legislation.

However, new compounds are always being added to the legislation whenever it is revised. It is necessary to develop new analytical methods to cover the monitoring of these new added compounds. That is the case of some of the compounds of this study.

Therefore, an analytical method was developed, optimized and validated for the analysis of organic components (pesticides) in different water matrices by gas chromatography coupled with mass spectrometry (GC-MS). The list of pesticides studied is composed by: biphenyl, dicofol, chlorpyrifos, aclonifen, quinoxyfen, cybutryne and the isomers of cypermethrine (alpha-cypermethrine, beta-cypermethrine, theta-cypermethrine and zeta-cypermethrine).

In order to concentrate and extract the pesticides from the water sample, two different extractions techniques - liquid-liquid extraction (LLE) and solid phase extraction (SPE) - were tested. The results showed that LLE gave successful recovery values, while the extraction using SPE showed many problems. Different tests were carried out in order to solve the problems and to optimize the extraction procedure, such as matrix effect, salting-out or sodium thiosulfate tests.

The method was validated, studying the working range, linearity, selectivity, precision, trueness and, LOD and LOQ. Uncertainty of the method was estimated following two different models, bottom-up and top down approach.

Then, these results were compared with the limits set by the legislation for drinking and surface water, concluding that the method is effective for the analysis of these

pesticides in drinking water and for some of them is also effective for their analysis in surface water, within the limits established by the legislation.

Keywords: water, pesticides, gas chromatography, mass spectrometry, liquid-liquid extraction, solid phase extraction, validation, legislation

Table of contents

List of tables

List of figures

List of symbols and abbreviations

BTEX - benzene, toluene, ethylbenzene, and xylenes

THMs – Trihalomethanes

PCBs – Polychlorinated biphenyls

DDT - Dichlorodiphenyltrichloroethane

IUPAC - International Union of Pure and Applied Chemistry

POPs – Persistent organic pollutants

EPA - US Environmental Protection Agency

WFD – Water Frame Directive

EU – European Union

EQSD - Environmental Quality Standards Directive

EPAL – Empresa Portuguesa das Águas Livres

Grupo AdP – Águas de Portugal

DAF - Dissolved air flotation

PCQA – Plan de Control da Qualidade da Água

APCER - Associação Portuguesa de Certificação

IPAC - Instituto Português de Acreditaçao

K_D – Distribution coefficient

a_s – Solute activity

[s] - Solute concentration

γ_s - Activity coefficient of the solute

K – Equilibrium constant

$\beta_{i,j}$ – Selectivity between analytes i and j

k_i – Distribution constant

LLE – Liquid-Liquid Extraction

E – Amount of extracted compound

V – Ratio between volumes (equation 3.6)

SPE – Solid Phase Extraction

FID – Flame Ionization Detector

ECD – Electron Capture Detector

MS – Mass Spectrometer

GC – Gas Chromatography

LC – Liquid Chromatography

NPD – Nitrogen Phosphorus Detector

R_S – Chromatography resolution

t_R – Retention time

Δt_R – Difference of retention times

$\bar{w}_b$ – Average peak width

w_b – Peak width at baseline

K – Retention constant (equation 3.8)

t_M – Holdup time

t'_R – Difference between retention time and holdup time

α – Separation factor (equation 3.9)

k – Retention factor (chromatography)

N – Number of theoretical plates

H – Plate height

L – Column length

A, B, C – Factors of Purnell Equation

u – Mobile phase velocity

d_c – Internal diameter of the column

d_f – Film thickness

K_C – Distribution constant

EI – Electron ionization

CI – Chemical ionization

FI – Field ionization

FD – Field desorption

ESI – Electrospray ionization

APCI – Atmosphere-pressure chemical ionization

DESI – Desorption electrospray ionization

DART – Direct Analysis in Real Time

FAB/FIB - Fast Atomic/Ion Bombardment

APPI - Atmospheric Pressure Photo Ionization

MALDI - Matrix-assisted Laser Desorption Ionization

TOF – Time-of-flight analyzer

FT – Fourier transform analyzer

RF – Radio frequency

DC -Direct current

QqQ – Triple quadrupole analyzer

q-TOF – Quadrupole with time-of-flight analyzer

FS – Full Scan

PIS – Product Ion Scan

SRM – Selected Reaction Monitoring

SIM – Selected Ion Monitoring

R^2 – Coefficient of determination

$S_{y/x}$ – Residual standard deviation for linear regression

S_{y2} – Residual standard deviation for second order regression

N – Number of concentration points

y_i – Obtained signal for a concentration i

y_i – Estimated signal for the linear regression

y_{i2} – Estimated signal for the second order regression

DS^2 – Difference between variances

TV – Test value

F – Tabulated value from Fischer-Snedecor table

LOD – Limit of Detection

LOQ – Limit of Quantification

ICH – International Conference on Harmonization

s_{xo} – Standard deviation of the fortified blanks

S – Standard deviation

x_i – Individual value of each measurement

$\overline{x}$ – Average value of measurements

n – Number of measurements

V_{xo} – Coefficient of variation

u_c – Combined uncertainty

u_{prec} – Precision uncertainty

$u_{stand.prep}$ – Standard preparation uncertainty

u_{interp} – Interpolation uncertainty

k – Coverage factor (uncertainty)

DCM – Dichloromethane

SALLE – Salting-out Liquid-Liquid Extraction

UPW – Ultra-Pure Water

TW – Tap Water

GW – Groundwater

SW – Surface Water

R - Recovery

SD – Standard Deviation

RSD – Relative Standard Deviation

MAC – Maximum Allowed Concentration

MA LOD – Maximum Allowed Limit of Detection

Objective

The aim of the thesis is developing a method that could be latter used in routine analysis of the determination of organic compounds in water intended for human consumption by gas chromatography coupled with mass spectrometry.

The optimization of the parameters of the sample preparation, the chromatography and mass spectrometry conditions were carried out, to assure the optimal conditions for the subsequent routine analysis.

Study the legal requirements stablished for the European Union related to water quality and for the organic compounds in water.

Calculate the validation parameters of the results in order to get reliable and objective results, proving that the analytical requirements for the method are fulfilled.

1. Introduction

Water is a fundamental resource for human life, as well as for other organisms. It covers around 70 % of the Earth's surface, finding the principal percentage in oceans as saline water. Only a small part of the Earth's water is freshwater, belonging to rivers and lakes and it is used for drinking water, in agriculture or in hygienic uses.

Regarding the importance of water, many legal requirements have been established in order to achieve a good quality of water. The number of possible organic contaminants in water is very large, as pesticides, hydrocarbons, phenols, BTEX, THMs, PCBs, etc. These contaminants could be originated from photochemical activities and introduced in the water by combustion and emission [1].

Pesticides are one of the biggest concerns for the environment because of its wide distribution. Drinking water, have to be monitored, analyzed and treated continuously in order to guarantee the good quality and to avoid any possible harmful effect on human health.

1.1. Organic compounds of interest: Pesticides

Pesticides along the history were always made of inorganic or organometallic compounds, having a high toxicity for humans and most of them containing also heavy metals, as mercury, arsenic or lead. DDT, dichlorodiphenyltrichloroethane, synthetized by Othmar Zeidler, was the first organic pesticide, but 65 years later Paul Hermann Muller discovered its insecticide properties. The discovery of DDT was very important because it replaced the use of arsenic pesticides, very toxic for human health. Later, organochlorines were substituted by organophosphates and carbamate pesticides.

Nowadays, pesticides are more than necessary for the developing of the world, because they allow an efficient agriculture, avoiding plagues and diseases and enhancing the production of crops.

Pesticides protect plants and fruits from the attack of weeds, diseases or insects. Pesticide is defined as a chemical or biological agent that kills and incapacitates pests. They

are divided in groups, depending on the target (herbicide, bactericide, insecticide, fungicide...)

The importance and potential to contaminate water with pesticides is well known. Pesticides can appear in water following different ways, but mainly via runoff, spray drift and drainage [2]. The use of pesticides is highly controlled, being some of the pesticides of this study already prohibited.

1.2. Description of the studied compounds

1.2.1. Pesticides

The pesticides of this study are included in the directive for priority substances in the field of water policy, and some of them are marked as priority hazardous substances [3].

- *Bifenox (IUPAC name Methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate)*

It is an organochlorine pesticide belonging to the family of herbicides. It is used for controlling broad-leaved weeds and some grasses. At room temperature it is a solid, yellow crystal, with a slightly aromatic odor. Its solubility in water is low (0,1 mg/L) but is quite soluble in organic solvents such acetone, dichloromethane, toluene, etc. Its molecular weight is 342.13096 [4].

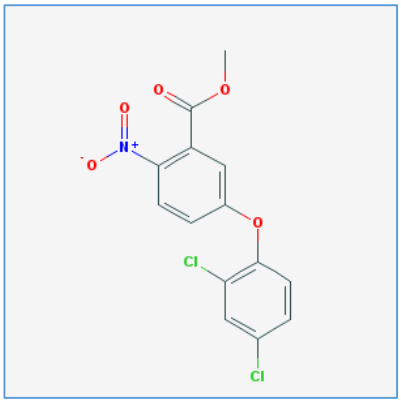


Figure 1. Structure of Bifenox [4]

- Dicofol (*IUPAC name 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol)*

It is an organochlorine pesticide with some chemical similarities to DDT. The structure is the same, but the Dicofol has a hydroxyl group in the C-1 instead of a hydrogen. It is a acaricide, very effective against spiders, but it is also use to mite control. It is a colorless solid at room temperature. Its solubility in water is around 1 mg/L and it is soluble in organic solvents. Its molecular weight is 370.48566 [5].

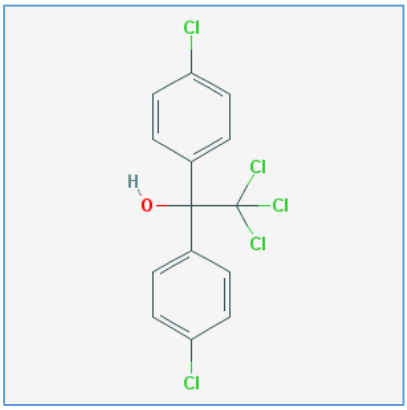


Figure 2. Structure of Dicofol [5]

- Aclonifen *(IUPAC name 2-chloro-6-nitro-3-phenoxyaniline)*

Aclonifen is a diphenyl ether pesticide used as an herbicide to control broad-leaves and grass weeds [6]. It is normally used as a pre-emergence herbicide. It is low soluble in water but high soluble in organic solvents. It is a white powder. Its molecular weight is 264.66446 [7].

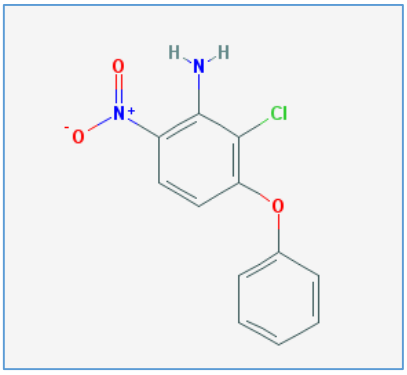


Figure 3. Structure of Aclonifen [7]

- Quinoxyfen *(IUPAC name 5,7-dichloro-4-(4-fluorophenoxy) quinoline)*

It is an organochlorine pesticide belonging to the family of fungicides. It is mainly used to control powdery mildew in cereals. It is an off-white solid, soluble in organic solvents but with a low solubility in water. Its solubility in water is low because it can be bounded and accumulated in sediments in water [8]. Its molecular weight is 308.13452 [9].

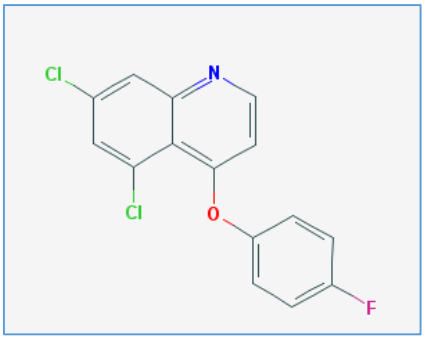


Figure 4. Structure of Quinoxyfen [9]

- Chlorpyrifos-ethyl *(IUPAC name diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl) oxy-{5}-phosphane)*

It is an organophosphate pesticide belonging to the insecticide family. It is used to control foliage and soil-borne insect pests on a variety of food and feed crops. The largest market in which it is used is corn [10]. It is also used in houses to control cockroaches, fleas, and termites. When chlorpyrifos come into contact with sediments, it usually sticks highly to the sediments, so it is difficult for chlorpyrifos to trespass to the water environment [11]. It is a white solid powder, with a mercaptan - like odor. It is quite soluble in organic solvents as acetone or dichloromethane. Its molecular weight is 350.58630 [12].

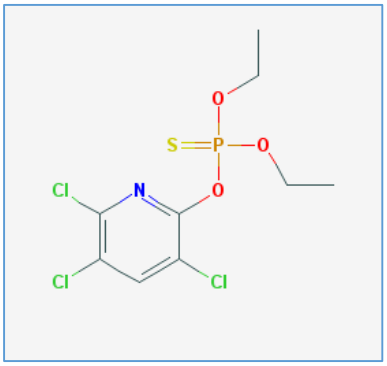


Figure 5. Structure of Chlorpyrifos [12]

- Deltamethrine *(IUPAC name [(S)-cyano-(3-phenoxyphenyl) methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate)*

It is a synthetic pyrethroid ester insecticide. It is only one stereoisomer of a possible of 8 stereoisomers, specifically prepared by esterification. It is one of the most used and popular insecticide. It is used in agriculture as well as in domestic products. It is a crystalline colorless to white power without any odor. Its molecular weight is 505.19916 [13].

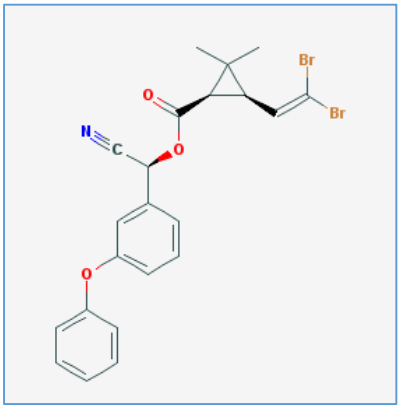


Figure 6. Structure of Deltamethrine [13]

- Cybutryn (*IUPAC name 2-N-tert-butyl-4-N-cyclopropyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine)*

Cybutryn is a s-triazine compound mainly used as algicide and biocide in buildings. It has also been used as an antifouling agent on ships, replacing the tributyltin, because this one was really toxic to non-target organisms [14]. Cybutryn is also known as Irgarol. It is a white to slightly yellow solid powder. Its molecular weight is 253.36706 [15].

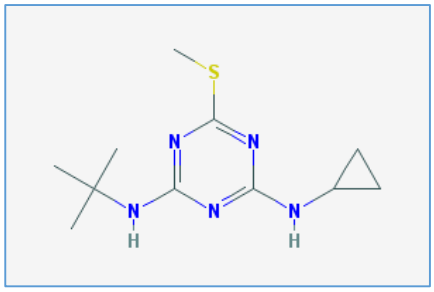


Figure 7. Structure of Cybutryn [15]

- Biphenyl *(IUPAC name 1,1'-biphenyl)*

Biphenyl is an aromatic hydrocarbon that is the main starting compound used to synthetize the widely known polychlorinated biphenyls (PCBs). It is not really reactive because it has not any functional group, for this reason it is very used. However, it can react by sulfonation and substitution, giving some fungicides such p-hydroxybiphenyl or the popular PCBs. It prevents also the growth of fungus, so it is used as a preservative, mainly in the citrus fruits. Its molecular weight is 154,20780 [16].

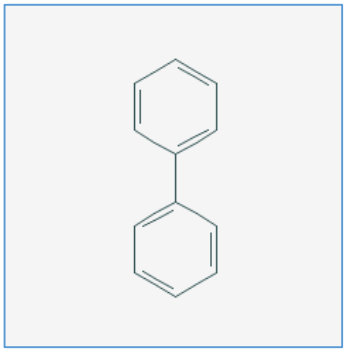


Figure 8. Structure of Biphenyl [16]

- Cypermethrine *(IUPAC name [cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate)*

It is a synthetic pyrethroid used as insecticide in a lot of agriculture applications and also in domestic insecticide products. It has 4 isomers alpha-cypermethrine, beta-cypermethrine, theta-cypermethrine and zeta-cypermethrine. These compounds can stick to the sediments, decreasing its mobility and its solubility in water. It can be a white solid or a yellow viscous liquid. They are soluble in organic solvents. Its molecular weight is 416.29716 [17].

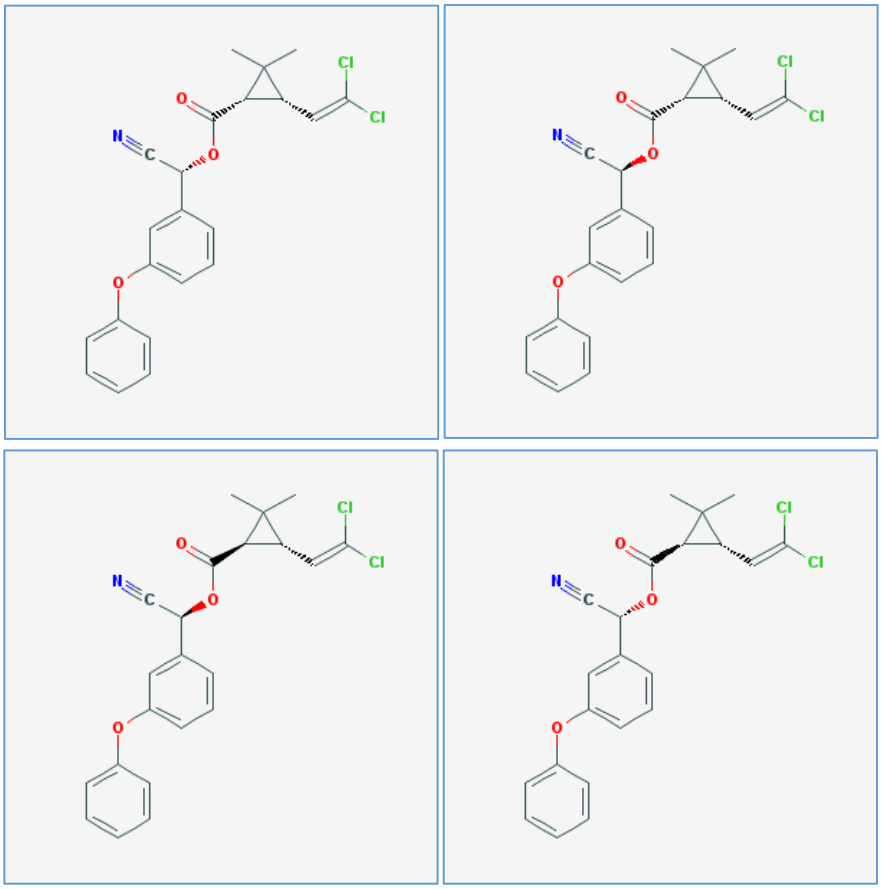


Figure 9. Structure of cypermethrine a) alpha b) beta c) theta d) zeta, isomers [17]

Name	Formula	Molecular Weight	Melting point	Boiling point	CAS No.
Bifenox	$C_{14}H_9Cl_2NO_5$	342.13096	85 °C	421 °C	42576-02-3
Dicofol	$C_{14}H_9Cl_5O$	370.48566	77-78 °C	225 °C	115-32-2
Quinoxyfen	$C_{15}H_8Cl_2FNO$	308.13452	105-106 °C	423 °C	124495-18-7
Aclonifen	$C_{12}H_9ClN_2O_3$	264.66446	81 °C	400 °C	74070-46-5
Chlorpyrifos-ethyl	$C_9H_{11}Cl_3NO_3PS$	350.58630	41-42 °C	160 °C	2921-88-2
Biphenyl	$C_{12}H_{10}$	154.20780	69 °C	256 °C	92-52-4
Cybutryne	$C_{11}H_{19}N_5S$	253.36706	128-133 °C	428 °C	28159-98-0
Deltamethrine	$C_{22}H_{19}Br_2NO_3$	505.19916	90 °C	300 °C	52918-63-5
Alpha - cypermethrine	$C_{22}H_{19}Cl_2NO_3$	416.29716	78-81 °C	200 °C (0,07 torr)	67375-30-8
Beta - cypermethrine	$C_{22}H_{19}Cl_2NO_3$	416.29716	78-81 °C	200 °C (0,07 torr)	65731-84-2
Theta - cypermethrine	$C_{22}H_{19}Cl_2NO_3$	416.29716	78-81 °C	200 °C (0,07 torr)	71697-59-1
Zeta - cypermethrine	$C_{22}H_{19}Cl_2NO_3$	416.29716	78-81 °C	200 °C (0,07 torr)	52315-07-8

Table 1. Characteristics of compounds of study

*Boiling and melting points at 760 torr

1.3. Source, behavior and fate of pesticides in the environment

The development of the pesticides has allowed to enhance and to obtain products more effective and selective. However, there are many negative aspects that can occur due to its use, because part of them can remain as residual pesticides in plants or in sediments, and can also be introduce into the food chain.

Pesticides can be present in plants, and therefore in the foodstuff, being a risk for human health. Hence, the presence of pesticides has to be controlled in the environment as well as in foodstuff. The behavior and source of the pesticides has to be studied to understand better where they can be found and its possible decomposition and effects.

When a pesticide is applied in the agriculture, there are different ways in which it can reach the environment (air, water or sediments). The first affected part are the plants and the zone around the application (organisms present in the land, air, sediments, humans, animals, etc). The pesticide can be volatilized and move through the air due to the wind, arriving far away from the place of its application and helped by the rain, contaminate surface waters, animals or humans. Another pathway of contamination, is the leaching of the pesticide through the soils and posterior contamination to the groundwater.

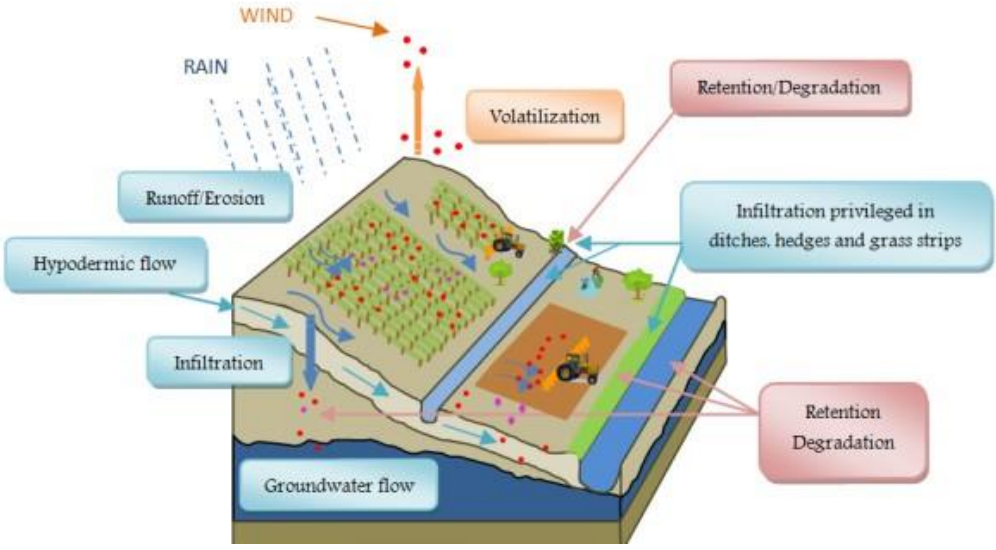


Figure 10. Pesticides contamination pathways

However, pesticides can be also degraded due to several conditions such as light, humidity, microorganism action, pH or temperature into others compounds with less toxicity, but sometimes can occur that the degradation product may be more toxic than the parent product.

Another important aspect to take into account is the bioaccumulation of pesticides. Bioaccumulation is defined as a process in which a chemical substance is absorbed in an organism by all routes of exposure, as occurs in the natural environment (dietary and ambient environment sources) [18].

For all of this, the use of pesticides requires a good knowledge of all its characteristics to try to minimize its possible spread in the environment, and all its negative effects in human health.

Some pesticides belong to the list of Persistent Organic Pollutants (POPs) due to its toxicity and persistence, because they are quite stable and can remain long time in the environment. In May of 2001, there was a meeting where more than 100 countries around the world signed, in the Stockholm convention, that POPs are really dangerous compounds for human health and for the environment and they established a list of dangerous and possible dangerous chemicals. The Stockholm convention requires the signatory countries to eliminate or reduce the release of POPs into the environment. The aim of the Stockholm convention was to protect human health and the environment from these dangerous compounds. This objective is carried out through the prohibition and elimination of the production and use of some of these compounds or in other cases just restricting their use [19].

1.4. Effects of pesticides in the human health

Humans can be poisoned by pesticides mainly in three different ways. The first, is due to a high level exposure in a short period of time for a certain pesticide. It can happen in suicide individuals or even in the use of pesticide formulations during the application. The second, is a high level exposure during long periods of time and usually happen in pesticide formulators or manufacturers. The third one, is due to the ingestion of products that

contain residual pesticides, that is, a continuously but low concentration ingestion of pesticides during long periods of time. Most of the cases that have been reported are due to the first mentioned exposure. The first and second are called *acute poisoning* and the third one is a *chronic poisoning*.

The study of the toxicology of the pesticides is an important goal. Toxicology is the scientific study of the adverse effects that occur in living organisms due to chemicals. The toxicological study involves the symptoms, ingestion pathways, mechanism of action, detection and treatment after the pesticide exposure to humans. The toxic effect of a pesticide depends on the exposure via and the time of exposure [20].

The pesticides studied in this research belong to different chemical groups, so they have different toxicities and for this reason they are used for different purposes. A pesticide is useful because of its toxicity to kill and eliminate insects, fungi, grass weeds [etc]. However, its toxicity can also affect other living organisms. A brief summary of its toxicity is shown below:

- Bifenox

It belongs to the family of the organochlorine pesticides. An acute toxicity is caused after an oral ingestion of more than 5000 mg/kg. Skin irritation, can appear after poisoning by an amount between 200 to 2000 mg/kg of bifenox. It may also cause eye irritation, damaging the cornea [21]. When bifenox enter into the water environment, it may be degraded by hydrolysis and photolysis, producing bifenox acid and 2.4-dichlorophenol, respectively.

- Dicofol

The main harmful effect for human health is that this pesticide can inhibit the acetylcholinesterase and cause accumulation dangers to the environment. It is very toxic to the aquatic environment, and can be accumulated in fish achieving the food chain. Dicofol can be decomposed producing toxic and corrosive fumes including chlorines [22]. This compound is being study by the Stockholm convention to be added to the list of POPs [23].

- Aclonifen

Studies in animals have stablished that aclonifen is not very toxic, because it can be extensively metabolized and eliminated via renal or via the feces [24]. However, it is suspected of causing cancer. It produces skin irritation and can produce an acute poisoning if high levels are inhaled [25].

- Quinoxyfen

Studies about hydrolysis, soil photolysis, aerobic/anaerobic soil metabolism, and field dissipation show that quinoxyfen has chemical properties that may cause its persistence in the environment under certain conditions. Quinoxyfen is highly accumulated in sediments, but its low solubility in water reduce its mobility. It is highly toxic to fish but presents low toxicity to terrestrial wildlife, so it is not very toxic to humans, either [8]. In contact with human can cause several eye irritation and if it is inhaled, can cause drowsiness and dizziness [26].

- Chlorpyrifos

Chlorpyrifos is widely used in many places, so it can be present in many different ways in our life. It degrades rapidly by sunlight, bacteria or chemical process, but low levels of it may persist for long time. It can cause dizziness, fatigue, runny nose or eyes, salivation, nausea, intestinal discomfort, sweating, and changes in heart rate, when a short-term oral exposure (one day) to low levels occurs. In a short-term exposure to high levels, chlorpyrifos may cause paralysis, seizures, loss of consciousness, and even death. However, chlorpyrifos was not classified as carcinogenic compound by the EPA [11].

- Cybutryn

Cybutryn is mainly used for antifouling paints, and for this reason can be in contact with the aquatic environment. It has strong adsorption and tends to accumulate in marine sediments and it can be degraded. It is an inhibitor of a photosynthetic system II, and for this reason it is also used as an herbicide in the agriculture [27]. It is toxic to fish and

invertebrates. In humans, it may cause eye irritation and skin sensitization in sensitive individuals [28].

- Biphenyl

Biphenyl is the base of PCBs but it can be also used with other goals as it is explained earlier. In workers, toxicity has been observed to cause eye and skin irritation and affect also the liver, kidney and nervous system. Biphenyl was not classified as carcinogenic compound by the EPA [29].

- Cypermethrines and Deltamethrine

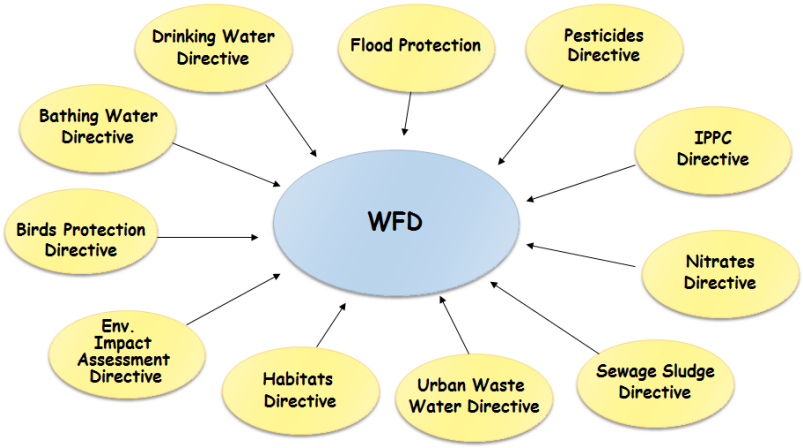
Studies show that people that handle with pyrethroids and pyrethrines, as cypermethrines and deltamethrine, can developed tingling, burning, dizziness and itching. EPA has classified pyrethrines as possible carcinogen. When they come in contact with the environment, are rapidly degraded by soil microbes, but they are stable to sunlight. If they are ingested accidentally, between 49-78 % of the total present in the body are rapidly excreted in the first 24h. The effect on human health, depends on the quantity and the time a human is exposed to the pyrethrines [30][31].

1.5. Legislation related to water quality

1.5.1. European legislation related to water quality

European legislation concerning water quality started in 1975 as a necessity of some European countries to develop standards to analyze the water destined for human consumption. In 1980, the first legislation was culminated, in which were included quality targets for drinking water. It was also included quality objective legislation on fish water, shellfish water, bathing water and groundwater. In 1988, the Frankfurt ministerial seminar of water reviewed the legislation, identifying improvements and gaps that could be fulfilled. In this moment, was born the second phase of the water legislation, and was created the Urban Waste Water Treatment Directive, the Nitrates Directive, a new Drinking Water Directive and a Directive for Integrated Pollution and Prevention Control.

While the legislation already done was really important, the citizens, other countries, authorities and organizations started to be more aware of the quality of their water. This is why, in 1996, 250 different delegates from all Member States, regional and local authorities, enforcement agencies, water providers, industry, agriculture and, not least, consumers and environmentalists met in the Water Conference to create a single coherent management frame for all water-related legislation [32].



The Water Frame Directive, is just a document that provides a framework for all the others pieces of legislation. The legislation that derive from it, contain more specific information about the scope, quality, limits, implementation, etc.

Therefore, the objectives of the Water Frame Directive [33] can be synthetized in a few points:

- Establishes a management structure for the European water policy
- Protect and improve the quality of aquatic ecosystems
- Promotes sustainable water use based on long-term protection of water resources, ensures that the right amount of water is available where and when it is needed
- Contributes to mitigating the effects of floods and droughts

1.5.2. European legislation related to drinking water quality

The 3rd of November of 1998 the European Union approved the Directive 98/83/CE [34] related to the water destined to human consumption, which contains the requirements that drinking water should fulfill inside the European Union. This Directive was born to upload the last Directive 80/778/CEE [35] which was built in 1980. The objective of it is to ensure the quality of drinking water and therefore, to protect human health from adverse effects of any contamination.

The scope of the Directive is water for human consumption, excluding mineral water (Directive 80/777/EEC) and medical products (Directive 65/65/EEC). The Directive applies to water with a minimum volume of 10 m^3 or to water destined for at least 50 people.

This directive establishes the basic quality standard at European level, including in it the analysis of microbiological, chemical, indicator and radioactivity parameters. However, each member of the EU can adapt it, only including, not eliminating, other parameters when they are relevant for the country. This is called the principle of subsidiarity.

According to the Drinking Water Directive [34], a water intended for human consumption is cleaned when:

- It does not have any microorganisms, any parasites or other substances that constitute a potential danger for human health
- Fulfill the minimum requirements present in the directive regarding the microbiological, chemical and indicators parameters

The directive also indicates requirements about planning, regulation, monitoring and information, and reporting. Each EU state have to provide regular information to the consumers. The analytical reports of drinking water have to be sent to the European Commission every three years to evaluate and analyze possible improvements [36].

Each member state has the responsibility to control that the directive is being fulfilled, and cannot allow that the water for human consumption might get degraded regarding its quality.

The directive establishes a maximum allowed amount of each pesticide and the maximum allowed amount for the sum of all the analyzed pesticides. Each individual pesticide cannot be present in water for human consumption in concentrations above 0,10 µg/L. The sum of total analyzed pesticides cannot exceed 0,5 µg/L [34].

The 6th of October of 2015, a review of the annexes of the Drinking Water Directive, was published, updating the previous document (Directive 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption). The drinking water directive also provides the allowed uncertainty of measurement for each analysis. The maximum allowed uncertainty associated with the analysis of pesticides is 30%. Also, trueness, precision and limit of detection are set up, being 25% in the case of pesticides, for all of them [37].

1.5.3. Legislation relative to compounds of interest

The first list of priority substances was elaborated and included in the Water Framework Directive, as the annex X, in 2001. The list was done taking into account the compounds that can show a significant risk to water environment. In 2008, the Directive 2008/105/EC on Environmental Quality Standards (EQSD) [38], replaced this list, establishing the environmental standards for water, and designing a classification of priority substances or priority hazardous substances for the listed compounds.

The EQSD identify 33 priority substances, 11 of them marked as hazardous priority substances. The directive also includes the possibility of using EQS for sediments and biota, instead of those for water. The EU member states have to control the emissions, discharge and losses of the substances mentioned on the list. Finally, the directive established the 13th of January of 2011 as a deadline to review the directive.

This revision added 15 new priority substances, 6 of them as priority hazardous substances, revised some environmental quality standards (EQS) for some of the existing substances, and established others actions related to monitoring and future reviews.

Recently, a new update of the legislation of priority substances has been done, amending the EQSD and the WFD. The new list and the information related to this compounds were unified in the Directive 2013/39/EU of the 12 of October 2013 regarding priority substances in the field of water policy [3]. In this last review, when most of the pesticides of this study were added [39].

1.5.4. Portuguese legislation related to water quality

Portugal is a European country belonging to the European Union since 1986, so it has to comply all the legislation established by the EU.

The 1st of August of 1998, the Portuguese legislation related to surface and ground waters destined to the production of water for human consumption was published. The document is the *Decreto - Lei nº 236/98* [40], and establish the standards, criteria, and quality purposes of water, trying to protect the aquatic environment and to assure the quality of water.

The 27th of August of 2007 the new Portuguese legislation for drinking quality water, *Decreto – Lei 306/2007* [41], was created. This document updated the *Decreto - Lei nº 236/98,* and it is the Portuguese law equivalent to the Drinking Water Directive.

2. Empresa Portuguesa das Águas Livres, EPAL

EPAL, Empresa Portuguesa das Águas Livres, S.A. is the successor of the Companhia das Águas de Lisboa, that was the company responsible of supplying water to the city of Lisbon since 1968 to 1974. After 1974, EPAL, named as Empresa Pública das Águas de Lisboa, was set up. In 1991, EPAL was converted into a private company with exclusively state capital. Then, it changed its name to Empresa Portuguesa das Águas Livres and it was included in the Grupo AdP – Águas de Portugal SGPS, SA [42].

The mission of EPAL, is to design, built, operate and manage the water supply system under its responsibility, within the applicable legislation. The objective of EPAL is to improve the quality of citizens´ lives and to enhance the social and economic development of the places in which it operates [43,44].

EPAL provides water approximately to 3 millions of people, all over 35 municipalities of the north region of the Tagus River, covering a total area of 7090 Km^2. In Lisbon, it is responsible of providing a door-to-door water supply to 564000 people [43,45].

The water supply system of EPAL involves abstraction, transportation and distribution. EPAL is responsible for the abstraction of 218 million of m^3 of water per year, from 3 main points, Zêzere River (Castelo de Bode, 70,9 %), Tagus River, (Valada do Tejo, 20,9 %) and several wells (Valada, Alenquer, Ota and Lezírias, 8,2 %) [43].

The abstracted water is treated from the moment it is taken until its distribution to the consumer. The water of Castelo de Bode is treated in the water treatment plant of Asseiceira. This treatment process includes pre-chlorination (when it is needed), re-mineralisation, flocculation, coagulation, clarification by the removal of suspended matter by dissolved air flotation (DAF), filtration, pH adjustment and finally disinfection using chlorine, leaving a residual of it in drinking water.

Water from Tagus River is treated in the water treatment plant of Vale da Pedra. This treatment process includes pre-chlorination, flocculation, coagulation, decantation,

intermediate chlorination, filtration, pH adjustment and disinfection with a final chlorination, leaving a residual chlorine in drinking water.

Groundwater are just disinfected using chlorine, except in one case, where the disinfection is carried out using sodium hypochlorite. In some cases, it is also needed to decrease water hardness and its alkalinity [46].

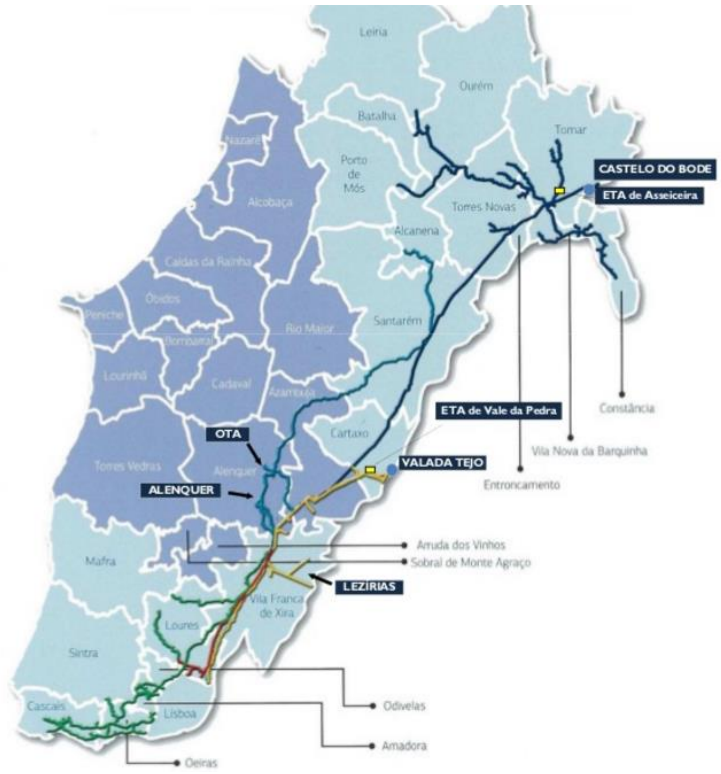


Figure 11. Water treatment plants and abstraction points [46]

The quality management system of the supply water system used by EPAL has a Plan to Control the Quality of Water, PCQW (PCQA in Portuguese). That plan controls that all the quality parameters defined by the legislation are fulfilled. The plan establishes different sampling points, from the abstraction to the distribution. There are 239 sampling points along the water supply system, 181 in the distribution network of Lisbon and 57 along the water pipe system that connect the catchment areas to the city of Lisbon.

The Water Analytical Laboratory is responsible for monitoring the quality of water along all the supply systems, from the abstraction points to the consumers' tap. This Laboratory also provides analytical services to others companies.

EPAL is certified for the supply of water for human consumption, including its collection, treatment, storage, transport, distribution, supply and related activities developed in EPAL's catchment area, by the Portuguese organism of certification (APCER, Associação Portuguesa de Certificação) in:

- Environmental Management, NP EN ISO 14001:2004
- Quality Management, NP EN ISO 9001:2008
- Occupational Health and Management, NP 4397:2008

The Water Analytical Laboratory is also an accredited laboratory since 1998 by the Portuguese accreditation organism, IPAC (Instituto Português de Acreditaçao). It is accredited for the ISO/IEC 17025, regarding the requirements for the competence of testing and calibration laboratories, to analyze 171 parameters/species, including the collection of samples.

The scope of accreditation is made up of a:

- Flexible scope, for the analysis of organic compounds using different chromatographic techniques (Annex 1).
- Fixed scope, for the analysis of inorganic and microbiological parameters, mainly. This scope also includes the accreditation for water sampling.

Annually, the Laboratory of EPAL participates in proficiency tests, to assure the continuous quality of its results.

3. Analytical methodology

The analysis and monitoring of water quality requires advanced and reliable analytical methods, because of levels of µg/L should be achieved. The analytical procedure begins with the sampling step and ends with the data analysis and report, but there are other important steps during the procedure such as sample preparation, extraction and analysis.

In the following study, all the steps were covered except sampling, even though it is a really important point, because it is where many errors can be done. Sampling is usually carried out by specific technicians, that take samples in the more possible representative way.

3.1. Sample preparation

The methodology of the analysis always depends on the kind of sample. In some cases, the sample can be analyzed directly in a chromatographic column with a detector. This is called direct analysis, but is not very usual because it needs a very specific kind of sample (enough cleaned and concentrated). In most of the cases a sample preparation, with and extraction and concentration step is needed. Sample preparation is one of the most important steps in the analytical procedure, because it requires around 60% of the time spent for the whole analysis [47].

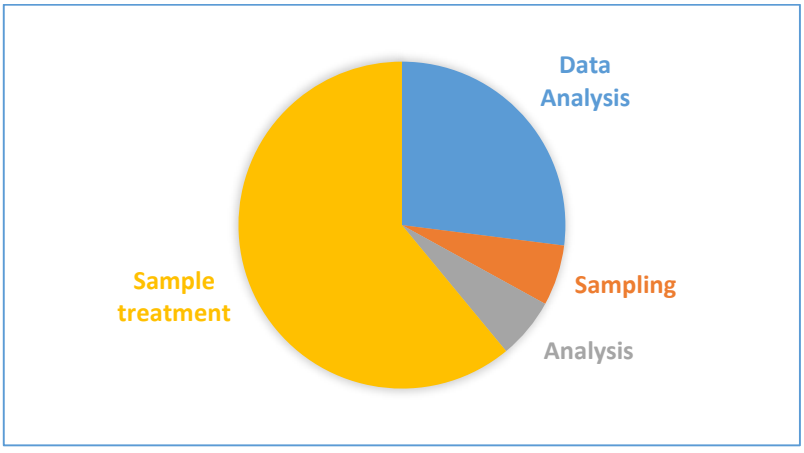


Figure 12. Distribution of time required for each step during the analytical procedure (adaptation from [47]*)*

For this reason, people are really interested in fast and automated sample preparation methods. Sample preparation is also really important because it has a big contribution to the total error, so it has to be controlled, and wherever possible, its automation can contribute to minimize the error.

The main objective of the sample preparation is to transfer the analytes of interest from the original matrix to a more adequate matrix in the most concentrated form possible, in order to being able to introduce them into a chromatographic column [47].

Pesticides are present in water in a low concentration so for this reason sample preparation is needed to concentrate and to eliminate other compounds that might interfere in the following separation and analysis steps.

3.1.1. Extraction methods

Extraction of an analyte from a solution A, consist in bringing the solution A into contact with a solution B, being phase A and B immiscible. The analyte distributes between both phases in a determine ratio, depending on its solubility in each solvent [48]. The equation 3.1. describes this equilibrium theory, being (s) a solute that can be distributed between A and B phases.

$$s_A \rightleftharpoons s_B \qquad \textit{Equation 3.1}$$

The distribution of the solute between phases is given by a thermodynamic equilibrium constant, called distribution coefficient (K_D):

$$K_D = \frac{a_S^{\beta}}{a_s^{\alpha}} \qquad \textit{Equation 3.2}$$

where α is the solute activity in each phase. The activity is defined by:

$$a_s = [s] * \gamma_s \qquad \textit{Equation 3.3}$$

where [s] is the solute concentration and γ_s is the activity coefficient of the solute.

The equilibrium constant expression, which follows a Nernst distribution law is:

$$K = \frac{[X]_B}{[X]_A} \qquad \textit{Equation 3.4}$$

being B always in the numerator of the equation and representing the concentration extracted in the new solution. The ideal situation is to have a K as large as possible, meaning a high degree of extraction from phase A to phase B.

Another important parameter is the selectivity (β) between analytes, especially when more than one analyte has to be extracted. The selectivity can be defined as the ratio between two distribution constants (k), and has to be higher than 1 to achieve a good extraction of both compounds.

$$\beta_{i,j} = \frac{K_i}{K_j} \qquad \textit{Equation 3.5}$$

Extraction methods are one of the most used sample treatment procedures in analytical chemistry. They are really important because:

- Increase the selectivity of the chromatographic methods by removing interferences
- Concentrate the sample, producing better limits of detection and quantification
- The chromatographic column suffers lower degradation, because undesirable compounds where removed
- It is completely needed with solid samples

There are many different methods that could be chosen to carry out an extraction procedure. The chosen one, will depend on the nature of the sample, matrix, time required, recoveries and many other characteristics that have to be studied in advance. However, most of the new methods are focused on minimizing the extraction time, using the less solvent possible, and trying to maintain a good recovery.

3.1.1.1. Liquid-Liquid Extraction (LLE)

The LLE has been the most used extraction technique in the past years, because it is really easy to use and its based in the solubility of the compounds in the extraction solvents, mainly. The phases are always two liquids, usually an aqueous phase and an organic phase, that are not miscible. The analyte is in the aqueous phase, and it is extracted by the organic

phase because its better solubility in it. This technique has been mainly used to extract organic compounds from water samples.

The most important characteristic to take into account before the extraction is the kind of solvent used. Good selectivity and efficiency must be achieved between two immiscible solvents [49]. There are some characteristics that a good solvent has to fulfill:

- Low solubility in the aqueous phase
- High volatility for its later concentration by evaporation
- Compatibility with the chromatographic analysis
- Polarity and hydrogen-bonding properties to improve the extraction recovery

LLE can be done in more than one step, increasing the efficiency. If a single step LLE is carried out, K_d >10 is needed to achieve a good recovery (>99%). However, the recoveries improve when two or three successive extraction with fresh organic solvent are done. There are other ways to increase the efficiency, for instance, varying the pH or adding a salt. The equation 3.6 explains the amount of extracted compound after successive multiple extractions:

$$E = 1 - \left[\frac{1}{(1+K_D V)}\right]^n \qquad \textit{Equation 3.6}$$

where V is the ratio between volumes and n is the number of extraction steps.

There are two different approaches for LLE, discontinuous and continuous LLE. In the first one, the equilibrium between phases is established, and in the second one, the equilibrium is never achieved [49].

Discontinuous LLE is carried out usually with a separatory funnel in which the aqueous sample is mixed with the organic solvent (volume depends on the number of extraction steps). The funnel is shaken to enhance the transfer of analytes to the organic phase. The equilibrium is reached and the phases are separated by density. This procedure can be repeated more times with fresh solvent to reach a better extraction [50].

The continuous extraction is usually used when the kinetics of the discontinuous LLE is not good, because K_D is small. Continuous extraction is also used for large amounts of aqueous sample. In this kind of LLE, there is always fresh solvent being introduced into the extraction tube that contain the sample. The organic solvent is heated and evaporated, then is condensate and re-introduced in the extraction tube. This method is more time consuming, but it achieves really good recoveries [49].

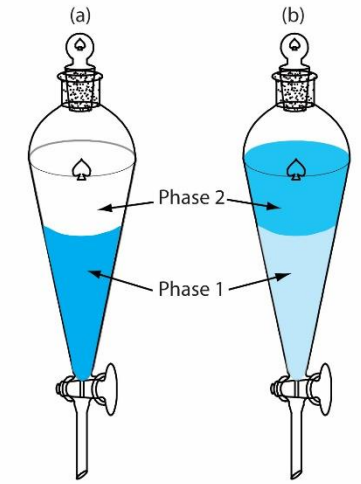


Figure 13. LLE before and after the equilibrium [50]

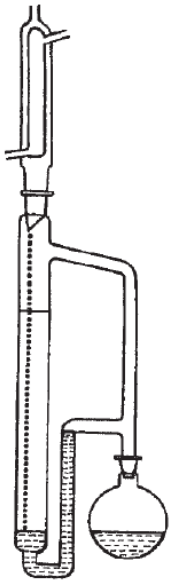


Figure 14. Continuous LLE (organic solvent heavier than water) [49]

LLE in both of the cases are a good method to extract compounds and gives very good recoveries, in fact, in many cases LLE is the reference method that new methods use to compare its recoveries. The main problem of LLE is that is a time consuming method and uses large amount of organic solvent.

3.1.1.2. Solid Phase Extraction (SPE)

Solid phase extraction is one of the most important sample preparation technique used in analytical chemistry for isolation, enrichment and clean-up of components in water samples [49]. It was born in the mid-1970s, and introduced in a laboratory in 1978. Nowadays, is a really common technique and disks and cartridges are available from many suppliers [51].

It is based in four mainly steps [52]:

1. Conditioning: The cartridge containing the sorbent is wetted and rinsed with the elution solvent. After that, water is passed to remove eluting solvent. This increase the reproducibility retention
2. Retention: The aqueous sample pass through the sorbent and the compounds are retained. Also, non-desirable compounds can be retained
3. Wash: The non-desirable compounds are eluted with a specific solvent (mix water/solvent)
4. Elution: The compounds of interest are eluted and collected by a suitable solvent. Many solvents can be used to elute more than one analyte

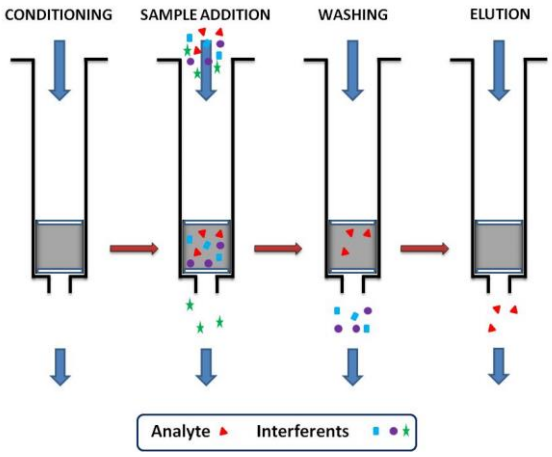


Figure 15. Solid Phase Extraction steps [52]

SPE solves many problems associated with LLE, such as an incomplete phase separation, less-quantitative recoveries, time consuming and use of large amounts of solvents. SPE is more efficient and can be easily automated [53].

The more important variables to take into account are the choice of the sorbent and the solvent used. The sorbent should be able to retain your compounds of interest in preference to the other undesirable compounds and the solvent has to elute them after the retention [49].

Sorbent can be divided in three main groups: normal phase, reverse phase and ion exchange phase. SPE and especially SPE with a resin-loaded membrane is used in

environmental analysis to determine low concentration of pesticides. This process is carried out in two main steps, (1) an effective extraction of the sample pesticides in which is important the sorbent and also the nature of the water sample, and (2) a desorption of the extracted analytes followed by a chromatographic analysis [54].

3.1.2. Clean-up and concentration

Sometimes, after the extraction procedure is still needed a clean-up or a concentration step. The clean-up consists mainly in an isolation of the analyte of interest from other possible compounds present in the matrix that the extraction could not eliminate [48].

Another sample treatment step that can be carried out is the concentration step. After the extraction, the extract is usually concentrated to reach a concentration of analyte that can be detected in the following analysis. However, sometimes is needed an extra concentration step to achieve this goal.

3.2. Analysis of the sample

The analysis of known or unknown compounds is sometimes a hard goal to achieve because of the complexity of some samples. For this reason, most of the people mainly carry out the analysis using chromatographic techniques coupled with a detector like Flame Ionization Detector (FID), Electron Capture Detector (ECD) or the widely used, Mass Spectrometer (MS). When the aim of the analysis is to determine non polar compounds, gas chromatography (GC) would be the best choice, because the separation is more influenced by the different temperatures of volatilization. In the other hand, with polar compounds, liquid chromatography (LC) can achieve better separation, dividing the compounds based on its polarity. Chromatography is a widely used technique in food control, food industry, in medical and pharmaceutical companies, in environmental studies, in cosmetics, fuel industries, etc.

In the analysis of pesticides, the most used methods are the gas chromatography coupled with the mass spectrometry (GC-MS) ([55][2][56][57][58][59]) and liquid

chromatography with mass spectrometry (LC-MS) ([57][60]. However, there are other techniques that can be applied to analyze pesticides, as electrochemical methods [61] or chromatography coupled with nitrogen phosphorus detector (NPD), in the case of organophosphorus pesticides [62].

The pesticides of study are both, polar and non-polar compounds and also volatile, so the method chosen will be the gas chromatography with a mass detector. The choice of gas chromatography instead of liquid chromatography allows the simultaneous analysis of polar and non-polar compounds, because it is only required a good volatilization of them.

3.2.1. Chromatographic process

The chromatographic process is based on the separation of the analytes due to its affinity between two phases, a stationary phase and a mobile phase.

Chromatography is built in two fundamental processes, one chemical that is the phase distribution and other physical, the diffusion. Diffusion is the migration - caused for a random movement of the molecules - of a particle from a region of high to a region of low concentration. It increases with high temperatures and decreases with the mass of the molecules and density. The chemical process is the distribution of the molecules between two phases in contact, due to their chemical interactions with the phases. It is always a continuous exchange of analytes between phases, until they achieve the equilibrium and make the concentration in each phase constant.

3.2.1.1. Gas chromatography

Gas chromatography is a very powerful and one of the most used techniques for analysis. It provides qualitative and quantitative information about the analytes present in a sample, after their separation. The compounds are separately due to the differences on its volatility and molecular structures, and for this reason many compounds are not suitable for gas chromatography. For a compound to be able to be analyzed by GC, it has to possess good volatility, under 350-400^{o}C, and has to be stable under high temperatures without

degradation or reaction with other compounds. It has been estimated that only 10% of all compounds can be analyzed using GC [63].

In order to carry out a separation using GC, a small amount of sample is injected in the gas at high temperature. An inert gas crosses the chromatography column in which the analytes are going to be separated due to the different volatility and affinity of each compound in the chromatographic column.

A gas chromatographic equipment is made of five principal components:

1. **Gas supply and flow controllers**: The pumps and the gases that are supplied to allow the sample to pass through the column. It is really important to regulate the pressure controlling the amount of gas that it is introduced in the column.
2. **Injector**: The injector is responsible for the introduction of the sample into the column. The most common is the split-splitless injector. With this injector, there are two possible modes to introduce the sample into the capillary column [49,64]:
    - Splitless injection mode: The sample is introduced with a syringe and rapidly evaporate at high temperature. The carrier gas is added to sweep the sample into the column. At some point, after the analyte is already transferred to the column, the split valve is opened to empty the injector.
    - Split injection mode: The first part of the injection is the same. The syringe introduces the sample in the injector where it evaporates. Then the carrier gas is added through the inlet gas supply with the split valve of the injector opened, taking part of the carrier gas. The proportion between the inlet gas flow and the split line flow is usually 1%. In this case, only a part of the sample is introduced, so it is not a good option in trace analysis. In the other hand, the split injection eliminates non-volatile compounds and ensure that they do not enter into the column. It also gives a better efficiency due to the decrease of the width of the peaks. The inlet residence time of the analyte is also reduced, so it implies less degradation.

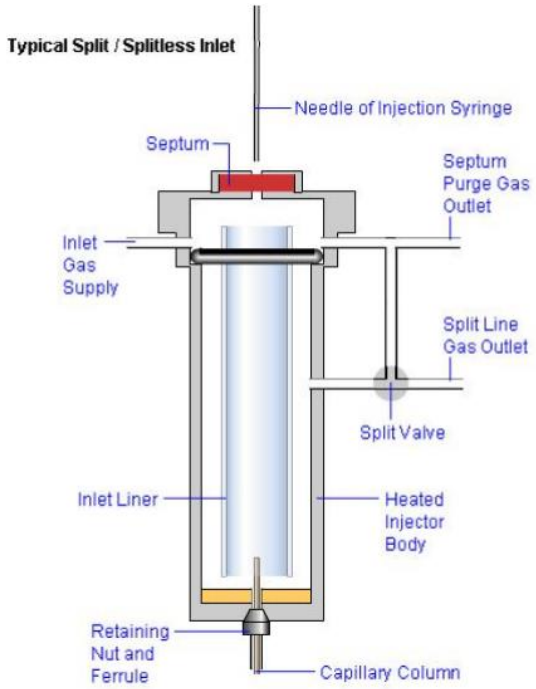


Figure 16. Draw of split/splitless injector [64]

3. **Capillary column and oven:** The column is responsible for the separation of the analytes of the sample. It resides inside an oven, in which the temperature is accurately controlled. The capillary column is made up for a thin film of polymeric material coating it, called the stationary phase, that interacts with each compound according to the different chemical properties of them. The stationary phase is made of substituted polysiloxanes. The stationary phase has to be resistant to high temperatures for prolonged times and be non-reactive. The thickness of the stationary phase, the column dimension and the diameter of the column are parameters that must be taken into account to achieve a good separation.
4. **Detector:** The compounds achieve the detector, interacting with it based on chemical or physical properties. This interaction is transformed into an electrical signal, that can be recorded and analyzed. There are detectors that interact with every compound, and other that are specific to some compounds. Nowadays, there are many detectors available in the market, but the most common are the Flame Ionized Detector (FID), Electron Capture Detector (ECD), and the Mass Spectrometer (MS).

5. **Data collector:** A device that record the data sent by the detector. It receives the data and usually plots the signal versus the time. The result is called chromatogram and appears as a series of peaks.

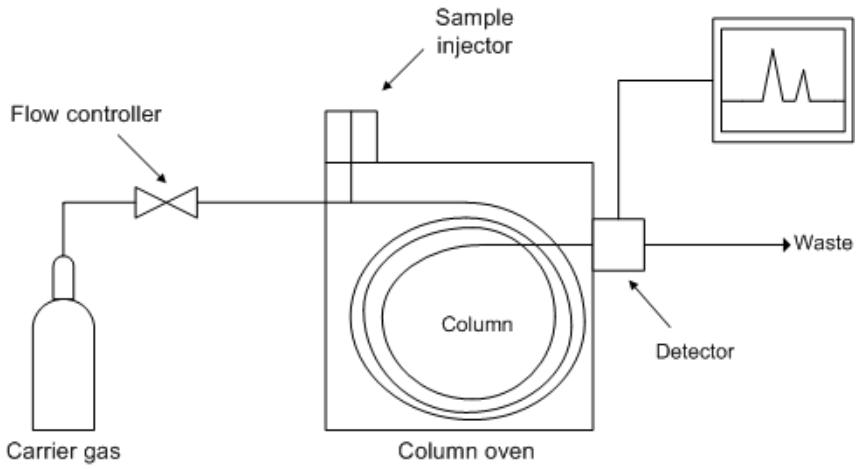


Figure 17. Diagram of a GC equipment

3.2.1.2. Gas chromatography parameters

Resolution is a parameter that represent how good two peaks (A and B) are separated. It is defined as the difference in retention time (t_R) divided by the average peak width at baseline (w_b) [65].

$$R_S = \frac{\Delta t_R}{\bar{w}_b} = \frac{t_{R(A)} - t_{R(B)}}{0{,}5(w_{b(B)} + w_{b(A)})} \qquad \textit{Equation 3.7}$$

A resolution of 1,5 is usually enough to achieve good accurate quantification. The resolution depends on three main factors:

- Chromatographic retention

The retention of each compound by the stationary phase. Retention also depends on the oven temperature. The retention constant is calculated with the retention time of each compound divided the holdup time (time an unretained analyte (k=0) uses through the column) [65].

$$k = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M} \qquad \textit{Equation 3.8}$$

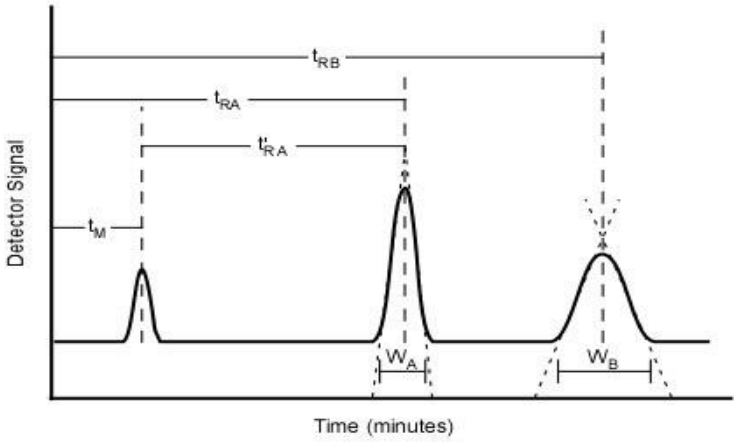


Figure 18. Chromatography retention times

- Chromatographic selectivity

The chromatographic selectivity is a measure of the difference between the retention factors of two different compounds. It is measured by the separation factor, α.

$$\alpha = \frac{K_B}{K_A} > 1 \qquad \textit{Equation 3.9}$$

- Chromatographic efficiency

The chromatographic efficiency is a measure of how much the peaks spread (w) compared to the amount of time they spend in the column (t_R). The efficiency is measured by the number of theoretical plates, N [65].

$$N = 16\left(\frac{t_R}{w_b}\right)^2 \qquad \textit{Equation 3.10}$$

High retention times and narrow peaks means a high number of theoretical plates, giving a good efficiency. Plate height (H) is related with the length of the column (L) and the number of theoretical plates (N).

$$H = \frac{L}{N} \qquad \textit{Equation 3.11}$$

These three factor are summarized in the Purnell equation, that shows their contribution to the resolution. Purnell equation is used to calculate the resolution between two peaks.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k_B}{k_B+1}\right) \qquad \textit{Equation 3.12}$$

where k_B is the retention factor of the most retained compound.

Understanding how these three factor change, chromatography retention can be explained.

The first factor, increase proportionally to the square root of the number of theoretical plates. That means, to double the resolution, the number of plates has to increase four times. The number of plates depends on the length of the column and on diffusion parameters. Increasing the resolution with the efficiency will require a change in the chromatographic column [65].

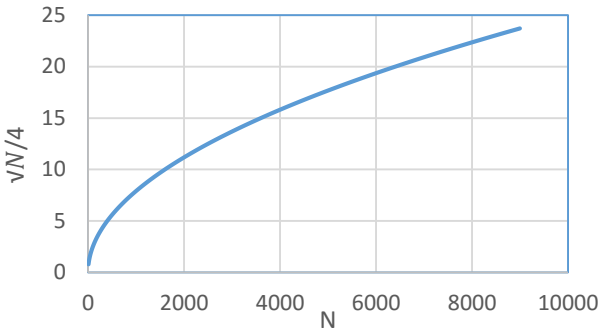


Figure 19. Factor 1 of the Purnell equation. Efficiency

The second factor is selectivity, that depends on the chemical properties of each phase and compounds. However, it is hard to increase the resolution altering this factor [65].

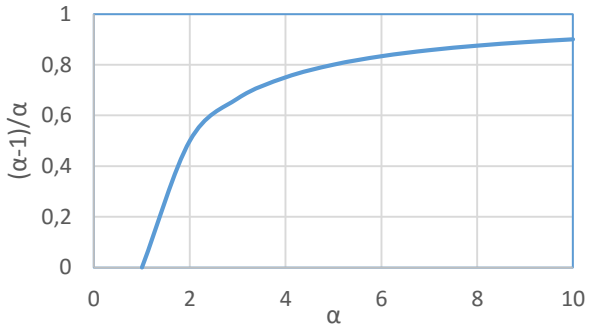


Figure 20. Factor 2 of Purnell equation. Selectivity

The third factor of the equation is the easiest to control because depends on the chemistry of the stationary phase and on the dimensions of the column. In theory, k should be as higher as possible, but it can be observed in the figure 18, that after k=2, the factor increases slowly [65].

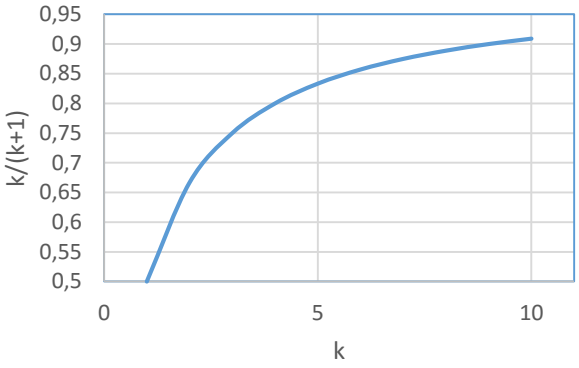


Figure 21. Factor 3 of Purnell equation. Retention

As it is explained above, the theoretical plate is related with the retention time and the peak width and can be calculated also with the column length and the plate height. The number of plates in a chromatogram is not similar for each peak, even if the peak width increases when the retention time increases. There are some factors that affect the height plate, and they are expressed in the Van Deemter equation [65].

$$H = A + \frac{B}{u} + C * u \qquad \textit{Equation 3.13}$$

The first factor of this equation, A, is related to the different paths that one molecule can take through the stationary phase. It does not depend on the mobile phase and does not affect the capillary columns [66].

The second factor, B, is affected by the longitudinal diffusion, which leads with the diffusion of molecules from high to low concentrate regions in the stationary phase. The result of a high term B is the broadening of the peaks. When the analyte spends more time in the mobile phase, much higher will be the effect of the B term, because diffusion is much higher in the mobile phase than in the stationary phase. This term varies inversely proportional to the mobile phase velocity (u) [66].

The third term, C, explains the resistance of the molecules to mass transfer between the mobile and the stationary phase. This term is also related with the phase mobile velocity, increasing proportionally to it [66].

With terms B and C of the Van Deemter equation, it is possible to calculate an optimum mobile phase velocity.

$$u_{opt} = \sqrt{\frac{B}{C}} \qquad \textit{Equation 3.14}$$

Figure 19 shows the contribution of each term to the mobile phase velocity and the height plate, giving a good view of how they vary and where is the optimal velocity.

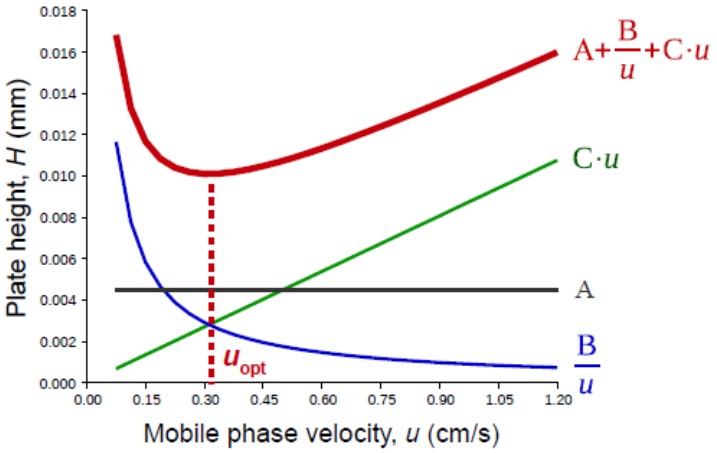


Figure 22. Van Deemter equation, contributions of each term

The length of the column has a big influence in the Purnell equation, the retention factor k and the number of plates (efficiency) N are related with the column dimension. The selectivity factor α is affected by the column dimension and the chemistry of the compounds [67].

The phase ratio is a parameter that relates the volume of the stationary phase with the volume of mobile phase. The phase ratio can be also calculated with the internal diameter and the film thickness [67].

$$\beta = \frac{V_M}{V_S} = \frac{0{,}25\, d_C}{d_f} \qquad \textit{Equation 3.15}$$

A high value of the phase ratio will give low retention, meaning that the mobile phase is much larger than the stationary phase. The diameter in GC columns is typically 1000 times the film thickness, so a typical phase ratio is around 250.

The distribution constant, K_c, is a parameter that relates the concentration of mobile and stationary phase [67]. This can be related with the retention factor k, assuming that the molecular mass of the analyte is the same in both phases:

$$K_c = \frac{[A]_s}{[A]_m} = k \cdot \beta \qquad \textit{Equation 3.16}$$

In order to achieve good separation, there are some parameters that can be controlled:

- The column length: Increases the number of plates, N and the retention factor, k

$$N \propto L; N \propto k \qquad \textit{Equation 3.17}$$

- Internal diameter of the column: The retention factor decreases when internal diameter increases

$$k \propto \frac{1}{d_c} \qquad \textit{Equation 3.18}$$

- Thickness of the stationary phase: The retention factor k increases proportionally with the thickness.

$$k \propto d_f \qquad \textit{Equation 3.19}$$

The whole theory of the chromatography, explained above, is based in a constant retention factor k, but in real cases that is not true. The analytes can interact between them - competing for the stationary phase or interacting between molecules - or the chromatography conditions can change deliberately during the run. That produces asymmetrical peaks, giving tailing peaks when it is due to competition for the stationary phase and fronting peaks when the molecules interact between them [68].

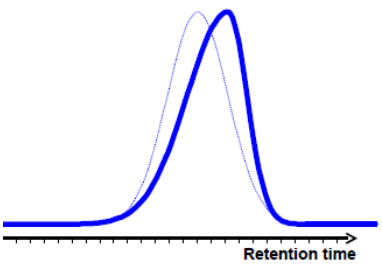


Figure 24. Fronting peak

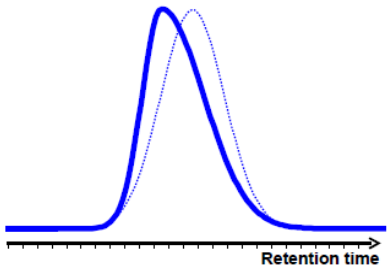


Figure 23. Tailing peak

3.2.2. Mass spectrometry

Mass spectrometry is one of the most used and versatile detectors in analytical chemistry for the identification and quantification of organic substances in complex matrices, due to its high sensitivity, detection limits, speed and diversity of its applications [69]. Mass spectrometry is an analytical technique that identifies the chemical composition of a molecule based on the mass-to-charge ratio (m/z) of charged particles. It is widely used in many different fields, as in pharmaceutical industry, drugs analysis, biochemistry, food analysis, environmental analysis, etc [55,57,58,62,70–72]. It gives structural information - because of the fragmentation of the ions – and the exact mass of the compound. For quantitative analysis, it can achieve limits of detection of ppt [73].

The principle of the technique is the ionization of the molecules, producing the fragmentation of them and detecting the mass of each ion.

The first step is to produce the gas phase ions of the compounds. This can be carried out by different mechanisms.

$$ABC + e^- \longrightarrow ABC^{*+} + 2e^- \longrightarrow (AB^+ + C^*)\ o\ (A + BC^{*+})\ o\ (A^* + BC^+)$$

As can be observed above, the molecular ion usually undergoes fragmentations because it is a radical cation with an odd number of electrons. It can fragment either a radical and an ion or a molecule and a new radical cation. All these ions are, lately, separated in the mass spectrometer, according with their mass-to-charge ratio and detected in proportion to their abundance [69]. The result mass spectrum has the ratio mass-to-charge in the x axis and the relative abundance of each ion in the y axis.

The mass spectrometer equipment is divided in 5 principal parts:

- **Vacuum pumps:** All mass spectrometers have to work under high vacuum conditions (low pressure), because it is necessary to allow ions pass through the system and reach the detector without having any collision with other gaseous molecules. These collisions may produce deviations of the trajectories, the ions could lose its charge by colliding with the walls of the instrument or unwanted reactions can happens [74].

That is which it is called ion free path, and the length and pressure of it should be at least 1 m and 1 torr, respectively. The pump system works in a gradually mode, to 10^{-3} torr at the beginning by the mechanical pumps, to 10^{-6} torr by the other pumping systems (diffusion and turbomolecular pumps) [69,74].

- **Ion source:** It is responsible for the ionization of the molecules before their analysis in the mass spectrometer. The ion source works transferring a certain energy to the molecule and causing the ionization and latter fragmentation of it. Techniques can be divided depending on the energy transferred, in hard ionization, causing extensive fragmentation, and soft ionization, producing only ions with the molecular weight of the compound.

  Some techniques are only suitable for a kind of sample, as electron ionization (EI) and chemical ionization (CI), that only work with gas-phase ionization, so are limited to volatile and thermally stable compounds. These techniques are the more used in gas chromatography. In the analysis of liquid samples, the analytes must be extracted directly from the liquid or solid to the gas-phase. Some of these direct ion sources are based on electric field evaporation, field ionization (FI), field desorption (FD) for solid samples, electrospray ionization (ESI), atmosphere-pressure chemical ionization (APCI) for liquid samples. Other ion sources, work by particle bombardment, as DESI (Desorption electrospray ionization) and DART (Direct Analysis in Real Time) in solid samples or FAB/FIB (Fast Atomic/Ion Bombardment) for liquids. There are other ion sources that can be used in some cases as APPI (Atmospheric Pressure PhotoIonization) and MALDI (Matrix-assisted Laser Desorption Ionization) used in the analysis of complex protein mixtures, carbohydrates and biochemical molecules [69,75].

  The ion source used in this work was an **electron ionization (EI)**, because of the high fragmentation, volatility and stable nature of the analytes. The mechanism of this ion source consists in a heated filament that produces electrons. These electrons are accelerated towards an anode and collide with the gaseous molecules of the analyte. The sample has to be always a gas, so samples with high vapor pressure and

gases can be directly introduced into the source. In the case of liquids and solids, they are heated to increase their vapor pressure [69]. The electron beam energy is determined by the potential used in the ionization source that is usually -70 eV, but it can vary from -20 to -100 eV. Almost all the formed ions are positive charge.

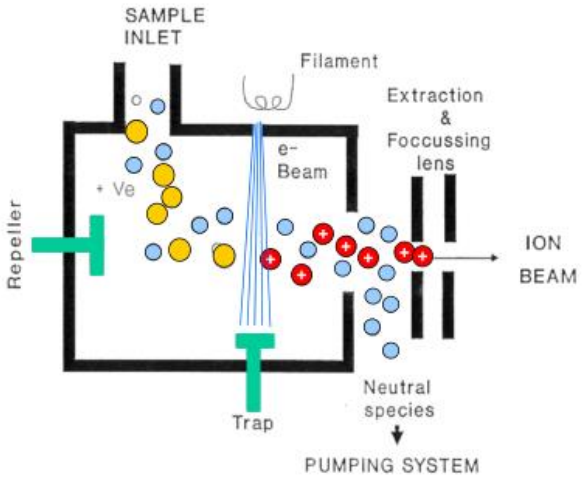


Figure 25. Electron Ionization Source [75]

This technique might provide structural information and the molecular weight, due to the big fragmentation of the analytes. This ion source cannot be used to analyzed isomers because the fragmentation path will be the same, and neither low volatile compounds.

- **Mass Analyzer:** After the production of the gas phase ions, they have to be separated according to their masses. The physical property that is measured is the mass-to-charge ratio (m/z). This separation is carried out by the mass analyzer, but the separation can be based on different principles depending on the kind of mass analyzer. Some of the most used analyzers are the quadrupole, ion trap, TOF (time of flight), FT (Fourier Transform) instruments or the more recent orbitrap. They can be divided in two groups regarding the resolution, being the quadrupole and ion trap tagged as low resolution, that means they are just able to differentiate between one unity of mass and the other group, TOF and FT, called high resolution analyzers, that can distinguish masses of 0.0001 unitis [76].

The mass analyzer used in this project is a **quadrupole**. It works separating the ions with oscillating electric fields according to the stability of its trajectories through the quadrupole. The trajectory of each ion depends on its mass-to-charge ratio (m/z). The quadrupole is made up of four hyperbolic or cylindrical rods (electrodes) in perfectly parallel position. Two of these rods are positive charge and the other two negative charge. The electrodes are linked to radio frequency (RF) and to direct current (DC) voltages. The combination of them creates a zone that focus and select the ions, called hyperbolic field. These two voltages, allow the selective transmission of the ions that enter from the ion source [77,78].

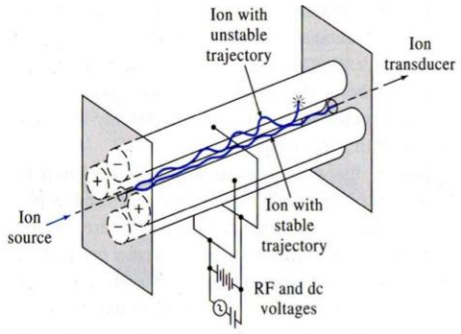


Figure 26. Quadrupole diagram [77]

Quadrupole analyzers can operate in full scan mode or in selected ion monitoring mode.

- Full Scan: The analyzer scans all the masses, one by one, inside a set range
- Selected ion monitoring: The analyzer only looks for certain ion masses (normally, 2 or 3 ions per peak). This mode increases the scan velocity and the sensitivity of the detector

- **Detector:** The function of the detector is to detect and transform the ions that arrive from the mass analyzer into analytical signals. They work generating an electrical signal from the incident ions, that is proportional to their abundance. Most of the detectors have to amplify the signal, before the detection, because the amount of ions that leave the mass analyzer is quite small. Indeed, the electrical signal that is produced by 10 ions per second arriving to the detector corresponds to an electrical current of $1.6 \cdot 10^{-18}$ A.

The detection is based on the mass, the charge or the velocity of the ions. Faraday cups detectors measure the direct charge current that is produced when the ions arrive, hit the surface and are neutralized. Electron multiplier or electro-optical ion (photomultiplier) detectors work generating secondary electrons from the kinetic energy transfer of incident ions that collide with a surface, which are latter amplified in a cascade effect [69].

The detector used in this study is an **electron multiplier detector**, so the ion collides with a surface and for this reason secondary electrons are released from atoms in this surface layer. There are two types of electron multiplier detectors that are used: a) the discrete-dynode electron multiplier, that has several dynodes that maintain and increase the potential resulting in a series of amplifications and b) the continuous-dynode electron multiplier, with a curved continuous dynode (horn shape) in which the amplifications occurs with several collisions with the dynode surface [79].

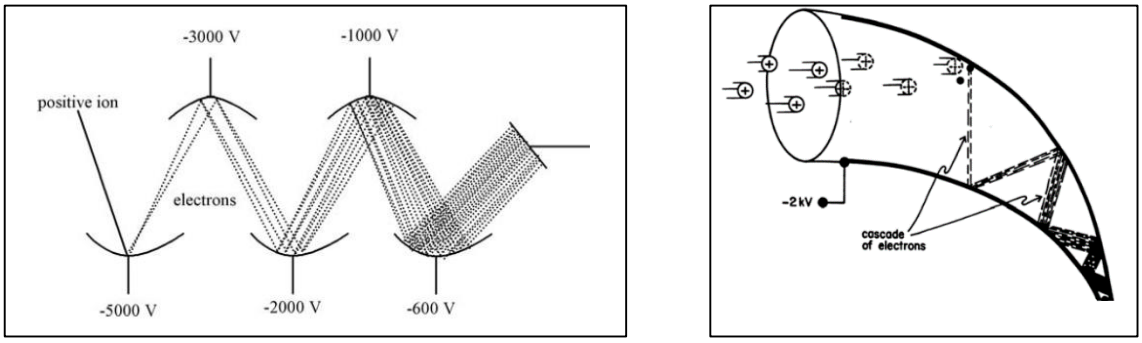


Figure 27. a) Discrete electron multiplier detector, b) Continuous electron multiplier detector [77]

- **Data system:**

Computer are always connected to the GC-MS equipment's, because it helps to analyze the obtained data. The main purposes of the data system, are:

1. Control of the mass spectrometer and the gas chromatography parameters.
2. Acquire and process the data from the gas chromatograph and the mass spectrometer: The data is given out and the computer converts it into values of masses and peaks intensities. It is also able to process data making some transformations.
3. Interpret and compare the data with mass libraries.

3.2.2.1. Tandem mass spectrometry

Tandem mass spectrometry is a variation of the traditional mass spectrometry which involves at least two stages of mass analysis. In tandem mass spectrometry, a first analyzer is used to isolate a precursor ion, which then undergoes a fragmentation usually caused by a collision gas that produces the product ions and neutral fragments. These product ions are analyzed by the second mass analyzer [69].

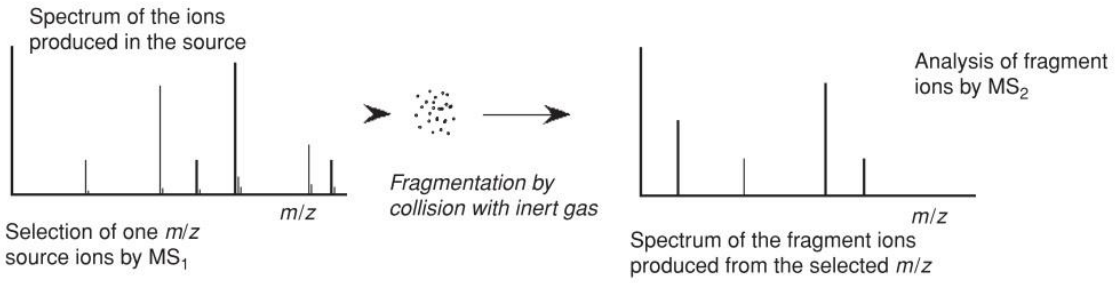


Figure 28. Tandem mass spectrometry principle [adapted from 69]

Tandem mass spectrometry can be performed in two different ways, using two physically distinct instruments coupled (Tandem mass spectrometry in space) or using only one instrument and performing several fragmentations in an ion storage device (Tandem mass spectrometry in time).

Time instruments can perform more than one fragmentation, taking the product ion and fragmenting it many times (MS^3). The most common time instruments are the ion-trap, orbitrap and FTICR (Fourier transform ion cyclotron resonance).

Space instruments have usually two analyzers, being triple quadrupole (QqQ) the most frequently used. Triple quadrupole detector is made up by three quadrupoles where the first one (Q) selects the precursor ion, the second one (q) is the reaction region and the third one (Q) analyze the product ions. However, there are other instruments that work in space tandem mass spectrometry, as can be the time-of-flight analyzer (TOF) that can work using a reflectron or in combination with a quadrupole (Q-TOF) [69,80].

Tandem mass spectrometry can work in different scan modes, product ion scan, precursor ion scan, neutral loss scan and selected reaction monitoring. The modes used in this study were:

1) Product Ion Scan (PIS): This mode consists of selecting a precursor ion of a chosen mass-to-charge ratio and determining all of the product ions resulting from a fragmentation with a certain collision energy.
2) Selected Reaction Monitoring (SRM): This method consists of selecting an ion to be fragmented, and analyze its selected product ions. Both quadrupoles are focused in the analysis of selected masses.

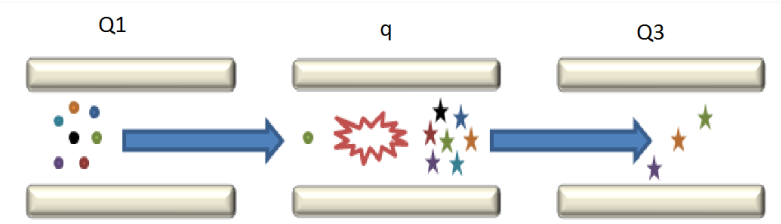


Figure 29. Selected reaction monitoring mode

Tandem mass spectrometry is actually used in many applications due to its high sensitivity in complex matrices, for instance it is very used in the analysis of proteins. It can also achieve low detection limits. The disadvantage of this mass analyzer is the high cost of the equipment. However, it is widely used for environmental, medical, food, proteins, cosmetics and pharmaceutical analysis [2,55–57,60,62,70,72].

3.3. Method validation

ISO/IEC 17025 specifies general requirements concerning method validation in laboratories. Laboratories shall use appropriate methods and procedures for all tests or calibrations within their scope. These requirements applied to sampling, handling, transport, storage and preparation of items to be tested or calibrated. [81]

Validation is defined by the ISO/IEC 17025 as *"confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled"* [81]. Method Validation can be explained as the process of defining an

analytical requirement, and confirming that the method of study has capabilities consistent with what the application requires [82].

Validation and verification are concepts that can be confused. A method can be validated, but when a laboratory wants to use and implement it, it is still needed to confirm the ability to apply the method, that is called verification.

Method validation plays an important role in the analytical measurements because reliable and accurate data are always needed. Method validation is used to judge the quality, reliability and consistency of analytical results, which are the base of any good analytical practice [83]. The laboratory that carries out the analysis has the responsibility to justify trust of the analytical measure. The method validation is the way to demonstrate that the method used fits to the purpose of the study and therefore, latter confidence decisions can be taken based on this result. It must always have a clearly uncertainty associated, at a determine confidence level. Besides the benefits explained above, carrying out a method validation also helps to understand and gain experience in all the practical details of performing the method, including awareness of any critical point of the process.

A method should be validated when it is needed to prove that its performance characteristics fits to a particular purpose. The validation shall be as extensive as is necessary to fulfil the needs of the purpose.

ISO/IEC 17025 establishes that a laboratory has to validate a method when it is going to apply [81]:

- non-standard methods
- laboratory-designed/developed methods
- standard methods used outside their intended scope
- amplifications and modifications of standard methods

There are different ways to validate a method, the interlaboratory comparison and the “in house” (single-laboratory) validation. When it is possible, the best procedure for method validation is the interlaboratory comparison, because it achieves good robustness,

precision and trueness. In this case, the method only has to be verified by the laboratory that wants to use it. That provides a full validation method of analysis, but sometimes it is not needed such performance to provide a method validation, in these cases, single-laboratory validation may be appropriate. Single-laboratory validation could be applied to ensure the viability of the method before the costly of an interlaboratory comparison, to provide evidence when interlaboratory trials does not exist for the method of study and to ensure that "off the shelf" methods are being used properly [81,82,84].

3.3.1. Range and linearity

Range is defined as the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [85]. The minimum concentration is usually above the limit of quantification of the chromatographic method.

The International Conference of Harmonization (ICH) defines **linearity** of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample [85].

Linearity should be validated using appropriate statistical methods, for example using the regression line by the least square method. Sometimes, before studying the linearity, results have to be mathematically transformed to achieve it [86].

The result of linearity is a regression line, with its regression equation and its plot, that is latter used to calculate the sample concentrations. For establishing a good linearity, a minimum of 5 concentrations points are required. Internal procedures of EPAL required at least 6 concentrations levels.

EPAL uses different methods to check linearity in its procedures [87]. In order to say that the method has a good linearity, a coefficient of determination higher than 0,99 (R^2>0,99), Mandel test, Residual test and RIKILT test should be passed.

1. Mandel test: According with the ISO 8466/1 [88], linearity can be validated using the Fisher-Snedecor test also called Mandel test. That test, only have to be used in the case of having homogeneity of variances in the working range. The test is based on the comparison of the linear regression model with a non-linear, second order function, model. The test assumes that large deviations of the linear regression are caused by a non-linearity and can be reduced fitting the data to a second order regression.

   a) In order to compare both regressions, $s_{y/x}$ and s_{y2} are calculated,

$$S_{y/x} = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \bar{y}_i)^2}{N-2}} \qquad \textit{Equation 3.20}$$

$$S_{y2} = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \overline{y_{i2}})^2}{N-3}} \qquad \textit{Equation 3.21}$$

   where:

   $S_{y/x}$ – Residual standard deviation for linear regression

   S_{y2} – Residual standard deviation for second order regression

   N – Number of concentration points

   y_i – obtained signal for a concentration i

   $\bar{y}_i$ – estimated signal for the linear regression

   $\overline{y_{i_2}}$ – estimated signal for the second order regression

   b) Calculate the difference between variances (DS^2)

$$DS^2 = (N-2) \times S_{y/x}^2 - (N-3) \times S_{y2}^2 \qquad \textit{Equation 3.22}$$

   c) Calculate the test value, TV

$$TV = \frac{DS^2}{S_{y2}^2} \qquad \textit{Equation 3.23}$$

   d) Compare the test value, TV, with the tabulated value from Fisher-Snedecor table, according with the degrees of freedom.

- If TV ≤ F: The function fits better the linear regression than the non-linear regression.
- If TV > F: The function fits better the non-linear regression, so the range should be reduced in order to achieve a better linear regression.

2. Residual analysis: Another approach to verify the linearity is the study of the residuals. The residuals are the vertical distance from the experimental values to the theoretical values given by the regression line. The residuals should be distributed randomly along the regression line in the case of good linearity. The residuals are plotted, and should be all between a specific interval of confidence.
3. RIKILT test: The objective of the RIKILT test is to investigate if the calibration could be done using a response factor instead of a calibration curve. The ratio y_i/x_i have to be calculated for each concentration, giving a percentage of 100% to the mean value. All the points shall fall into a specified percentage range (90-110 %) when compared with the mean value.

3.3.2. Limit of detection (LOD)

Detection limit of an individual analytical procedure is defined by the ICH as *"the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value"* [85].

Quantitatively, is the minimum amount that it is possible to distinguish from the blank. The LOD can be calculated with [87]:

- Signal-Noise ratio: By comparing measured signals from known low concentrations samples with blank samples, and establishing the minimum concentration at which the analyte can be detected. The value of LOD is 3 times the signal-noise ratio in concentration units.
- Residual Standard Deviation of the calibration: LOD can be calculated using the residual standard deviation of the calibration curve.

$$LOD = \frac{|3 \times s_{y/x}|}{b} \qquad \textit{Equation 3.24}$$

$s_{y/x}$ – Residual standard deviation of the calibration curve

b – slope of the calibration curve

- Fortified low concentration samples: Fortifying blank samples with known low concentrations standards, and measuring the deviations. Calculate the standard deviation of the fortified blanks, S_{xo}. The LOD can be calculated using this deviation.

$$LOD = 3 \times s_{xo} \qquad \textit{Equation 3.25}$$

3.3.3. Limit of quantification (LOQ)

Quantification limit of an individual analytical procedure is defined by the ICH as "*the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy*" [85].

The limit of quantification can be calculated in the same way as the LOD, but with some modifications:

- Signal-to-noise ratio: Multiplying the signal-noise value per 10, in units of concentrations.
- Residual Standard Deviation of the calibration: LOQ can also be calculated with the residual standard deviation of the calibration curve.

$$LOQ = \frac{|10 \times s_{y/x}|}{b} = 3 \times LOD \qquad \textit{Equation 3.26}$$

- Fortified low concentration samples: the samples are carried out in the same way as in the LOD.

$$LOQ = 10 \times s_{xo} \qquad \textit{Equation 3.27}$$

3.3.4. Precision

The ICH defines **precision** as *"the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions"* [85].

It is often expressed as the standard deviation, variance or coefficient of variation of a series of measurements.

$$S = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}} \qquad \textit{Equation 3.28}$$

where,

S– Standard deviation

x_i – individual value of each measurement

$\bar{x}$ – average value of measurements

n – number of measurements

The coefficient of variation (V_{xo}) can be calculated from the standard deviation,

$$V_{xo}(\%) = \frac{S}{\bar{x}} \times 100 \qquad \textit{Equation 3.29}$$

where,

S – standard deviation of the measurements

$\bar{x}$ – average value of measurements

Precision is divided into repeatability, intermediate precision and reproducibility.

3.3.4.1. Repeatability

Repeatability involves precision under the same conditions over a short period of time. It could also be called intra-assay precision.

3.3.4.2. Intermediate precision

Intermediate precision involves the same method in the same laboratory but in different days or analyst.

3.3.4.3. Reproducibility

Reproducibility examines the precision between laboratories and it is tested in collaborative studies.

3.3.5. Trueness

According to ICH, **trueness** is *"the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found"* [85]. It is an indicator of systematic errors.

Trueness can be determined in different ways [87]:

- Comparison of the results with Certified Reference Material
- Participating in proficiency tests
- Comparison with a reference analytical method
- Testing the recovery by adding a known amount of a standard into a blank sample

In this study, trueness was measured testing the recoveries by adding a known standard into different kind of water matrices.

3.3.6. Selectivity and specificity

The **selectivity** is the ability of the method to analyze particular analytes in a complex mixture, without interferences of other compounds in the mixture. When a method has a good selectivity it is called a **specific** method.

Selectivity has to be achieved in different kind of samples. In the case of this project, it was tested in ultra-pure water, tap water, surface water and groundwater. Studies of recovery are carried out in order to test the selectivity of the method.

3.3.7. Uncertainty

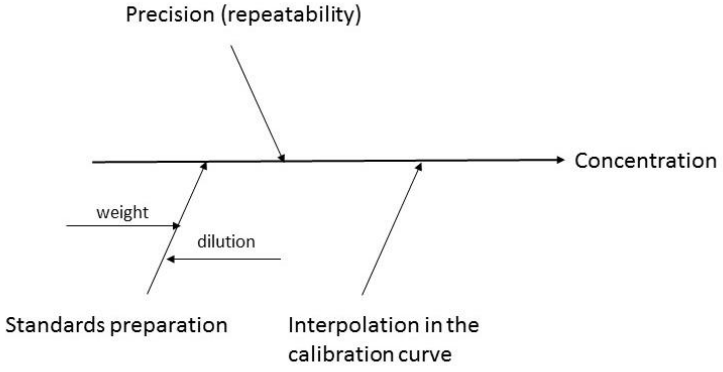
ISO 17025 established that any result has to be reported with its associated measurement uncertainty [81]. Every measurement implies certain error associated with the procedure, that affects the result. When a laboratory is giving a result, it means that is making an estimation of the true value of the measurand. This estimation must have always an uncertainty associated, which indicates the quality of the result. The uncertainty is calculated within a confidence interval, meaning the allowed interval where a true value falls [89].

Uncertainty may be caused by different sources, such as sampling, interferences in the matrix, environmental conditions, error due to the instruments, human errors, or dilution and weighting errors. Some sources of uncertainty should be taken into consideration to calculated the total uncertainty, others are negligible.

Calculate the uncertainty of a method is a useful tool to know the method deeply, to identify any possible error and to control every single step. The procedure to determine the uncertainty involves [90]:

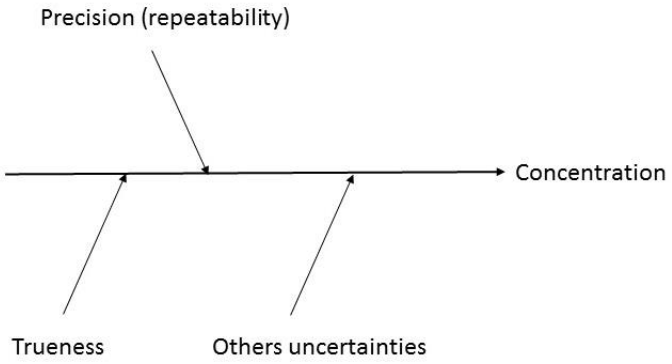
- The identification of every source of uncertainty and quantification of it.
- Convert every uncertainty component to standards deviations.
- Combined all the uncertainties and calculate the expand uncertainty according to the confidence level established.

In this study, the components that make up the uncertainty are taken from, the preparation of the standards, the standard deviation of the precision studies and the calibration curve. These three components build up the **bottom-up approach**, that involves the quantification and combination of all individual uncertainty components responsible for the occurrence of random and systematic effects on the measurement result.



Another approach to estimate the uncertainty is the **top-down approach** based on in-house validation data. This approach allows the estimation of the uncertainty using the data

collected during an in-house validation procedure. Trueness and precision are used to calculate the total uncertainty.



The combined uncertainty for the bottom-up approach is calculated using the following equation:

$$u_c = \sqrt{u_{prec}^2 + u_{stand.prep}^2 + u_{interp}^2} \qquad \textit{Equation 3.30}$$

And for the top-down approach:

$$u_c = \sqrt{u_{prec}^2 + u_{trueness}^2} \qquad \textit{Equation 3.31}$$

The expanded uncertainty is calculated taken a confidence level of 95% according to the legislation [37], and using the coverage factor K = 2.

$$U = k * u_c = 2 * u_c \qquad \textit{Equation 3.32}$$

4. Experimental procedure

4.1. Materials

- Hirschmann 1 ml, 2 ml capacity pipettes
- Volumetric flask: 10, 50,100, 1000 and 2000 ml
- eVol XR digital analytical syringe: 20 – 500 µl
- Graduate cylinder: 50 ml
- Beakers: 10, 50, 100 and 500 ml
- Separatory funnels
- TurboVap flasks
- Chromatography vials of 1.5 ml capacity

4.2. Equipment

GC-MS

- Gas Chromatography, Agilent Technologies 7890B:
    - Capillary column: Agilent HP 5MS, 60m x 0,25mm x 0,25µm
    - Automatic sampler Agilent Technologies 7693
    - *Software* Agilent MassHunter Quantitative Analysis for GC systems
- Mass Spectrometer, Agilent Technologies 5977A:
    - Mass analyzer: Quadrupole
    - Ion source: Electron Impact (EI)
    - Electron multiplier detector

GC-MS-MS

- Gas Chromatography, Agilent Technologies 6890N:
    - Capillary Column : Agilent HP 5MS, 30m x 0,25mm x 0,25µm
    - Automatic Sampler : Gerstel Multipurpose Sampler
    - *Software* Agilent MassHunter Quantitative Analysis for GC systems

- Mass Spectrometer, Agilent Technologies 5975B Inert XL EI/CI :
    - Mass Analyzer : Triple quadrupole
    - Ion source : Electron Impact (EI)
    - Electron multiplier detector
- Liquid-Liquid Extraction shaker (Agitelec) for 4 simultaneous extractions
- Automatic Solid Phase Extraction equipment, Caliper AutoTrace SPE Workstation
- Evaporation system: TurboVap II, Zymark
- Milli-Q Advantage A10 system, Millipore
- Analytical balance, XS204, Metler Toledo
- Vortex, VELP Scientific

4.3. Reagents and standards

4.3.1. General reagents

- Acetone, ≥ 99,8%, Analytical Reagent Grade, Fisher Chemical
- Dichloromethane (DCM), ≥ 99,8%, ChromaSolv, Sigma-Aldrich
- Methanol, ≥ 99,99%, HPLC Reagent Grade, Fisher Chemical
- Ultrapure water, Millipore
- Anhydrous Sodium Sulfate
- Sodium Chloride, Merck
- Sodium Thiosulfate Pentahydrate, Merck
- Solid Phase Extraction cartridges, Waters OASIS HLB.

4.3.2. Gases

- Nitrogen, used during the sample preparation by the TurboVap to evaporate the solvent
- Argon, used by the mass spectrometer to produce the collision
- Helium, used by the gas chromatograph as carrier gas

4.3.3. Standards

- Bifenox ($C_{14}H_9Cl_2NO_5$), 98%, Dr. Ehrenstorfer
- Aclonifen ($C_{12}H_9ClN_2O_3$), 99%, Dr. Ehrenstorfer
- Quinoxyfen ($C_{15}H_8Cl_2FNO$), 99%, Dr. Ehrenstorfer
- Dicofol ($C_{14}H_9Cl_5O$), 99%, Dr. Ehrenstorfer
- Cybutryne ($C_{11}H_{19}N_5S$), 98,3%, ChemService
- Biphenyl ($C_{12}H_{10}$), 99,5%, Dr. Ehrenstorfer
- Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$), 98,5%, Dr. Ehrenstorfer
- Deltamethrine ($C_{22}H_{19}Br_2NO_3$), 99,5%, Dr. Ehrenstorfer
- alpha – Cypermethrine ($C_{22}H_{19}Cl_2NO_3$), 97,5%, Dr. Ehrenstorfer
- beta – Cypermethrine, ($C_{22}H_{19}Cl_2NO_3$), 99%, Dr. Ehrenstorfer
- theta – Cypermethrine, ($C_{22}H_{19}Cl_2NO_3$), 10 ng/ml, Dr. Ehrenstorfer
- zeta – Cypermethrine, ($C_{22}H_{19}Cl_2NO_3$), 96%, Dr. Ehrenstorfer

4.4. Standards solution preparation

Standards solution were prepared following the internal procedures of EPAL [91].

Primary standard solution

Weight approximately 0.02 g of each original pesticide and transfer to a 50 ml volumetric flask, and make up to the mark with acetone. The result are 12 primary solutions, because theta – cypermethrine standard is already a solution. These standards solutions should be stored in a cold environment (< 8^{o}C). Primary standards solutions have a validation date of one year.

Intermediate standard solution

Take 1 ml of the primary standard solution into a 100 ml volumetric flask. Make up to the mark with acetone. Repeat the process for each pesticide. These solutions have a validation date of six months and should be stored below 8^{o}C.

Pesticide	Weight(g)	Purity (%)	Volumetric flask (mL)	Primary standard solution concentration (mg/L)	Intermediate standard solution/Mixed standard solution concentration (mg/L)
Bifenox	0,0200	98,0	50	392	3,9
Dicofol	0,0190	99,0	50	376	3,8
Quinoxyfen	0,0200	99,0	50	396	4,0
Aclonifen	0,0219	99,0	50	434	4,3
Chlorpyrifos	0,0201	98,5	50	396	4,0
alpha - cypermethrine	0,0198	97,5	50	386	3,9
beta - cypermethrine	0,0199	99,0	50	394	3,9
theta - cypermethrine	-	-	-	-	-
zeta - cypermethrine	0,0202	96,0	50	388	3,9
Deltamethrine	0,0203	99,5	50	404	4,0
Biphenyl	0,0197	99,5	50	392	3,9
Cybutryne	0,0196	98,3	50	385	3,9

Table 2. Primary, intermediate and mixed standards solutions preparation

Mixed standard solution

Take 1 ml of each primary standard solution and transfer it to a 100 ml volumetric flask. Make up to the mark with acetone. This mixed standard solution has a validation date of 6 months and should be stored below 8^{o}C.

Calibration standard solutions

Take the required amount of the mixed standard solution into 10 ml volumetric flasks. Theta – cypermethrine is not included in this solution, so it is necessary to add the required amount of the original pesticide standard into the calibration standard solutions. The final solvent could be acetone or DCM. These solutions have to be prepared each time the analysis is carried out.

The volumes added are shown in the table below:

Calibration standards	Estimated concentration (µg/L)	Volume Mixed Standard Solution (µl)	Volume theta - cypermethrine (µl)
P1	40	100	40
P2	60	150	60
P3	80	200	80
P4	100	250	100
P5	120	300	120
P6	160	400	160
P7	200	500	200

Table 3. Calibration standards preparation

The Table 4, shows the real concentration in each calibration standard

Pesticide	P1 (µg/L)	P2 (µg/L)	P3 (µg/L)	P4 (µg/L)	P5 (µg/L)	P6 (µg/L)	P7 (µg/L)
Bifenox	39,2	58,8	78,4	98,0	117,6	156,8	196,0
Dicofol	37,6	56,4	75,2	94,0	112,9	150,5	188,1
Quinoxyfen	39,6	59,4	79,2	99,0	118,8	158,4	198,0
Aclonifen	43,4	65,0	86,7	108,4	130,1	173,5	216,8
Chlorpyrifos	39,6	59,4	79,2	99,0	118,8	158,4	198,0
alpha - cypermethrine	38,6	57,9	77,2	96,5	115,8	154,4	193,1
beta - cypermethrine	39,4	59,1	78,8	98,5	118,2	157,6	197,0
theta - cypermethrine	40,0	60,0	80,0	100,0	120,0	160,0	200,0
zeta - cypermethrine	38,8	58,2	77,6	97,0	116,4	155,1	193,9
Deltamethrine	40,4	60,6	80,8	101.0	121,2	161,6	202,0
Biphenyl	39,2	58,8	78,4	98,0	117,6	156,8	196,0
Cybutryne	38,5	57,8	77,1	96,3	115,6	154,1	192,7

Table 4. Real concentration of each calibration standard

4.5. Sample preparation: Procedures and parameters

All the procedures followed in the project were according to the EPAL internal procedures.

4.5.1. Liquid-Liquid extraction

The procedure can be divided in several steps, going from 1 L of water sample to 1 ml:

1. Locate the separatory funnels in the liquid-liquid extraction apparatus

2. Wash with a small quantity of organic solvent, in this case dichloromethane (DCM)
3. Add 1 L of water sample
4. Add 30 ml of DCM, shake 2 or 3 times and liberate the pressure (2-3 times)
5. Shake for 4-5 min
6. Wait 7-8 min until the phases are well separated
7. Collect the organic phase into a TurboVap tube, passing it through a funnel containing glass wool and sodium sulfate anhydrous to eliminate the residues of water.
8. Repeat steps 4,5,6 and 7, three times
9. Evaporate the solvent using TurboVap to a final volume of 0,4-0,6 ml
10. a) In the case the final solvent is Acetone: Add 3-4 ml of acetone, let them evaporate and make up the solution to 1 ml with acetone
    b) In the case the final solvent is DCM: Make up the solution to 1 ml with DCM
11. Transfer to a chromatographic vial

Liquid-liquid extraction can be also carried out adding NaCl before the extraction procedure. This method is called Salting-out Liquid-Liquid Extraction (SALLE). Different amounts of salt (10, 15 and 20%) were added after step 3, trying to improve the efficiency of the extraction.

Sodium thiosulfate can be used to eliminate the chlorine present in drinking water samples, when it is interfering with the pesticides of study. It has to be added before the extraction, just after step 3.

4.5.2. Solid Phase Extraction

Solid Phase Extraction procedure uses 0,5 L of sample, but it is needed a little more to avoid some errors.

1. Place the sample in its adequate position in the SPE equipment
2. Assure that the solvent flasks have enough solvent to carry out the separation
3. Place the cartridges in its positions
4. Run the method

a) Condition the cartridge with 6 ml of DCM + 6 ml Methanol + 6 ml ultra-pure water
b) Retention of the sample by passing 500 ml of sample through the cartridge
c) Wash the cartridge with 4 ml of ultra-pure water
d) Drying the cartridge during 55 min with nitrogen
e) Elute the compounds with 2 + 2 + 2 ml of DCM

5. Evaporate the solvent using TurboVap to a final volume of 0,2-0,4 ml
6. a) In the case the final solvent is Acetone: Add 3-4 ml of acetone, let it evaporates to 0,2-0,4 ml and make up the solution to 0,5 ml with acetone
   b) In the case the final solvent is DCM: Make up the solution to 0,5 ml with DCM
7. Transfer to a chromatographic vial

4.6. Gas chromatography conditions

- **Injector conditions**
  - Inlet temperature: 250 ^{o}C
  - Volume injection: 1 µL
  - Septum Purge flow: 3 ml/min
  - Purge Flow to split vent: 25 ml/min at 1,5 min
- **Column conditions**
  - Column Flow: 1 ml/min
  - Pressure: 16.086 psi = 1,094 atm = 110909,066 Pa
  - Oven program:

Rate, ^{o}C/min	Temperature, ^{o}C	Hold time, min
-	40	1
40	190	1
10	280	20

Table 5. GC program

4.7. Mass spectrometry conditions

Mass spectrometer works in two possible modes, Full Scan and Selected Ion Monitoring (SIM). However, the conditions are the same in both cases.

- Detector: MS detector, quadrupole
- Source Temperature: 230 ^{o}C
- Quadrupole Temperature: 150 ^{o}C
- Ionization: Electron Impact
- Ionization energy: -70 ev

Full Scan works scanning the whole mass spectrum, whilst Selected Ion Monitoring only scan the specific ions for each compound. The Table 6 shows 1) retention time in which the compounds are found, 2) ions of each compound that were chosen, after running a Full Scan analysis.

Compound	Retention time, min	Ion 1	Ion 2	Ion 3
Biphenyl	8,963	154*	76	-
Chlorpyrifos	13,949	314	258	197*
Dicofol	14,143	250	139*	111
Cybutryne	14,664	253	182*	-
Aclonifen	16,771	264*	212	183
Quinoxyfen	17,426	307	272	237*
Bifenox	19,268	343*	341	311
Cypermethrines	25,166	181	163*	-

Table 6. Retention times and selected ions in SIM mode

*Most intense ion

5. Results and discussion

In the optimization of a method, the first part should be always the study of the validation parameters. Selectivity, range, linearity, precision, limit of detection, limit of quantification and trueness were studied for the pesticides: Biphenyl, Chlorpyrifos, Dicofol, Aclonifen, Cybutryne, Quinoxyfen and Cypermethrines. The initial list of pesticides also included Bifenox and Deltamethrine, but they did not show good signal in our GC-MS system, so the method validation could not be developed for them.

The aim of developing this method, is to use it for routine analysis, so trueness was studied in different kind of water samples as ultra-pure water, tap water, surface water, and groundwater. EPAL is already responsible for the analysis of a large number of pesticides in different kind of water matrices, but some of the pesticides of study were included in the most recent legislation, having EPAL the responsibility to develop a method to analyse them. The objective is to include these pesticides in an accredited method that it is already being used, but some modifications can be done if needed, to adequate it to the specific targets.

5.1. Method Validation GC-MS-MS

The first step in the development of a method to be used in GC-MS-MS (SRM), is the study of the transitions of each compound. An injection in Full Scan mode was done for each pesticide, in order to choose the precursor ion, which will be lately fragmented in the SRM mode. The precursor ion is usually the most intense mass of the Full Scan chromatogram. Once the precursor ion was chosen, other two masses were chosen as possible product ions, that is, two ions that appear due to the fragmentation of the precursor ion. The product ions are usually the second and third most intense masses, due to the fact that the energy used to fragment the molecule (-70 eV) also produces fragmentation of the precursor ion.

After that, it was necessary to check these transitions, and study the optimal collision energy used to produce these transitions, from the precursor ion to the product ions. That studied was developed using Product Ion Scan mode.

Table 7 shows the precursor ion and product ions chosen for each studied pesticide. Three different collision energies were tested for each transition, trying to determine which one was the optimal collision energy, that is, the collision energy that gave higher peak intensity. Optimal collision energies for each transition are shown in Table 7.

Pesticide	Precursor ion	Product ion 1	Product ion 2	Optimal collision energy, eV	
				1st transition	2nd transition
Chlorpyrifos	314	286	258	-10	-10
Dicofol	250	215	139	-10	-20
Aclonifen	264	212	194	-10	-10
Quinoxyfen	272	237	208	-10	-20
Deltamethrine	253	172	93	-10	-10
Bifenox	341	310	281	-10	-10
Cypermethrines	181	152	127	-20	-20

Table 7. Precursor, product ions and optimal collision energy used in selected reaction monitoring

Range and linearity were being studied, when the equipment suddenly broke without any known reason. We were waiting until the technical services came to repair it but they were not able to do that. After the Christmas break, we decided to start again all the study in a different equipment (GC-MS), in which I have had to develop the whole method again.

5.2. Method Validation GC-MS

5.2.1. Selectivity

An accredited chromatography method that is used for the determination of pesticides by GC-MS was taken, trying to fit this method to our pesticides. Each pesticide, from each intermediate standard solution, was injected in full scan mode to observe its retention time, intensity and shape of the peak. The results of this first injections did not show good results for all the compounds, so the oven program was changed in order to get all the peaks with the properly shape and intensity. The final GC program was explained above in the point 4.6.

Once the study of each compound individually was done, the mixed standard solution was injected in full scan mode, checking that there were not overlapping peaks and interferences between them.

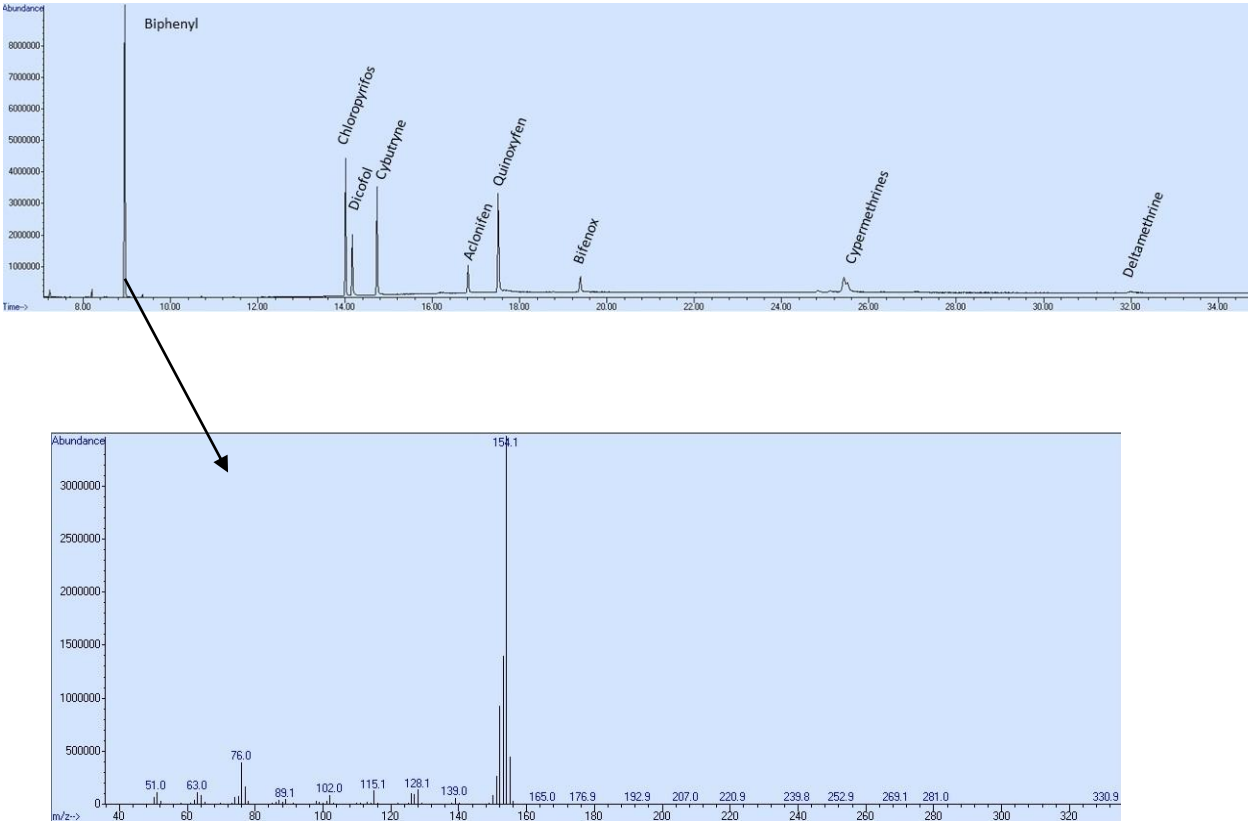


Figure 30. a) Full Scan chromatogram for the mix of compounds, b) Mass Spectrum for Biphenyl

A Full Scan chromatogram for the mix of the pesticides is shown above, with a mass spectrum of one of them, biphenyl. This mass spectrum shows the most intense and characteristics masses of each compound. That masses, 2 or 3 depending on the pesticide, were selected to build up the Selected Ion Monitoring program. The chosen masses of each compounds are organized in Table 6 in the point 4.7.

All the cypermethrines appear as a cluster of four peaks in the chromatogram, not being possible the differentiation of each isomer, but can be quantified as a group of cypermethrines. Each isomer of cypermethrine studied had also other isomer as impurities. In the following parameters, cypermethrines were taken as a group, doing the calibration curve and all the other measurements as a single compound.

The Figure 31 shows the shape of the peaks of the cypermethrine group, containing the four isomers of study.

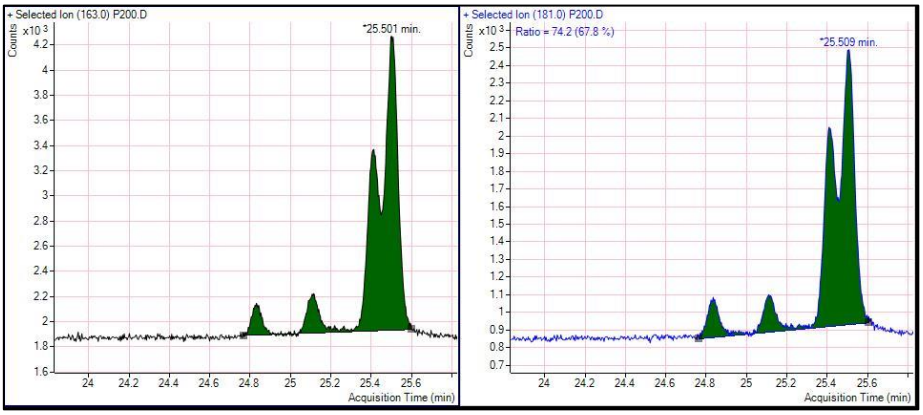


Figure 31. Cluster of Cypermethrines. a) Ion 163, b) Ion 181

An injection in Selected Ion Monitoring mode were conducted to assure the selectivity of the method. Deltamethrine peak was really weak in Full Scan mode, and it was not present when SIM mode was done. After many probes (changing the oven program, changing the selected ions) deltamethrine was eliminated as one of our pesticides of study, due to its complications of being analyzed using GC-MS.

All the following injections were done using Selected Ion Monitoring mode.

5.2.2. Working range and linearity

The working range of study was set up according to the maximum allowed concentration for each pesticide established in the drinking water directive, being 0,10 µg/L for each individual pesticide and 0,5 µg/L for the total pesticides [34]. The range of study was set up in 0,04-0,2 µg/L. That concentration of these pesticides is not possible to be detected in GC-MS, but the sample preparation concentrates the sample 1000 times, allowing the detection of them in a more suitable range, 40-200 µg/L.

Linearity has to be demonstrated in the working range of study. In order to study the linearity, several concentrations of the mixed standard solution were injected.

Pesticides	P40, µg/L	P50, µg/L	P60, µg/L	P70, µg/L	P80, µg/L	P90, µg/L	P100, µg/L	P120, µg/L	P160, µg/L	P180, µg/L	P200, µg/L
Bifenox	39,2	49,0	58,8	68,6	78,4	88,2	98,0	117,6	156,8	176,4	196,0
Dicofol	37,6	47,0	56,4	65,8	75,2	84,6	94,1	112,9	150,5	169,3	188,1
Quinoxyfen	39,6	49,5	59,4	69,3	79,2	89,1	99,0	118,8	158,4	178,2	198,0
Aclonifen	43,4	54,2	65,0	75,9	86,7	97,6	108,4	130,1	173,4	195,1	216,8
Chlorpyrifos	39,6	49,5	59,4	69,3	79,2	89,1	99,0	118,8	158,4	178,2	198,0
alpha - cypermethrine	38,6	48,3	57,9	67,6	77,2	86,9	96,5	115,8	154,4	173,7	193,1
beta - cypermethrine	39,4	49,3	59,1	69,0	78,8	88,7	98,5	118,2	157,6	177,3	197,0
theta - cypermethrine	40,0	50,0	60,0	70,0	80,0	90,0	100,0	120,0	160,0	180,0	200,0
zeta - cypermethrine	38,8	48,5	58,2	67,9	77,6	87,3	97,0	116,4	155,1	174,5	193,9
Total cypermethrines	40,4	50,5	60,6	70,7	80,8	90,9	101,0	121,2	161,6	181,8	202,0
Biphenyl	39,2	49,0	58,8	68,6	78,4	88,2	98,0	117,6	156,8	176,4	196,0
Cybutryne	38,5	48,2	57,8	67,4	77,1	86,7	96,3	115,6	154,1	173,4	192,7

Table 8. Concentration of the standand solutions for the study of linearity

The criteria used to validate the linearity of the compounds was explained in the point 3.3.1. Coefficient of determination, Residuals Test (Anexa 2 – Residual test), Mandel Test (Annex 3 – Mandel Test) and Rikilt Test (Annex 4 – Rikilt Test) were used to demonstrate the linearity in the established range.

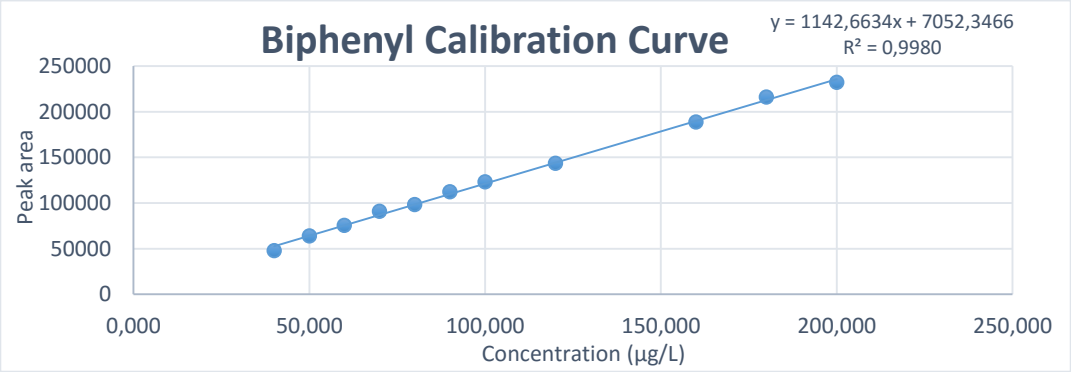


Figure 32. Calibration curve for biphenyl

The coefficients of determination were acceptable for all the compounds for the range of study and are shown in Table 9. In this table, the parameters of the linear regression are also reported.

Pesticide	Range, µg/L	N	R^2	b	a
Dicofol	37,62 - 188,10	11	0,9962	221,8	635,4
Quinoxyfen	39,60 - 198,00	11	0,9985	220,8	-1214,4
Aclonifen	43,36 - 216,81	10	0,9971	35,5	-726,2
Chlorpyrifos	39,60 - 197,99	11	0,9951	1,0	-3,2
Total cypermethrines	156,80 - 783,98	10	0,9985	41,6	-1213,9
Biphenyl	39,20 - 196,02	11	0,9980	1165,9	7052,3
Cybutryne	38,53 - 192,67	11	0,9981	173,1	-3135,9

Table 9. Range and Linearity parameters

All the compounds gave satisfactory results in the Residual Analysis Test for the range of 40-200 µg/L, except for Bifenox. Bifenox signal peaks were very weak and unstable, not passing the Residual Test, neither the Mandel Test.

Residual Test calculates the coefficient of variation (V_{xo}) from each point using the ratio between its experimental peak area and its estimated peak area, where each V_{xo} should fall between an interval of ± 20%. Below in the Figure 33, Residual Test for biphenyl is plotted, showing a randomly distribution of the V_{xo}.

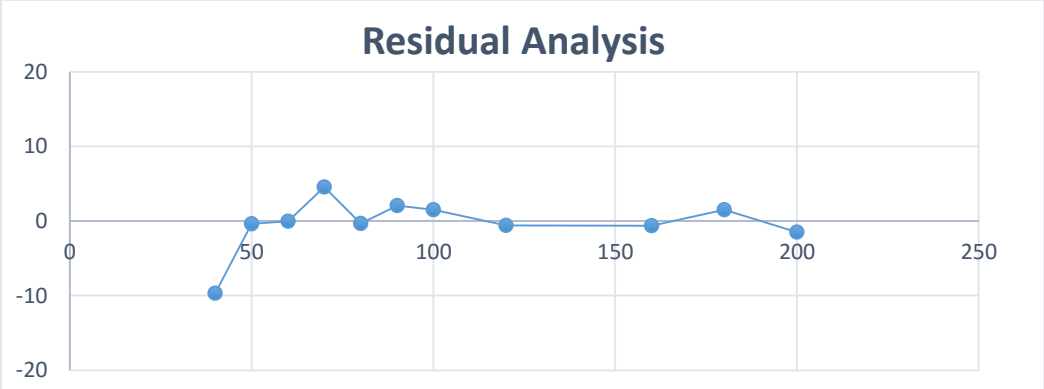


Figure 33. Residual Test for Biphenyl

Mandel Test is used to check linearity, trying to fit the data to a non-linear calibration curve and comparing it with the linear calibration. The complete procedure is explained in point 3.3.1. All the studied compounds were accepted using Mandel Test and Residual Test, meaning that all of them show a good linearity in the interval of study (40-200 µg/L).

Rikilt Test was carried out in order to check if a response factor could be used instead the whole calibration curve. Not all the pesticides of study gave good results for this test. Cybutryne, Aclonifen and Quinoxyfen did not pass satisfactorily Rikilt Test for the whole range of study. The results indicate that response factor cannot be used for the analysis of all the pesticides, so a calibration curve must be done in every sequence analysis.

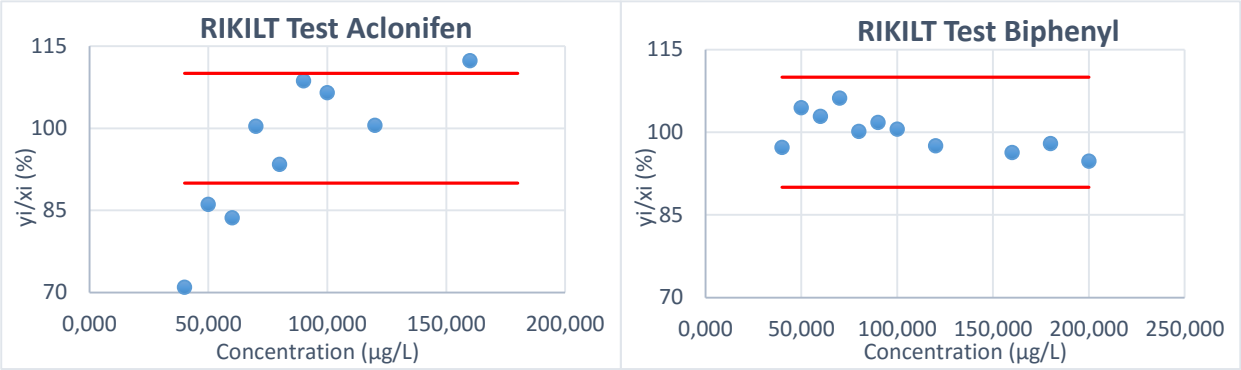


Figure 34. a) Rikilt test non satisfactory for Aclonifen, b) Rikilt Test satisfactory for biphenyl

5.2.3. Limit of detection and limit of quantification (LOD and LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) of the instrument were calculated using the residual standard deviation of the calibration curve as it is described in point 3.3.2. and 3.3.3. The criteria to accept these parameters is that LOQ have to be lower (or very close) than the less concentrated standard in each calibration curve. That is because LOQ is the lowest concentration that the instrument is able to determine quantitatively, so a concentration in the calibration curve under the LOQ would not have any veracity. The table below shows the LOD and LOQ for each pesticide:

Pesticide	Range, µg/L	N	LOD, µg/L	LOQ, µg/L
Dicofol	37,62 - 188,10	11	10,0	33,3
Quinoxyfen	39,60 - 198,00	11	6,5	21,8
Aclonifen	43,36 - 216,81	10	10,6	35,5
Chlorpyrifos	39,60 - 197,99	11	11,9	39,8
Cypermethrines	156,80 - 783,98	10	27,1	90,4
Biphenyl	39,20 - 196,02	11	7,4	24,8
Cybutryne	38,53 - 192,67	11	7,2	23,9

Table 10. LOD and LOQ for the studied pesticides

5.2.4. Precision

Precision was measured in repeatability conditions for the most and less concentrated standard of the calibration curve. According to the legislation related to pesticides in water intended by human consumption, precision can have a maximum value of 25% [37].

Repeatability was calculated as the relative standard deviation (RSD %) of a 10 independent measurements.

Pesticide	N	RSD, % (40 µg/L)	RSD, % (200 µg/L)
Biphenyl	10	2,5	2,3
Chlorpyrifos	10	2,2	1,9
Dicofol	10	6,6	6,4
Cybutryne	10	5,0	2,0
Aclonifen	10	5,3	3,3
Quinoxyfen	10	6,5	2,2
Cypermethrines	10	7,0	2,8

Table 11. Repeteability for 40 and 200 µg/L

5.3. Optimization of the extraction techniques

The aim of the study was trying to develop a method to analyze these pesticides in water intended for human consumption. In order to analyze, as it is explained above it is necessary to carry out a sample treatment to extract the pesticides to a more suitable solvent and to concentrate them to a measurable concentration. Liquid-Liquid Extraction and Solid-Phase Extraction were studied in the four possible matrices, ultra-pure water, tap water, surface water and groundwater. The study was carry out testing the recoveries at different concentrations for each matrix using both extraction techniques.

In order to improve the data obtained, different procedure modifications were tested. The parameters that were optimized in the LLE and the SPE were:

- Solvent used to fortify the blank samples and final solvent of injection
- TurboVap tests
- Salting Out test for LLE
- Adding Thiosulfate to eliminate chlorine in the tap water samples
- Matrix effect studies in SPE

A minimal of 6 extractions were needed to have a significant estimation of the extraction recovery. Relative standard deviation cannot be higher than 25%, according to the drinking water legislation.

5.3.1. Optimization of Liquid-Liquid Extraction

Liquid-Liquid extraction is a well-known extraction technique based on the solubility of the compound in different solvents. To extract the pesticides was used dichloromethane (DCM), that is capable to extract many organic compounds and its solubility in water is really low. That provides a good phase separation and good extraction recoveries.

The analytical procedure used was according to the EPAL internal procedures and it is detailed in the point 4.5.1. Recoveries were carried out fortifying with a standard solution at low and high concentration (60 or 80 µg/L and 200 µg/L) just before the extraction.

The mixed standard solution, from which the calibration standards were prepared, was done and stored in acetone, because of the good stability of the pesticides in this solvent. Acetone is soluble in water, so the fortification of the standard did not show any inconvenient. If the standards from the calibration curve are prepared with acetone, the sample should be also prepared using this solvent, so during the TurboVap concentration the solvent should be changed from DCM to acetone. There is no problem on that, except for the degradation that acetone produces dissolving the septum of the gas chromatography injector.

In the other hand, preparing the calibration standards using DCM, presents the complication of the low solubility of DCM in water, because the fortification is done with the standard in DCM. But, despite the low solubility, 1 ml of DCM can be dissolved in 1 L of water.

Studies with **ultra-pure water** were carried out testing the recoveries with both solvents. The chart below compares the sample treatment recoveries using both solvents for injection.

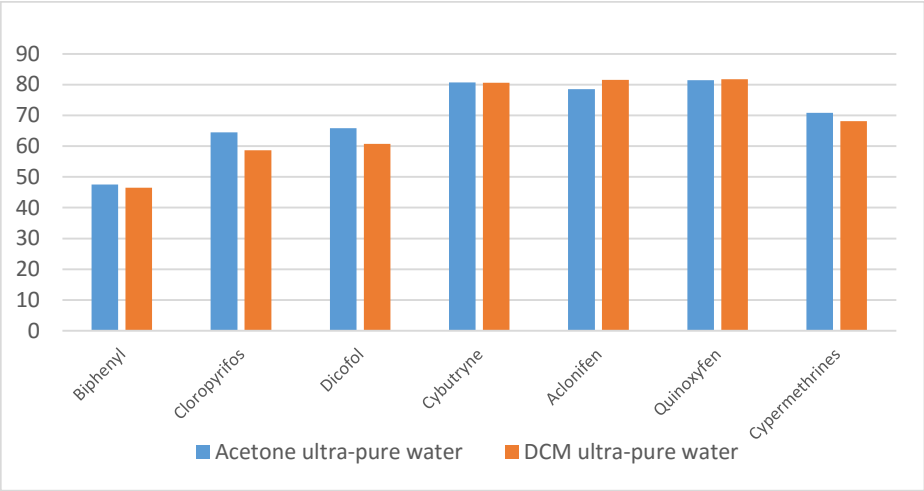


Figure 35. Test to determine the solvent of injection in liquid-liquid extraction

As it can be observed in the chart, both solvents present really similar recoveries for all the pesticides. DCM was used as final solvent to inject the standards and samples, due to its less degradation of the GC septum and because a step in the TurboVap concentration was also eliminated.

With the purpose of minimize and correct any possible error, TurboVap recoveries were also studied. In order to test TurboVap, 90 mL of DCM were mixed with aliquots of 1 mL of standards solution in TurboVap tubes just before its concentration, to verified its recovery after that step.

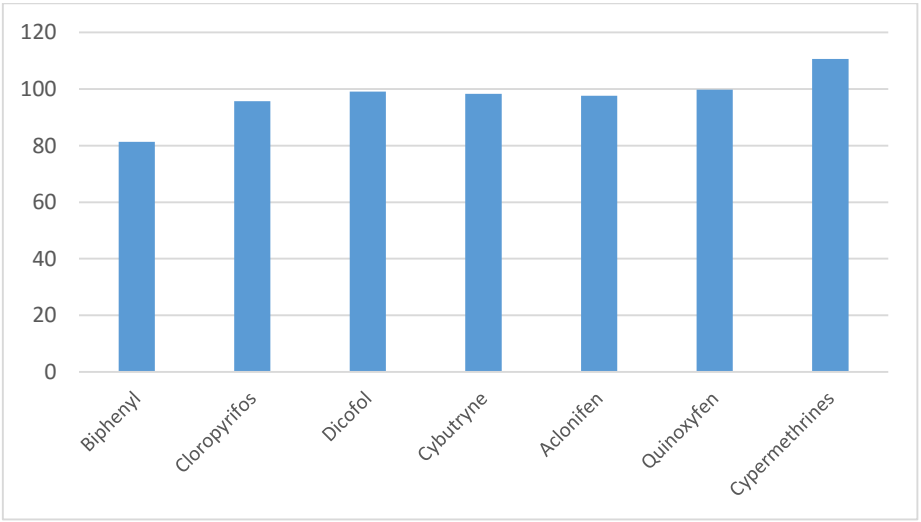


Figure 36. TurboVap Test

As the Figure 36 shows, all the TurboVap recoveries fall into a range of ± 10 % from the estimated value of 100%, except the recovery of biphenyl. Its recovery was 81 %, slightly below the estimated value. That demonstrates that it suffers a volatilization during the concentration step, and can be explained because biphenyl is a compound with a low boiling point and not very soluble in DCM, due to its lower polarity.

After studying these two parameters, it was performed the recovery study with different standards concentrations. The extractions with ultra-pure water were the first to study.

The following tables shows the experimental extraction recoveries for each compound, with their standard deviation. The concentration studied in ultra-pure water were 60 and 200 µg/L of standard solution.

Pesticide	%R (200 µg/L)	SD (200 µg/L)	RSD, % (200 µg/L)	%R (60 µg/L)	SD (60 µg/L)	RSD, % (60 µg/L)
Biphenyl	51,1	7,5	14,8	54,6	6,1	11,1
Chlorpyrifos	73,2	13,4	18,3	77,9	7,1	9,1
Dicofol	82,9	14,3	17,3	94,2	4,3	4,5
Cybutryne	97,0	10,0	10,3	100,4	3,3	3,3
Aclonifen	91,3	9,6	10,5	99,5	3,9	3,9
Quinoxyfen	93,8	8,4	8,9	102,6	4,5	4,3
Cypermethrines	93,8	10,2	10,8	95,9	14,4	15,0

Table 12. Ultra-pure water recoveries for Liquid-Liquid Extraction

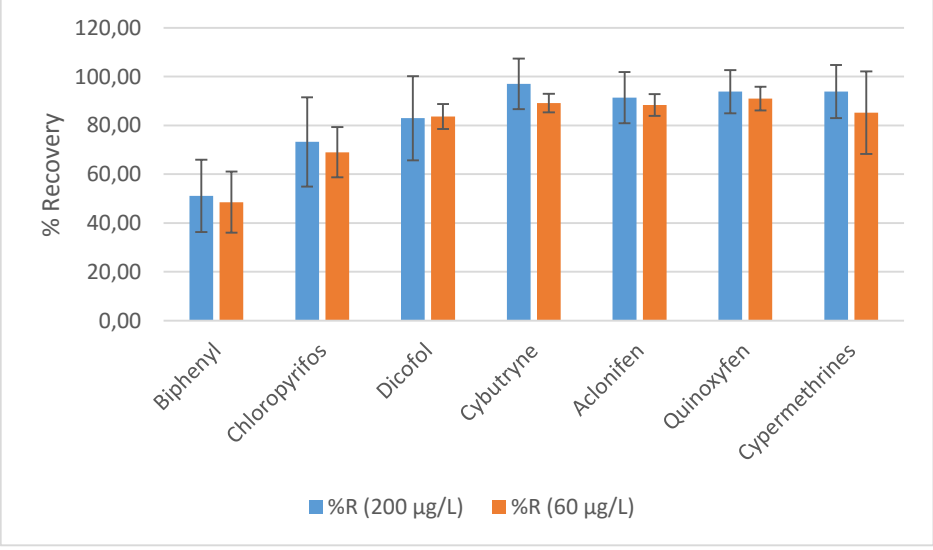


Figure 37. Ultra-pure water recoveries using liquid-liquid extraction

The recoveries were acceptable for all the compounds, even for biphenyl that showed the lowest recovery, around 50%. As it is explained below, that recovery is influenced by the TurboVap concentration.

The recoveries for **tap water** were studied for the concentration of 80 µg/L and 200 µg/L of the standard solution.

Pesticide	%R, (200 µg/L)	SD, (200 µg/L)	RSD, % (200 µg/L)	%R, (80 µg/L)	SD, (80 µg/L)	RSD, % (80 µg/L)
Biphenyl	44,4	5,7	12,8	54,6	10,1	18,5
Chlorpyrifos	65,9	10,2	15,5	85,0	8,5	10,1
Dicofol	74,3	11,0	14,9	75,8	14,0	18,4
Cybutryne	77,7	10,1	13,0	92,3	13,5	14,6
Aclonifen	93,6	13,3	14,3	95,9	12,4	12,9
Quinoxyfen	85,9	13,0	15,2	90,4	12,0	13,2
Cypermethrines	82,2	11,5	14,0	88,7	16,8	18,9

Table 13. Tap water recoveries using Liquid-Liquid Extraction

The Table 13 and the Figure 38 show the recoveries obtained from the fortification of tap water with standard solutions, using liquid-liquid extraction. The results were similar to the ultra-pure water recoveries, but tap water had slightly lower recoveries.

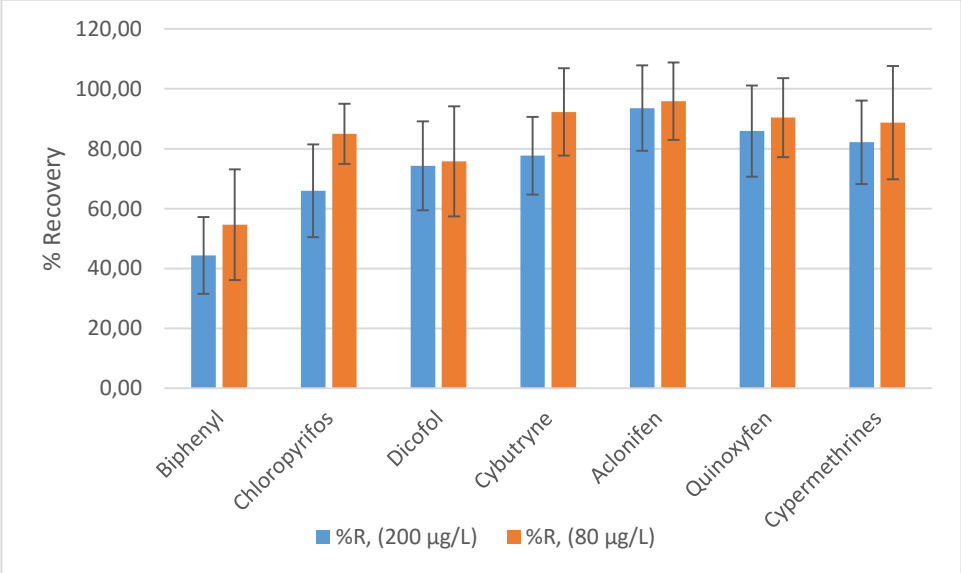


Figure 38. Tap water recoveries using liquid-liquid extraction

In order to improve biphenyl recovery, salting out liquid-liquid extraction (SALLE) were tested, adding different amount of NaCl to the water sample. The NaCl is dissolved in

water, decreasing the solubility of pesticides in water and facilitating the extraction of them to the organic phase, the dichloromethane. The concentrations of NaCl tested were 10 %, 15 % and 20 % and the results are shown below:

Pesticide	%R, 10% NaCl	%R, 15% NaCl	%R, 20% NaCl
Biphenyl	51,27	38,48	48,83
Chlorpyrifos	94,63	79,67	96,95
Dicofol	119,16	104,25	134,78
Cybutryne	113,32	107,96	116,46
Aclonifen	107,41	97,43	110,37
Quinoxyfen	106,43	101,83	108,02
Cypermethrines	100,41	94,63	99,38

Table 14. Salting out liquid-liquid extraction tests

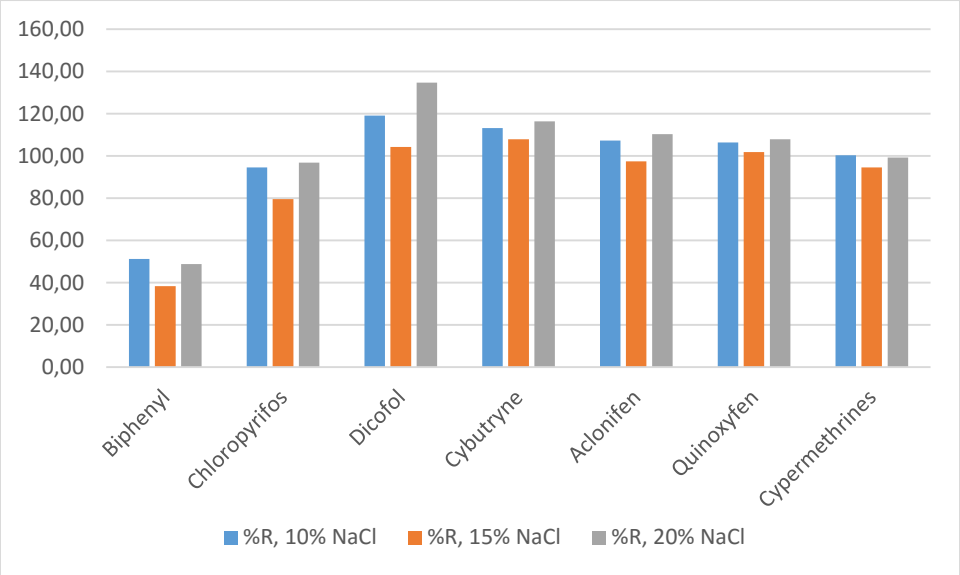


Figure 39. Salting out liquid-liquid extraction recoveries

The Figure 39 shows that adding NaCl before the extraction procedure, did not improve significantly the biphenyl recoveries, so salting out extraction were not carried out any more.

The **groundwater** used in this study was taken from Lezirias' well (Figure 11). The standards added to study the recoveries were 80 µg/L and 200 µg/L.

Table 15 shows the recoveries in groundwater with its relative standards deviations for the pesticides of study.

Pesticide	%R, (200 µg/L)	SD, (200 µg/L)	RSD, % (200 µg/L)	%R, (80 µg/L)	SD, (80 µg/L)	RSD, % (80 µg/L)
Biphenyl	43,5	2,2	5,1	46,2	3,5	7,7
Chlorpyrifos	58,0	4,7	8,2	85,2	2,9	3,5
Dicofol	67,3	11,9	17,6	84,9	7,6	9,0
Cybutryne	74,7	12,2	16,4	88,7	3,8	4,2
Aclonifen	89,6	11,6	13,0	109,1	7,3	6,7
Quinoxyfen	76,0	12,3	16,1	84,8	6,9	8,2
Cypermethrines	70,8	7,2	10,1	80,8	8,1	10,0

Table 15. Groundwater recoveries for Liquid-Liquid Extraction

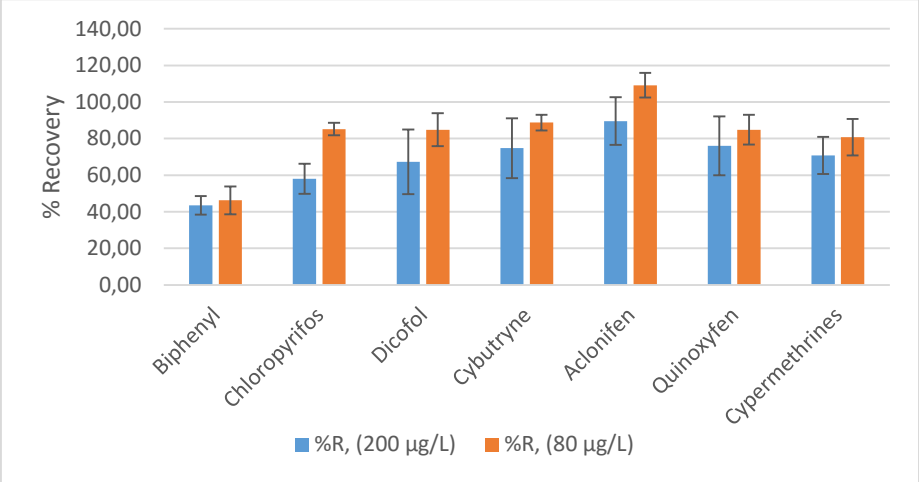


Figure 40. Groundwater recoveries for liquid-liquid extraction

In Figure 40 can be observed that recoveries when a standard of 200 µg/L was used were slightly lower than recoveries with a standard of 80 µg/L. Moreover, recoveries with 80 µg/L standard showed lower precision. However, recoveries using the lower concentrated standard matched better the expected values, considering the previous studies in tap and ultra-pure water.

The **surface water** used came from the Tagus River, that it is usually dirtier than water from Zêzere River. Tagus water showed turbidity and suspended soils, causing difficulties to the extraction step and the following analysis.

The fortifications were done with standards of 80 µg/L and 200 µg/L, and are shown in the following table.

Pesticide	%R, (200 µg/L)	SD, (200 µg/L)	RSD, % (200 µg/L)	%R, (80 µg/L)	SD, (80 µg/L)	RSD, % (80 µg/L)
Biphenyl	38,6	4,0	10,5	43,4	3,4	7,8
Chlorpyrifos	65,0	6,9	10,6	90,4	4,6	5,0
Dicofol	143,3	16,3	11,4	142,0	14,9	10,5
Cybutryne	63,9	8,3	13,1	61,5	7,6	12,4
Aclonifen	123,1	17,7	14,4	125,2	12,7	10,1
Quinoxyfen	81,2	9,8	12,0	83,3	7,4	8,8
Cypermethrines	54,7	6,7	12,2	52,0	3,0	5,8

Table 16. Surface water recoveries for Liquid-Liquid Extraction

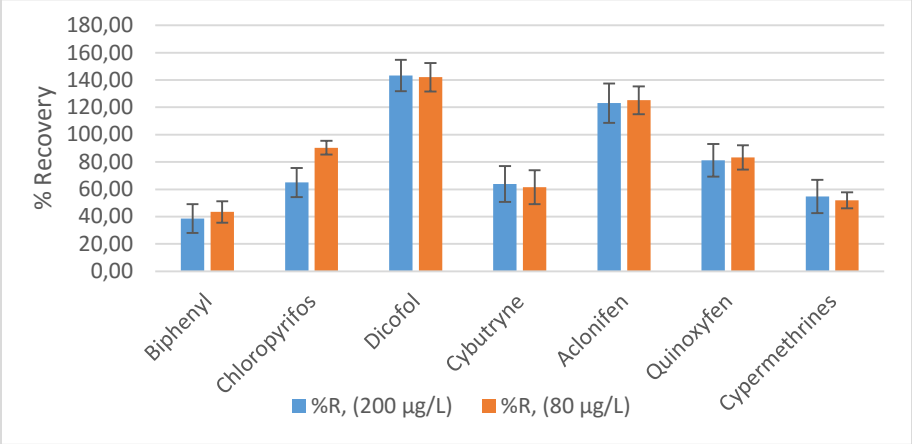


Figure 41. Surface water recoveries for liquid-liquid extraction

The results obtained from the surface water showed reasonable recoveries for biphenyl, chlorpyrifos, cybutryne, quinoxyfen and cypermethrines. Moreover, some of the recoveries were quite low comparing with the results from others water matrices. Aclonifen and dicofol revealed recoveries above 100 %, suggesting matrix effect or any other interference. Studies should be repeated to confirm these high recoveries. Water from Tagus river interferes completely in the ion 258 of chlorpyrifos, blinding it and avoiding the use of this ion for quantification.

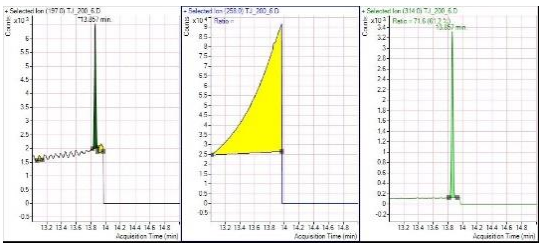


Figure 42. Chlorpyrifos peak in Tagus water

An option to improve these recoveries could be carrying out a filtration step previous the extraction procedure, in order to clean the water from the suspended soils and eliminate the turbidity. However, it does not assure anything, because the interferences can be caused by compounds that cannot be eliminated during the filtration step.

The comparison of recoveries shows a constant value of recoveries for biphenyl, chlorpyrifos and quinoxyfen. Dicofol and aclonifen also gave constant recoveries, without taking into account surface water recoveries. Cybutryne and cypermethrines had recoveries near 100 % in ultra-pure water, decreasing in tap and groundwater, and ending near 50-60 % in surface water. However, recoveries in all the water matrices were acceptable.

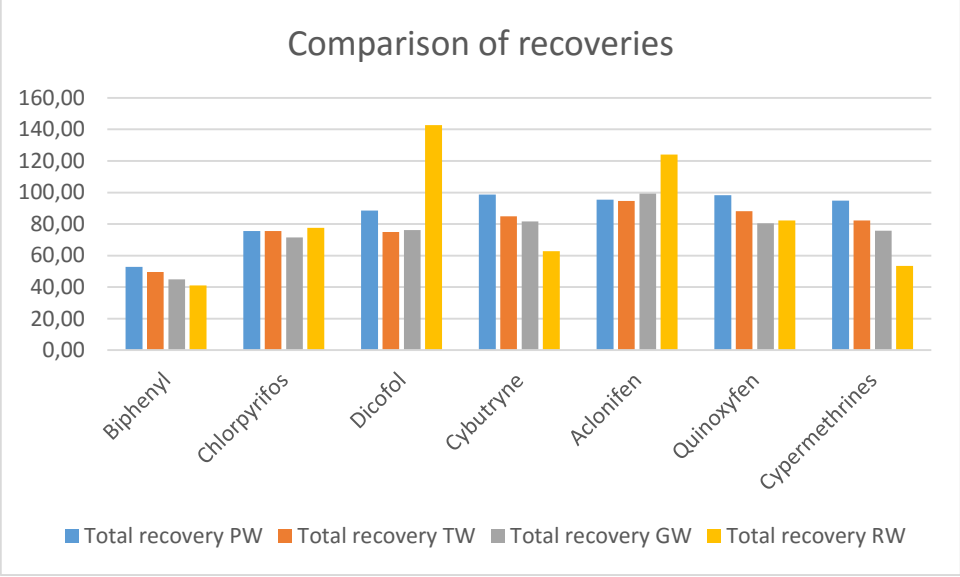


Figure 43. Comparison of recoveries in different water matrices

5.3.2. Optimization of the Solid Phase Extraction

One of the core aims of the study is to compare both extraction techniques, liquid-liquid extraction and solid phase extraction in order to determine which one gives better recoveries and should be used to analyze these pesticides in the different water matrices.

A priori, solid phase extraction is a better choice to prepare and extract the sample because it uses less amount of solvent and it can be easily automated. However, recoveries were analyzed to check and compare both techniques.

Solid phase extraction procedure was explained in 3.1.1.2, containing 5 steps, conditioning, retention, washing, drying and eluting. It took around 2h to complete the whole procedure that was carried out using the solid phase extraction equipment, totally automatic. After that, the TurboVap concentration took around 20 min, because the amount of solvent after SPE was just 6 ml of DCM to concentrate to 0,5 ml.

First of all, ultra-pure water was studied using solid phase extraction, fortifying in the same way as in the liquid-liquid extraction. Standards of 200 µg/L were fortified in order to check the recoveries in ultra-pure water.

Results using solid phase extraction in **ultra-pure water** showed successful recoveries, better for some compounds but worst for others than the liquid-liquid extraction. Recovery of biphenyl improved from 50% to 70%, but in the other hand, cypermethrines were less recovered than in liquid-liquid extraction. Table 17 and Figure 44 show the recoveries achieved with the solid phase extraction for the concentration standard of 200 µg/L.

Pesticide	%R (200 µg/L)	SD (200 µg/L)	RSD, % (200 µg/L)
Biphenyl	68,1	7,4	10,9
Chlorpyrifos	98,9	6,5	6,6
Dicofol	108,7	11,6	10,6
Cybutryne	109,9	2,1	1,9
Aclonifen	102,3	13,4	13,1
Quinoxyfen	86,1	15,1	17,5
Cypermethrines	60,3	6,1	10,8

Table 17. Recoveries from solid phase extraction in ultra-pure water

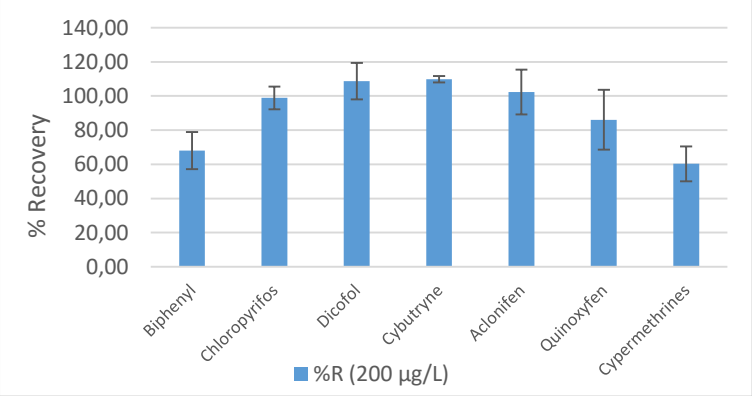


Figure 44. Ultra-pure water recoveries in solid phase extraction

The studies continued with the recoveries in **tap water** with the highest concentration standard. The results were not satisfactory, giving unexpected results, because some of the recoveries were significantly higher than 100%, and that is impossible.

In order to solve this problem and understand what happened, 3 different causes were studied: 1) the solvent, acetone or DCM, used to fortify the water sample, 2) adding sodium thiosulfate to eliminate the chlorine present in tap water and 3) matrix effect tests.

- Acetone or dichloromethane as solvent for the preparation of the standards

Pesticide	%R, (Acetone)	RSD, % (Acetone)	%R, (DCM)	RSD, % (DCM)
Biphenyl	56,0	18,7	72,3	5,6
Chlorpyrifos	70,1	52,1	86,6	35,0
Dicofol	131,8	5,4	144,8	4,5
Cybutryne	155,0	-	173,5	-
Aclonifen	139,5	3,2	151,8	10,7
Quinoxyfen	147,0	2,7	158,7	13,8
Cypermethrines	63,0	17,9	88,4	21,5

Table 18. Comparison of solvent used for preparation of the standards in solid phase extraction

*There no RSD for cybutryne because only one measure showed a reasonable result

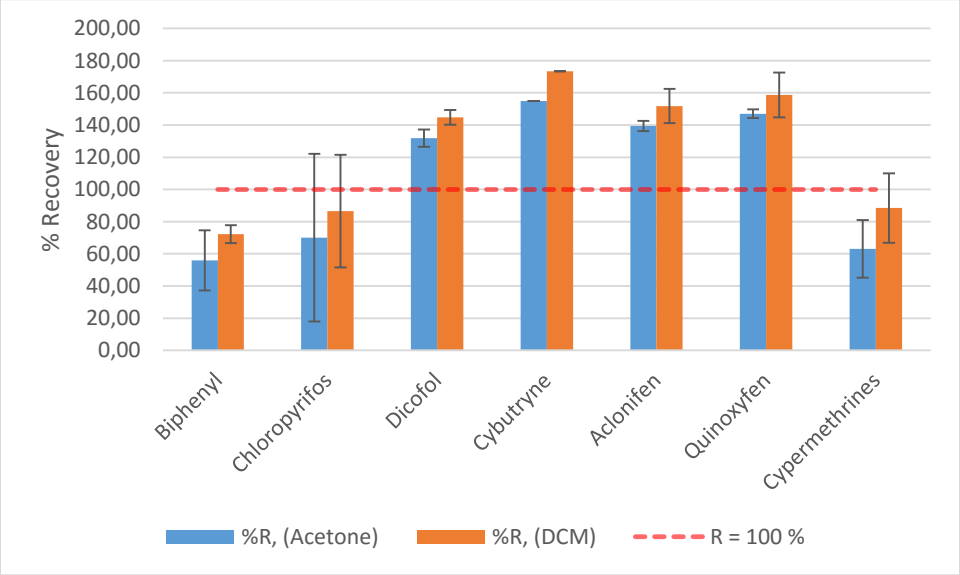


Figure 45. Comparison of tap water recoveries using solid phase extraction

Recoveries results were slightly better when using DCM, they have lower relative standard deviations, and higher values of recoveries. However, recoveries using DCM also shown really high recoveries, above 100 %.

- Sodium thiosulfate test

The chlorine present in the tap water, used to disinfect it, can cause some interferences in the sample, usually decreasing the signal of the pesticides peaks. Thiosulfate eliminates the chlorine present in the sample avoiding the possible interferences. In order to check if the high values achieved in the tap water recoveries were caused by chlorine, studies adding different amount of sodium thiosulfate were done. The sodium thiosulfate (250 mg, 500 mg and 800 mg) was added before the extraction procedure.

Pesticide	%R (250 mg ThioS)	%R (500 mg ThioS)	%R (800 mg ThioS)
Biphenyl	72,1	61,0	68,3
Chlorpyrifos	103,4	91,2	111,1
Dicofol	154,2	130,9	160,4
Cybutryne	151,2	136,2	162,7
Aclonifen	140,9	108,9	155,2
Quinoxyfen	182,2	141,5	162,6
Cypermethrines	54,0	49,4	77,1

Table 19. Recoveries for the Thiosulfate test in solid phase extraction

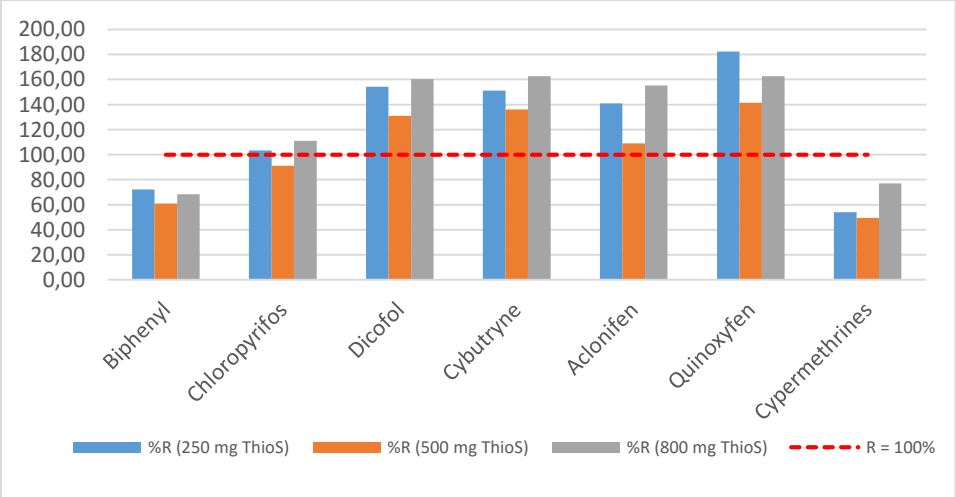


Figure 46. Thiosulfate tests using solid phase extraction

These test did not show any improvement when compared the previous extraction recoveries, so chlorine was not the cause of the high values of recoveries.

- Matrix effect studies

Due to the unexpected recoveries above 100 %, and taking into account that in the blank samples pesticides were not present, matrix effects studies were carried out in order to identify the causes of these high recoveries.

The matrix effect test was done comparing two vials that were prepared, 1) taking 200 µL of 200 µg/L standard and mixing with 200 µL of DCM, and 2) taking 200 µL of 200 µg/L standard and mixing with 200 µL of the extract of a blank sample. It was supposed to have a concentration signal of 100 µg/L for both vials.

Pesticide	%R (P200+DCM)	%R, (P200+B)
Biphenyl	97,4	135,2
Chlorpyrifos	99,4	140,9
Dicofol	90,1	143,1
Cybutryne	103,4	137,5
Aclonifen	94,1	156,3
Quinoxyfen	99,4	165,0
Cypermethrines	112,0	175,6

Table 20. Matrix effect in tap water using solid phase extraction

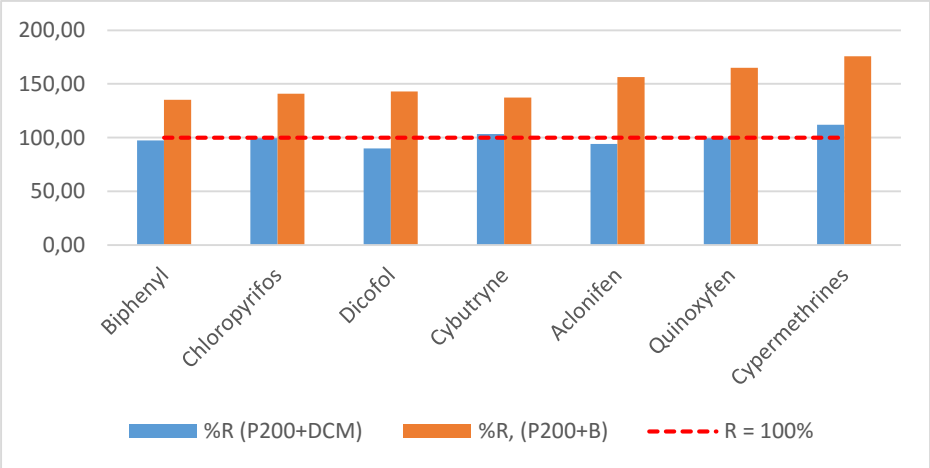


Figure 47. Matrix effect test for solid phase extraction

The expected recoveries were only achieved in the case of the standard fortified with DCM as solvent. The other injection showed some matrix effect in the tap water sample when a solid phase extraction was done.

5.4. LOD and LOQ of the method

Limit of detection and limit of quantification have to be calculated taking into account the recoveries achieved in the studies with different matrices, because the legislation establishes different maximum allowed concentration depending on the pesticide and the water matrix.

Table 21 shows the maximum allowed concentration (MAC) the legislation establishes according to the Directive 2015/1787 (directive that amends the annex II and II of the Drinking Water Directive 98/83/EC) [37] and the Directive 2013/39/EU (regarding the list of priority substances in the field of water policy) [3].

Pesticide	MAC Drinking water, µg/L	MAC Surface water, µg/L
Dicofol	0,1	not applicable
Quinoxyfen	0,1	2,7
Aclonifen	0,1	0,12
Chlorpyrifos	0,1	0,1
Total cypermethrines	0,4	0,0006
Biphenyl	0,1	no information
Cybutryne	0,1	0,016

Table 21. Maximum allowed concentration in drinking and surface water for the studied pesticides

The maximum allowed concentration for pesticides in drinking water is 0,1 µg/L and 0,5 µg/L for the sum of pesticides. The drinking water directive does not specify the pesticide, while the priority substances directive specify a maximum allowed concentration for each pesticide. In the case of dicofol, the directive says that there is not enough information to set a maximum allowed concentration. There is no information regarding biphenyl in the priority substances directive.

Table 22 shows LOD and LOQ calculated using the recoveries of each pesticide in each water matrix. The drinking water directive establishes that LOD must be lower than 25 % of the parametric value, that is, LOD < 0,025 µg/L for drinking water. All the compounds (tap

water) fulfil the requirements, having LOD below the limit. Total cypermethrines had a LOD of 0,033 µg/L, but there are a group of four isomers, so the parametric value is 0,4 µg/L, and the LOD must be below 0,1 µg/L.

	Ultra-pure water (UPW)		Tap water (TW)		Groundwater (GW)		Surface water (SW)	
Pesticide	**LOD UPW, µg/L**	**LOQ UPW, µg/L**	**LOD TW, µg/L**	**LOQ TW, µg/L**	**LOD GW, µg/L**	**LOQ GW, µg/L**	**LOD SW, µg/L**	**LOQ SW, µg/L**
Dicofol	0,011	0,038	0,013	0,044	0,013	0,044	0,007	0,023
Quinoxyfen	0,007	0,022	0,007	0,025	0,008	0,027	0,008	0,026
Aclonifen	0,011	0,037	0,011	0,037	0,011	0,036	0,009	0,029
Chlorpyrifos	0,016	0,053	0,016	0,053	0,017	0,056	0,015	0,051
Cypermethrines	0,029	0,095	0,033	0,110	0,036	0,119	0,051	0,169
Biphenyl	0,014	0,047	0,015	0,050	0,017	0,055	0,018	0,061
Cybutryne	0,007	0,024	0,008	0,028	0,009	0,029	0,011	0,038

Table 22. LOD and LOQ for the different water matrices

Table 23 shows the maximum allowed LOD (MA LOD) in surface water for each pesticide studied according with the priority substances legislation. Quinoxyfen and chlorpyrifos gave LOD below the set limit, fulfilling the requirements of the directive. Cypermethrines and cybutryne did not fulfill the requirements established by the legislation, having LODs below the set limits. Dicofol and aclonifen also fulfilled the requirements for LOD but the recoveries studies should be repeated due to the high value shown.

Pesticide	**MA LOD surface water, µg/L**
Dicofol	-
Quinoxyfen	0,675
Aclonifen	0,03
Chlorpyrifos	0,025
Cypermethrines	0,00015
Biphenyl	-
Cybutryne	0,004

Table 23. Maximum allowed LOD in surface water according to the legislation

5.5. Uncertainty

Uncertainty was estimated following two different approaches, bottom-up and top-down. According to the legislation, uncertainty of measurement in the case of pesticides cannot be higher than 30 % [37].

The bottom-up approach takes into account the precision, preparation of the standards and the interpolation in the calibration curve. It was calculated following equation 3.30 and 3.32 explained in the point 3.3.7.

Pesticide	U_c , %
Biphenyl	16,7
Chlorpyrifos	7,0
Dicofol	21,8
Cybutryne	14,6
Aclonifen	23,4
Quinoxyfen	14,3
Cypermethrines	16,6

Table 24. Combined uncertainties calculated using the bottom-up approach

All the uncertainties were below the established limit of 30 %, fulfilling the requirements of the drinking water directive.

In the other hand, the top-down approach uses the trueness of the recoveries and the precision. That approach gives an associated uncertainty for each type of sample matrix. Expanded uncertainties were calculated with the equations 3.31 and 3.32 explained in point 3.3.7.

Pesticide	U ultra-pure water, %	U tap water, %	U groundwater, %	U surface water, %
Biphenyl	9,1	12,0	6,9	6,5
Chlorpyrifos	7,8	13,1	4,0	4,8
Dicofol	6,3	15,8	10,2	9,3
Cybutryne	5,3	11,1	5,3	10,2
Aclonifen	5,6	10,6	6,9	8,7
Quinoxyfen	6,2	12,4	8,2	8,2
Cypermethrines	28,0	25,8	21,4	20,1

Table 25. Combined uncertainties calculated using the top-down approach

Expanded uncertainties calculated with both approaches fell into the allowed limits established by the legislation. Dicofol and aclonifen uncertainties were calculated with the recoveries obtained, even when they were too high. These recoveries should be calculated again when the laboratory repeats the studies.

6. Conclusions

Monitoring water quality is a necessary step in order to control that the water supplied to the consumers' home is not harmful for human health. To assure that, water has to be carefully treated and analyzed.

A LLE-GC-MS and a SPE-GC-MS methods were studied, optimized, compared and validated for the analysis of organic compounds which can be present in drinking water, well water or river water. The objective of the study was developing a method for the study of 10 pesticides in different water matrices.

Deltamethrine was removed from the list due to difficulties in the detection of it by our GC-MS system. Bifenox was lately removed because it did not pass the Residual and Mandel test for linearity for the established working range. The rest of the compounds of the list passed satisfactorily the linearity tests for the established working range. Rikilt test was not successful for some of the compounds, so a calibration curve has to be done every time a sequence is analyzed.

Instrumental limit of detection and limit of quantification were calculated using the linear regression for each compound. LOD and LOQ from all the compounds fulfill the criteria. Precision studies were made using two different concentrations, the lowest and the highest of the working range. In both cases, precision never exceed the allowed value set by the legislation, 25 %. They, indeed, were always below 7,5 %.

In the comparison of both extraction methods, liquid-liquid extraction and solid phase extraction, the results showed acceptable recoveries in ultra-pure water for both of them. In tap water many problems were found when SPE was used, achieving really high recoveries with also large standard deviations. Solvent, thiosulfate and matrix effect tests were carried out in order to improve these recoveries. The results showed:

- The solvent used did not influence the recoveries, having high recoveries also with DCM.

- Thiosulfate test did not improve recoveries.
- Matrix effect test proved the existence of some matrix effect during the extraction procedure, that could be caused for some interferences due to the cartridge.

Liquid-liquid extraction did not have these matrix effect problems, giving acceptable recoveries for all the compounds in tap water. However, in order to improve the recoveries, optimization of the method was carried out performing salting-out and solvent tests. DCM was also chosen as solvent and salting-out test did not improve significantly the recoveries.

Therefore, due to the good recoveries obtained in liquid-liquid extraction and the problems found with solid phase extraction, liquid-liquid extraction was chosen to continue studying the recoveries in ground and surface water. The groundwater studied was from Lezirias well, and results from the recoveries were successful for all the compounds. Water from Tagus river was studied, having reasonable recoveries for all the compounds except for aclonifen and dicofol. Aclonifen and dicofol showed really high recoveries, being necessary to double check their recoveries.

LOD and LOQ of the method were calculated using the results from the recoveries and were compared with the maximum allowed concentrations established by the legislation. All the compounds fulfilled the requirements set by the drinking water legislation, being this method able to analyze theses pesticides. In surface water, quinoxyfen and chlorpyrifos satisfied the set limits, while cybutryne and cypermethrines exceed these limits. Hence, this method cannot be used to analyze cybutryne and cypermethrines in surface water. In the case of biphenyl and dicofol, the priority substances legislation did not give information regarding the allowed limits.

To estimate the associated uncertainty of the method, two different approaches were carried out, bottom-up and top-down. Uncertainties calculated with both approaches gave results within the limit established by the legislation (U_c < 30%). The bottom-up approach gave highest values from the expanded uncertainties than the top-down approach.

However, bottom-up approach has better detailed balance of the contributions to the measurement uncertainty and more complete way to measure the uncertainty magnitude.

It has been observed during the study, that relative standard deviations increased when samples were prepared in different days, so it is preferable to make always a recovery sample for each sequence analysis.

To continuing the study and trying to fit as more number of pesticides as possible for surface water analysis, some studies can be done:

- Recoveries in surface water have to be double check for aclonifen and dicofol, confirming the existence of matrix effect.
- Add a filtration step before the extraction in surface water analysis, in order to eliminate turbidity and suspended soils and study if the recoveries improve.
- Cybutryne LOD fell near the required value set by the legislation. To improve that, working range and concentration factor can be increased.
- Study Bifenox and Deltamethrine in a different equipment. These pesticides could be better analyzed using LC-MS.

LLE-GC-MS was found to be effective in extracting and analyzing the pesticides of this study, except for biphenyl and deltamethrine. This method could be used to analyze these pesticides in drinking water, and for some of the pesticides also in surface water.

7. Bibliography

[1] X. Chen, Q. Luo, D. Wang, J. Gao, Z. Wei, Z. Wang, et al., Simultaneous assessments of occurrence, ecological, human health, and organoleptic hazards for 77 VOCs in typical drinking water sources from 5 major river basins, China, Environ. Pollut. 206 (2015) 64–72. doi:10.1016/j.envpol.2015.06.027.

[2] E.-N. Papadakis, A. Tsaboula, A. Kotopoulou, K. Kintzikoglou, Z. Vryzas, E. Papadopoulou-Mourkidou, Pesticides in the surface waters of Lake Vistonis Basin, Greece: Occurrence and environmental risk assessment., Sci. Total Environ. 536 (2015) 793–802. doi:10.1016/j.scitotenv.2015.07.099.

[3] The European Parlament and the Council of the European Union, Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, Off. J. Eur. Union. 2013 (2013) 1–17.

[4] National Center for Biotechnology Information. PubChem Compound Database; CID=39230, https://pubchem.ncbi.nlm.nih.gov/compound/39230 (accessed Oct. 30, 2015)., (n.d.).

[5] National Center for Biotechnology Information. PubChem Compound Database; CID=8268, https://pubchem.ncbi.nlm.nih.gov/compound/8268 (accessed Nov. 23, 2015)., (n.d.).

[6] Ö. Kilinc, R. Grasset, S. Reynaud, The herbicide aclonifen: The complex theoretical bases of sunflower tolerance, Pestic. Biochem. Physiol. 100 (2011) 193–198. doi:10.1016/j.pestbp.2011.04.001.

[7] National Center for Biotechnology Information. PubChem Compound Database; CID=92389, https://pubchem.ncbi.nlm.nih.gov/compound/92389 (accessed Nov. 24, 2015)., (n.d.).

[8] CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION, PUBLIC REPORT 2004-01 Quinoxyfen, 53 (1989) 160. doi:10.1017/CBO9781107415324.004.

[9] National Center for Biotechnology Information. PubChem Compound Database; CID=3391107, https://pubchem.ncbi.nlm.nih.gov/compound/3391107 (accessed Nov. 23, 2015)., (n.d.).

[10] D.. United States Environmental Protection Agency (EPA). Washington, Interim reregistration eligibiltity decision for Chlorpyrifos, (2002).

[11] Agency for Toxic Substances and Disease Registry, Toxicological Profile for Chlorpyrifos, (1997) 1 – 287. http://www.atsdr.cdc.gov/toxprofiles/tp84.pdf.

[12] National Center for Biotechnology Information. PubChem Compound Database; CID=2730, https://pubchem.ncbi.nlm.nih.gov/compound/2730 (accessed Nov. 24, 2015)., (n.d.).

[13] National Center for Biotechnology Information. PubChem Compound Database; CID=40585, https://pubchem.ncbi.nlm.nih.gov/compound/40585 (accessed Nov. 24, 2015)., (n.d.).

[14] V.W.W. Bao, K.M.Y. Leung, G.C.S. Lui, M.H.W. Lam, Acute and chronic toxicities of Irgarol alone and in combination with copper to the marine copepod Tigriopus japonicus, Chemosphere. 90 (2013) 1140–1148. doi:10.1016/j.chemosphere.2012.09.022.

[15] National Center for Biotechnology Information. PubChem Compound Database; CID=91590, https://pubchem.ncbi.nlm.nih.gov/compound/91590 (accessed Dec 11, 2015), (n.d.).

[16] National Center for Biotechnology Information. PubChem Compound Database; CID=7095, https://pubchem.ncbi.nlm.nih.gov/compound/7095 (accessed Apr. 14, 2016), (n.d.).

[17] National Center for Biotechnology Information. PubChem Compound Database; CID=2912, https://pubchem.ncbi.nlm.nih.gov/compound/2912 (accessed Nov. 24, 2015), (n.d.).

[18] J. a Arnot, F.A. Gobas, A review of bioconcentration factor (BCF) and bioaccumulation

factor (BAF) assessments for organic chemicals in aquatic organisms, Environ. Rev. 14 (2006) 257–297. doi:10.1139/a06-005.

[19] Stockholm Convention on Persistent Organic Pollutants (POPs), 2010. doi:10.1351/goldbook.S06019.

[20] D.. United States Environmental Protection Agency (EPA). Washington, Toxicity assessment, (n.d.) Region 8. http://www2.epa.gov/region8/hh-toxicity-assessment.

[21] A. Pharm, Bifenox - MATERIAL SAFETY DATA SHEET, (2008) 1–3.

[22] P.I. Park, L. District, Dicofol - MATERIAL SAFETY DATA SHEET, (n.d.) 1–5.

[23] Stockholm Convention on Persistent Organic Pollutants, Proposal to list dicofol in Annexes A, B and/or C to the Stockholm Convention on Persistent Organic Pollutants, 2013. UNEP/POPS/POPRC.9/2.

[24] C. for R.A. RAC, ANNEX 1 – Background document to RAC opinion on Aclonifen, (2011).

[25] S. Road, N. Lincolnshire, Aclonifen - MATERIAL SAFETY DATA SHEET, 2006 (2006) 1–7.

[26] L. SPEX CertiPrep, Quinoxyfen - MATERIAL SAFETY DATA SHEET, (2000) 1–6.

[27] V.W.W. Bao, K.M.Y. Leung, G.C.S. Lui, M.H.W. Lam, Acute and chronic toxicities of Irgarol alone and in combination with copper to the marine copepod Tigriopus japonicus., Chemosphere. 90 (2013) 1140–8. doi:10.1016/j.chemosphere.2012.09.022.

[28] Ciba Specialty Chemicals Corporation, Irgarol 1051 - MATERIAL SAFETY DATA SHEET, (2010) 1–8.

[29] Biphenyl | Technology Transfer Network Air Toxics Web site | US EPA www3.epa.gov, (n.d.). http://www3.epa.gov/airtoxics/hlthef/biphenyl.html#ref2.

[30] National Pesticide Information Center, Cypermethrin, (1998). http://npic.orst.edu/factsheets/cypermethrin.pdf.

[31] T. Daniel, D. Wohlers, M. Citra, Toxicological Profile for Pyrethrins and Pyrethroids, U.S. Dep. Heal. Hum. Serv. Agency Toxic Subst. Dis. Regist. (2003) 328.

[32] European Commission, Introduction to the new EU Water Framework Directive, accessed Dec. 03, 2015, (2015). http://ec.europa.eu/environment/water/water-framework/info/intro_en.htm.

[33] The European Parlament and the Council of the European Union, Directive 2000/60/EC of the European Parliament and of the Councils of 23 October 2000 establishing a framework for Community action in the field of water policy, Off. J. Eur. Communities. 2000/60/EC (2000) 1–73.

[34] The Council of the European Union, COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, Off. J. Eur. Communities. L 330 (1998) 32–54.

[35] The Council of the European Communities, COUNCIL DIRECTIVE of 15 July 1980 relating to the quality of water intended for human consumption (80/778/EEC), J. Eur. Communities. (1980).

[36] European Commission, An overview of the Drinking Water Directive, accessed Dec. 03, 2015, (2015). http://ec.europa.eu/environment/water/water-drink/legislation_en.html.

[37] The European Commission, COMMISSION DIRECTIVE (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption, Off. J. Eur. Union. (2015) 11–12.

[38] The European Parlament and the Council of the European Union, DIRECTIVE 2008/105/EC of 16 December 2008 on environmental quality standards in the field of water policy, Off. J. Eur. Union. (2008) 84–97.

[39] European Commission, Priority substances under the Water Framework Directive, accessed Dec. 04, 2015, (2015). http://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm.

[40] Ministério Do Ambiente, Decreto-lei n.º 236/98 de 1-8-1998, Diário Da República. 176 (1998) 3676–3722.

[41] Ministério do Ambiente, Decreto-Lei n.º 306/2007 de 27 de Agosto, Diário Da República. (2007) 5747–5765.

[42] Empresa Portuguesa das Águas Livres, About us, (n.d.). http://www.epal.pt/EPAL/en/menu/epal/about-us.

[43] A. Report, Annual report & accounts, (2011).

[44] Empresa Portuguesa das Águas Livres, Mission, vision and values, (n.d.). http://www.epal.pt/EPAL/en/menu/epal/about-us/mission-vision-and-values.

[45] Empresa Portuguesa das Águas Livres, What we do, (n.d.). http://www.epal.pt/EPAL/en/menu/epal/about-us/what-we-do.

[46] Empresa Portuguesa das Águas Livres, Qualidade da água para consumo humano-Relatório Anual, (2013).

[47] R.E. Majors, LCGC 9(1), 16-20 (1991) (accessed Apr. 14), (n.d.). http://www.chromatographyonline.com/overview-sample-preparation.

[48] W. J.D., Sample Preparation Techniques in Analytical Chemistry, 2003. doi:10.1002/0471457817.

[49] J.R. Dean, Extraction Techniques in Analytical Sciences, 2009. doi:10.1002/9780470682494.

[50] Classifying Separation Techniques - ChemWiki, (n.d.). http://chemwiki.ucdavis.edu/Core/Analytical_Chemistry/Analytical_Chemistry_2.0/07%3A_Collecting_and_Preparing_Samples/7F%3A_Classifying_Separation_Techniques.

[51] Y. Picó, M. Fernández, M.J. Ruiz, G. Font, Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment., J. Biochem. Biophys. Methods. 70 (2007) 117–31. doi:10.1016/j.jbbm.2006.10.010.

[52] P. Lucci, D. Pacetti, O. Núñez, N.G. Frega, Current Trends in Sample Treatment Techniques for Environmental and Food Analysis., Chromatogr. - Most Versatile Method Chem. Anal. (2012) 127–164. doi:DOI: 10.5772/47736.

[53] S. Sigma-Aldrich, Guide to Solid Phase Extraction - bulletin 910, Bull. 910. (1998) -. http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf.

[54] J.S. Fritz, Analytical Solid-Phase Extraction, Wiley-VCH, 1999.

[55] C. Rasche, B. Fournes, U. Dirks, K. Speer, Multi-residue pesticide analysis (gas chromatography–tandem mass spectrometry detection)—Improvement of the quick, easy, cheap, effective, rugged, and safe method for dried fruits and fat-rich cereals—Benefit and limit of a standardized apple purée calibr, J. Chromatogr. A. 1403 (2015) 21–31. doi:10.1016/j.chroma.2015.05.030.

[56] A. Kouzayha, A.R. Rabaa, M. Al Iskandarani, D. Beh, F. Jaber, Multiresidue Method for Determination of 67 Pesticides in Water Samples Using Solid-Phase Extraction with Centrifugation and Gas Chromatography-Mass Spectrometry, 2012 (2012) 257–265.

[57] D. de Almeida Azevedo, S. Lacorte, T. Vinhas, P. Viana, D. Barceló, Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography–mass spectrometry and liquid chromatography–atmospheric pressure chemical ionization mass spectrometry, J. Chromatogr. A. 879 (2000) 13–26. doi:10.1016/S0021-9673(00)00372-1.

[58] S.-L. McManus, C.E. Coxon, K.G. Richards, M. Danaher, Quantitative solid phase microextraction – Gas chromatography mass spectrometry analysis of the pesticides lindane, heptachlor and two heptachlor transformation products in groundwater, J. Chromatogr. A. 1284 (2013) 1–7. doi:10.1016/j.chroma.2013.01.099.

[59] N.S. Chary, A.R. Fernandez-Alba, Determination of volatile organic compounds in drinking and environmental waters, TrAC Trends Anal. Chem. 32 (2012) 60–75. doi:10.1016/j.trac.2011.08.011.

[60] J. Robles-Molina, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Monitoring of

selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain, Sci. Total Environ. 479-480 (2014) 247–257. doi:10.1016/j.scitotenv.2014.01.121.

[61] M. Hromadová, P. Mořkovská, L. Pospíšil, S. Giannarelli, Decomposition reactions of bifenox anion radical involving intramolecular electron transfer, J. Electroanal. Chem. 582 (2005) 156–164. doi:10.1016/j.jelechem.2004.12.013.

[62] P.A. Souza Tette, L. Rocha Guidi, M.B. de Abreu Gloria, C. Fernandes, Pesticides in honey: A review on chromatographic analytical methods, Talanta. 149 (2015) 124–141. doi:10.1016/j.talanta.2015.11.045.

[63] D. Rood, A Practical Guide to the Care, Maintenance, and Troebleshooting of Capillary Gas Chromatography Systems, Third Edit, Wiley-VCH, 1999.

[64] J. V Hinshaw, T. Taylor, C. Trainer, T. Director, C.S. Moderator, D. Walsh, Split / Splitless Injection for Capillary GC.

[65] S. Mjøs, Lecture 2 of Gas Chromatography Module of EMQAL – Factors leading to separation, (2015).

[66] S. Mjøs, Lecture 3 of Gas Chromatography Module of EMQAL – Chromatographic efficiency, (2015).

[67] S. Mjøs, Lecture 4 of Gas Chromatography Module of EMQAL – Column dimensions, (2015).

[68] S. Mjøs, Lecture 5 of Gas Chromatography Module of EMQAL – Limits of classical chromatographic theory, (2015).

[69] E. De Hoffmann, V. Stroobant, Mass Spectrometry - Principles and Applications., 2007. doi:10.1002/mas.20296.

[70] M.C. Pietrogrande, G. Basaglia, GC-MS analytical methods for the determination of personal-care products in water matrices, TrAC Trends Anal. Chem. 26 (2007) 1086–1094. doi:10.1016/j.trac.2007.09.013.

[71] H. Shang, Y. Li, T. Wang, P. Wang, H. Zhang, Q. Zhang, et al., Chemosphere The presence of polychlorinated biphenyls in yellow pigment products in China with emphasis on 3 , 3 0 -dichlorobiphenyl (PCB 11), Chemosphere. 98 (2014) 44–50. doi:10.1016/j.chemosphere.2013.09.075.

[72] B.R. Ramaswamy, G. Shanmugam, G. Velu, B. Rengarajan, D.G.J. Larsson, GC–MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers, J. Hazard. Mater. 186 (2011) 1586–1593. doi:10.1016/j.jhazmat.2010.12.037.

[73] E. Moyano, Lecture of Mass Spectrometry Module of EMQAL. Introduction, (2015) 1–9.

[74] E. Moyano, Lecture of Mass Spectrometry Module of EMQAL. Components, (2015).

[75] E. Moyano, Lecture of Mass Spectrometry Module of EMQAL. Sample Inlet-Ion sources, (2015).

[76] E. Moyano, Lecture of Mass Spectrometry Module of EMQAL. Mass Analyzers, (2015).

[77] D.A. Skoog, F.J. Holler, T.A. Nieman, Principios de Análisis Instrumental, Principios Análisis Instrum. (2001) 607–843. doi:10.1017/CBO9781107415324.004.

[78] What-when-how.com, Quadrupole mass analyzers: theoretical and practical considerations, (n.d.). http://what-when-how.com/proteomics/quadrupole-mass-analyzers-theoretical-and-practical-considerations-proteomics/ (accessed 11 March 2016).

[79] F. Restek, Electron Multipliers for Mass Spectrometry The ETP Electron Multiplier Advantage.

[80] E. Moyano, Lecture of Mass Spectrometry Module of EMQAL. Tamdem Mass Analyzers, (2015).

[81] ISO/IEC 17025:2005(E) - General requirements for the competence of testing and

calibration laboratories, (2005).

[82] B. Magnusson and U. Örnemark (eds.), The Fitness for Purpose of Analytical Methods, Eurachem Guid. (1998) 1–61. doi:978-91-87461-59-0.

[83] L. Huber, A Primer: Validation of Analytical Methods, (2009).

[84] M. Thompson, S.L.R. Ellison, R. Wood, Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), Pure Appl. Chem. 74 (2002) 835–855. doi:10.1351/pac200274050835.

[85] European Medicines Agency ICH, ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, 2 (2006) 1–15. doi:10.1136/bmj.333.7574.873-a.

[86] International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology Q2(R1), (2005).

[87] Empresa Portuguesa das Águas Livres, SISTEMA DE GESTÃO DA QUALIDADE DOS LABORATÓRIOS DE ENSAIO PROCEDIMENTO TÉCNICO - PT 29.00 Validação de métodos de ensaio cromatográficos.

[88] ISO 8466-1:1990 - Water quality -- Calibration and evaluation of analytical methods and estimation of performance characteristics -- Part 1: Statistical evaluation of the linear calibration function.

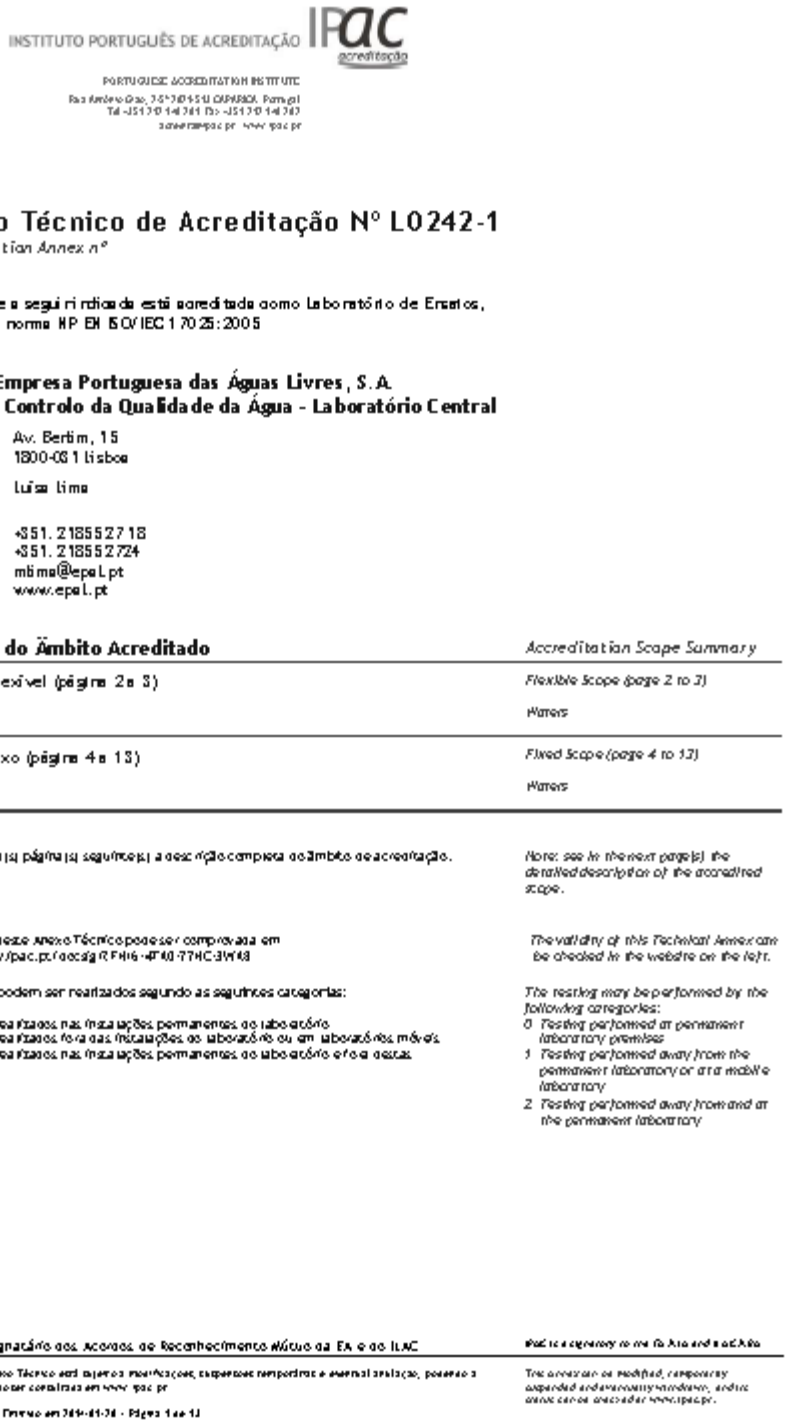
[89] ISO 11352:2002 Water quality - Estimation of measurement uncertainty based on validation and quality control data, (2002).

[90] Eurachem CITAC Guide CG, Quantifying Uncertainty in Analytical Measurement, English. 2nd (2000) 126. doi:0 948926 15 5.

[91] Empresa Portuguesa das Águas Livres, Determinação Quantitativa de Pesticidas por Cromatografía Gasosa associada à Espectrometria de Massa tandem (GC-MS/MS), (2014).

8. Annexes

8.1. Annex 1 – Accreditation Annex nº L0242-1, EPAL, 2014 (organic chemistry part)



INSTITUTO PORTUGUÊS DE ACREDITAÇÃO IPAC acreditação

PORTUGUESE ACCREDITATION INSTITUTE
Rua Antero de Quental, 135 3030-153 COIMBRA, Portugal
Tel. +351 239 140 360 Fax +351 239 140 367
secretariado@ipac.pt www.ipac.pt

Anexo Técnico de Acreditação Nº L0242-1
Accreditation Annex nº

A entidade a seguir indicada está acreditada como Laboratório de Ensaios, segundo a norma NP EN ISO/IEC 17025:2005

EPAL - Empresa Portuguesa das Águas Livres, S.A.
Direção Controlo da Qualidade da Água - Laboratório Central

Endereço / *Address*: Av. Berlim, 15, 1800-031 Lisboa
Contacto / *Contact*: Luísa Lima
Telefone: +351. 218552718
Fax: +351. 218552724
E-mail: mlima@epal.pt
Internet: www.epal.pt

Resumo do Âmbito Acreditado	*Accreditation Scope Summary*
Âmbito Flexível (página 2 a 3)	*Flexible Scope (page 2 to 3)*
Águas	*Waters*
Âmbito Fixo (página 4 a 13)	*Fixed Scope (page 4 to 13)*
Águas	*Waters*

Nota: ver na(s) página(s) seguinte(s) a descrição completa do âmbito de acreditação.

Note: see in the next page(s) the detailed description of the accredited scope.

A validade deste Anexo Técnico pode ser comprovada em http://www.ipac.pt/acesso/RFH16-4T40-77HC-3W48

The validity of this Technical Annex can be checked in the website on the left.

Os ensaios podem ser realizados segundo as seguintes categorias:

0 Ensaios realizados nas instalações permanentes do laboratório
1 Ensaios realizados fora das instalações do laboratório ou em laboratórios móveis
2 Ensaios realizados nas instalações permanentes do laboratório e fora delas

The testing may be performed by the following categories:
0 Testing performed at permanent laboratory premises
1 Testing performed away from the permanent laboratory or in a mobile laboratory
2 Testing performed away from and at the permanent laboratory

O IPAC é signatário dos Acordos de Reconhecimento Mútuo da EA e do ILAC

IPAC is a signatory to the EA and ILAC Mutual Recognition Agreements

O presente Anexo Técnico só pode ser objeto de modificações, suspensões ou retirada, parcial ou total, mediante decisão do IPAC, publicada em www.ipac.pt

This annex can be modified, temporary suspended or withdrawn, partially or totally, under decision of IPAC, published at www.ipac.pt.

Emissão nº 14 - Emitido em 2014-01-31 - Página 1 de 13

INSTITUTO PORTUGUÊS DE ACREDITAÇÃO IPAC acreditação
PORTUGUESE ACCREDITATION INSTITUTE
Rua António Gião, 2 2829-513 CAPARICA, Portugal
Tel. +351 210 948 100 Fax +351 210 948 101
geral@ipac.pt www.ipac.pt

Anexo Técnico de Acreditação Nº L0242-1

Accreditation Annex nº

EPAL - Empresa Portuguesa das Águas Livres, S.A.
Direção Controlo da Qualidade da Água - Laboratório Central

Este laboratório está acreditado para efectuar as determinações correspondentes à implementação da Matriz de competências a seguir apresentada.

O laboratório possui uma "Lista de Ensaios Acreditados" discriminando os ensaios abrangidos pela descrição flexível, permanentemente actualizada, e que será disponibilizada ou consultada a pedido.

Para cada tipo de ensaio é assinalado o tipo de flexibilidade aceite de acordo com os seguintes códigos:

Tipo A - Capacidade para implementar métodos normalizados
Tipo B - Capacidade para implementar métodos desenvolvidos e validados pelo laboratório

Nº	Tipo de Ensaio *Test Type*	Tipo de Produto *Product Type*			Categoria *Category*
		Águas Naturais	Águas de Consumo Humano	Águas de Processo	
ÁGUAS *WATERS*					
1	Análise de Compostos Orgânicos Voláteis por "Purge & Trap" e Cromatografia Gasosa associada à Espectrometria de Massa	B	B	-	0
2	Análise de Compostos Orgânicos Voláteis por Microextracção em Fase Sólida e Cromatografia Gasosa associada à Espectrometria de Massa	B	B	-	0
3	Análise Quantitativa de Compostos Orgânicos não específicos por Cromatografia Gasosa associada à Espectrometria de Massa	B	B	-	0
4	Determinação Quantitativa de Pesticidas: Método de Extracção em Fase Sólida e Cromatografia Gasosa associada à Espectrometria de Massa (SPE-GC-MS)	B	B	-	0
5	Determinação Quantitativa de Pesticidas: Método de Extracção em Fase Sólida e Cromatografia Líquida de Alta Eficiência associada à Espectrometria de Massa (SPE-LC-MS/MS)	B	B	-	0
6	Análise de Compostos Orgânicos Voláteis responsáveis pelo cheiro e Sabor por Microextracção em Fase Sólida e Cromatografia Gasosa associada à Espectrometria de Massa	B	B	-	0
7	Determinação Quantitativa de Pesticidas por Cromatografia Gasosa associada à Espectrometria de Massa tandem (GC-MS/MS)	B	B	-	0

INSTITUTO PORTUGUÊS DE ACREDITAÇÃO IPAC acreditação
PORTUGUESE ACCREDITATION INSTITUTE
Rua Antero de Quental, 136, 3000-033 COIMBRA, Portugal
Tel. +351 239 780 440 Fax +351 239 780 499
geral@ipac.pt www.ipac.pt

Anexo Técnico de Acreditação Nº L0242-1

Accreditation Annex nº

EPAL - Empresa Portuguesa das Águas Livres, S.A.
Direção Controlo da Qualidade da Água - Laboratório Central

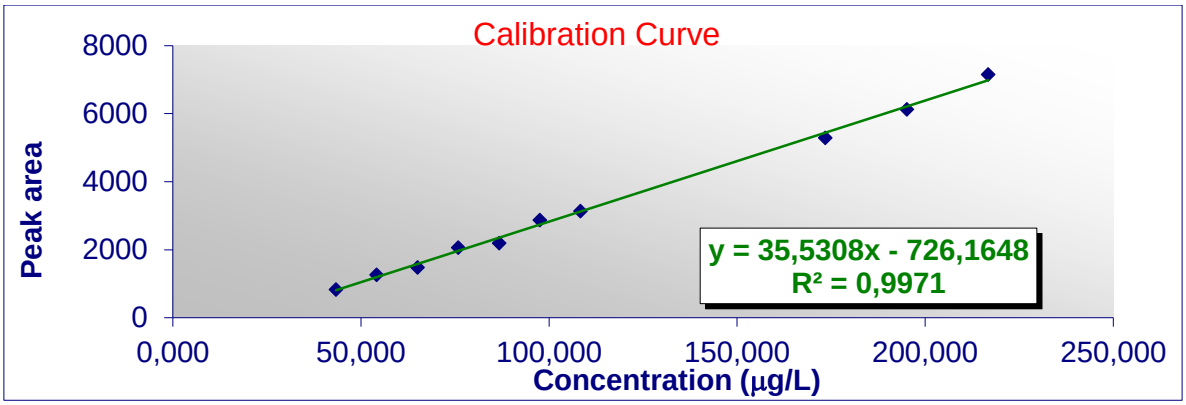
Nº	Tipo de Ensaio *Test Type*	Tipo de Produto *Product Type*			Categoria *Category*
		Águas Naturais	Águas de Consumo Humano	Águas de Processo	
ÁGUAS *WATERS*					
8	Determinação Quantitativa de Desreguladores Endócrinos por Cromatografia Líquida de Ultra Eficiência associada à Espectrometria de Massa (SPE-UPLC-ESI-MS/MS)	B	B	-	Q
9	Análise Quantitativa de Desreguladores Endócrinos por Cromatografia Gasosa associada à Espectrometria de Massa (GC-MS)	B	B	-	Q
10	Determinação Quantitativa de Trihalometanos e outros Haletos de Alquilo Detector de captura electrónica e sistema Head-Space	B	B	B	Q
11	Análise semi-quantitativa de compostos orgânicos não específicos por Cromatografia Gasosa associada à Espectrometria de Massa (GC-MS)	B	B	B	Q

O responsável pela gestão e implementação técnica desta matriz, nomeadamente pela aprovação da "lista de Ensaios Acreditados" é a Eng.ª Maria João Bertolet.

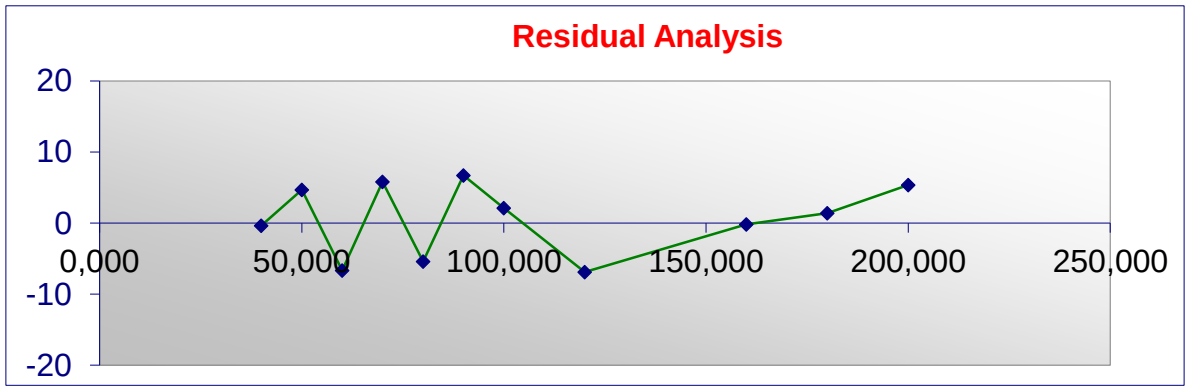
8.2. Anexa 2 – Residual test

- **Aclonifen**

Concentration (µg/L)	Peak area
43,36	835,54
54,20	1266,65
65,04	1476,20
75,88	2066,19
86,72	2198,58
97,56	2876,95
108,41	3132,62
173,45	5287,26
195,13	6124,26
216,81	7146,79
43,36	835,54

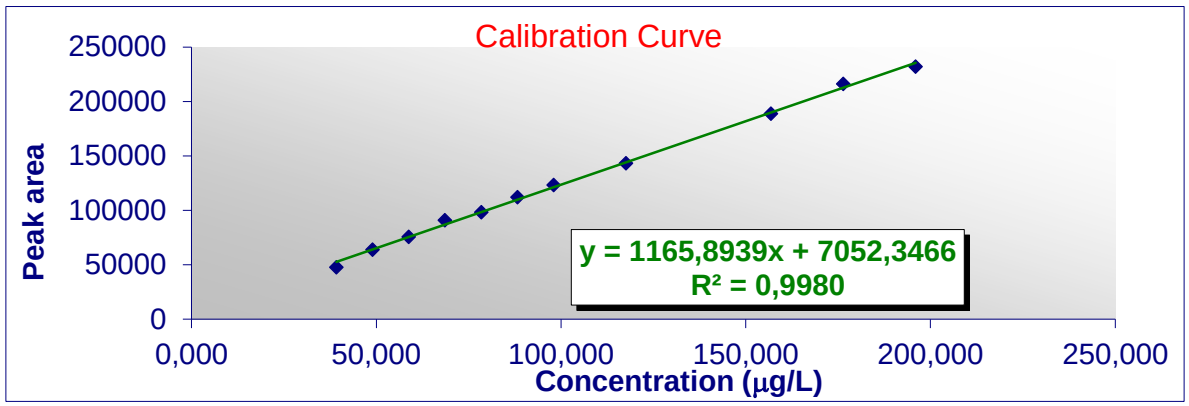


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
814,5	1,03	43,362	2,6
1199,7	1,06	54,203	5,6
1584,9	0,93	65,043	-6,9
1970,0	1,05	75,884	4,9
2355,2	0,93	86,724	-6,7
2740,4	1,05	97,565	5,0
3125,6	1,00	108,405	0,2
5436,6	0,97	173,448	-2,7
6206,9	0,99	195,129	-1,3
6977,3	1,02	216,810	2,4

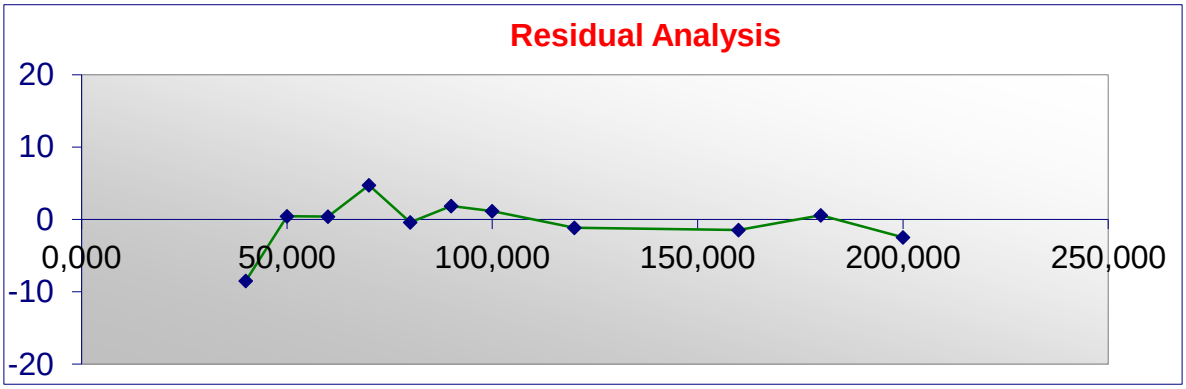


- **Biphenyl**

Concentration (µg/L)	Peak area
39,20	47641,36
49,00	63949,99
58,80	75586,69
68,61	91013,96
78,41	98134,86
88,21	112179,32
98,01	123137,80
117,61	143306,13
156,81	188691,02
176,41	215924,18
196,02	232073,46

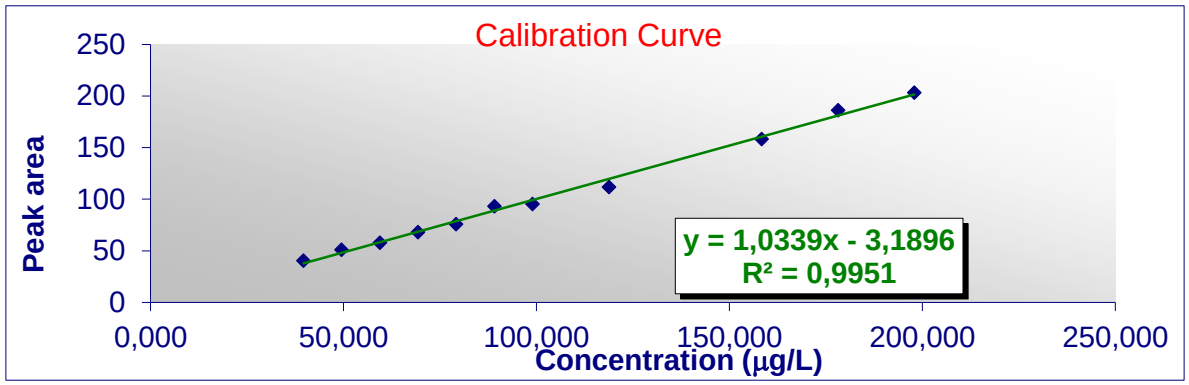


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
52758,9	0,90	39,203	-9,7
64185,5	1,00	49,004	-0,4
75612,2	1,00	58,805	0,0
87038,8	1,05	68,605	4,6
98465,4	1,00	78,406	-0,3
109892,1	1,02	88,207	2,1
121318,7	1,01	98,008	1,5
144172,0	0,99	117,609	-0,6
189878,5	0,99	156,812	-0,6
212731,8	1,02	176,414	1,5
235585,0	0,99	196,015	-1,5

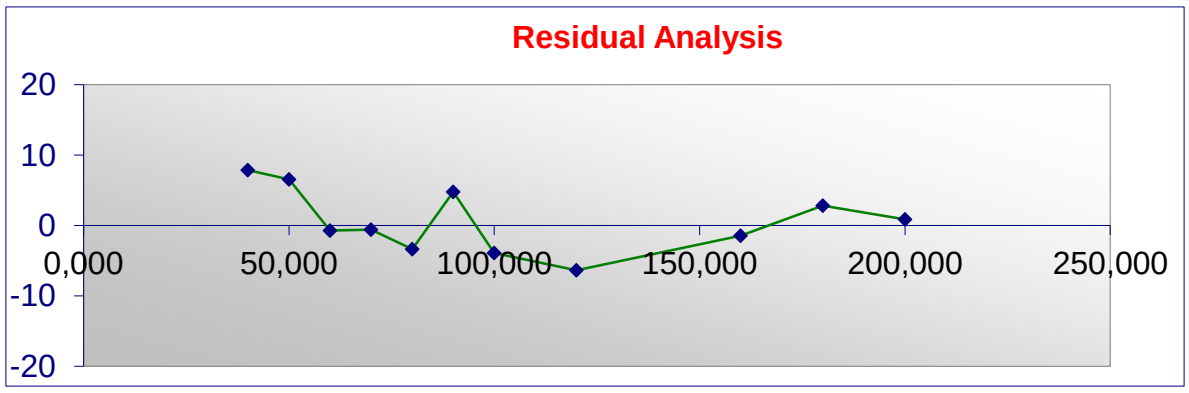


- **Chlorpyrifos**

Concentration (µg/L)	Peak area
39,597	40,71225916
49,49625	51,12516646
59,3955	57,80550264
69,29475	68,04850868
79,194	76,0398866
89,09325	93,18040099
98,9925	95,28618712
118,791	111,9996299
158,388	158,2546551
178,1865	186,1676424
197,985	203,2611143

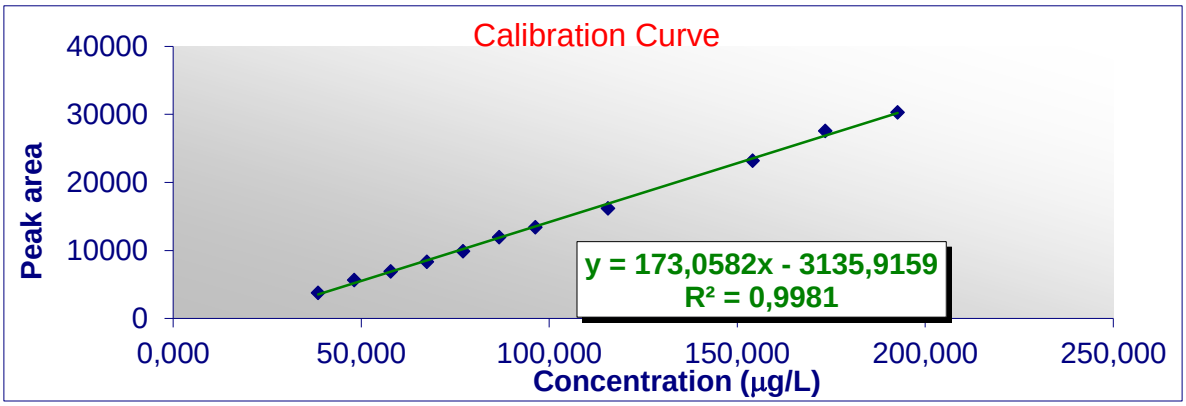


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
37,7	1,079	39,597	7,9
48,0	1,065	49,496	6,5
58,2	0,993	59,396	-0,7
68,5	0,994	69,295	-0,6
78,7	0,966	79,194	-3,4
88,9	1,048	89,093	4,8
99,2	0,961	98,993	-3,9
119,6	0,936	118,791	-6,4
160,6	0,986	158,388	-1,4
181,0	1,028	178,187	2,8
201,5	1,009	197,985	0,9

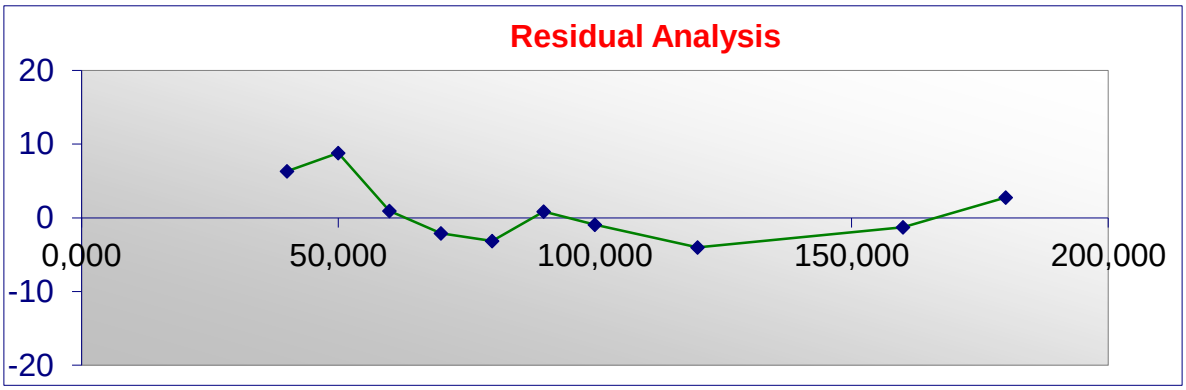


- **Cybutryne**

Concentration (µg/L)	Peak area
38,53	3771,33
48,17	5668,53
57,80	6938,39
67,43	8356,89
77,07	9881,45
86,70	11964,92
96,33	13403,05
115,60	16177,52
154,13	23207,01
173,40	27579,51
192,67	30277,27

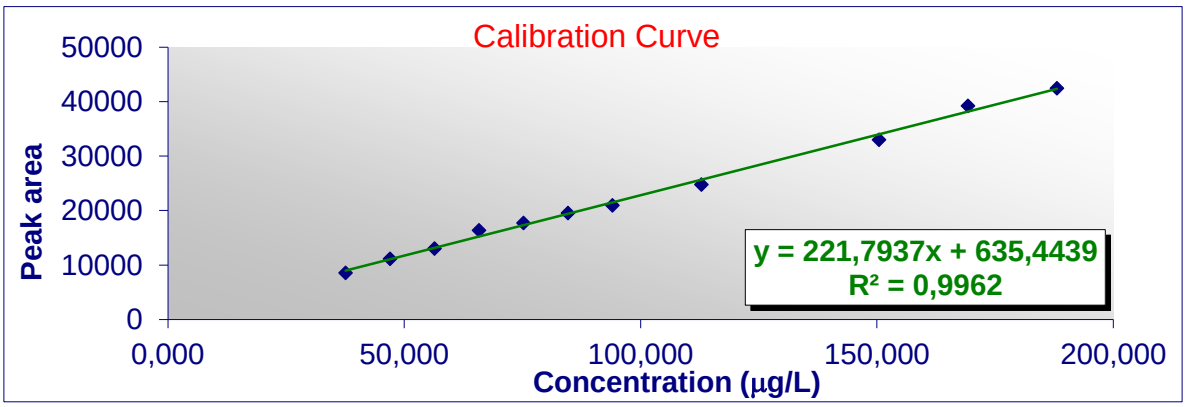


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
3532,6	1,07	38,534	6,8
5199,8	1,09	48,167	9,0
6866,9	1,01	57,800	1,0
8534,1	0,98	67,434	-2,1
10201,2	0,97	77,067	-3,1
11868,3	1,01	86,701	0,8
13535,5	0,99	96,334	-1,0
16869,7	0,96	115,601	-4,1
23538,3	0,99	154,134	-1,4
26872,6	1,03	173,401	2,6
30206,9	1,00	192,668	0,2

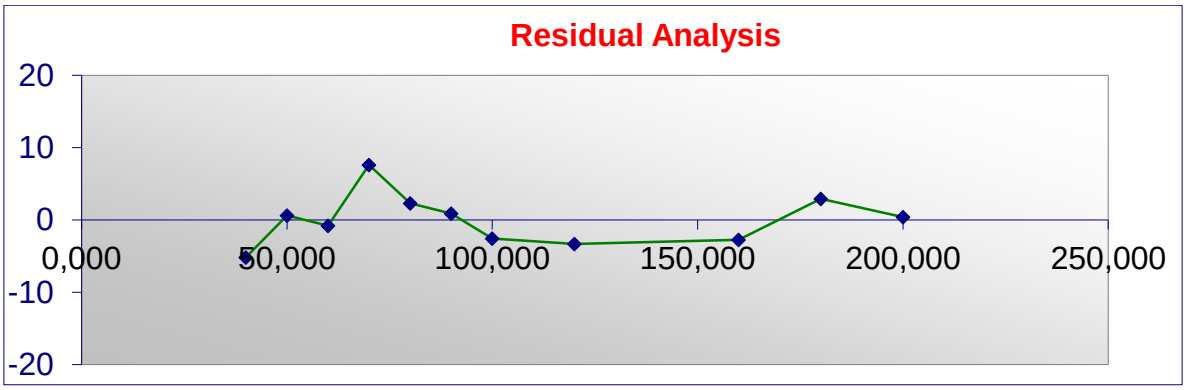


- **Dicofol**

Concentration (µg/L)	Peak area
37,62	8534,33
47,03	11146,87
56,43	13057,04
65,84	16397,44
75,24	17720,95
84,65	19575,35
94,05	20933,63
112,86	24787,05
150,48	33032,08
169,29	39234,35
188,10	42457,36

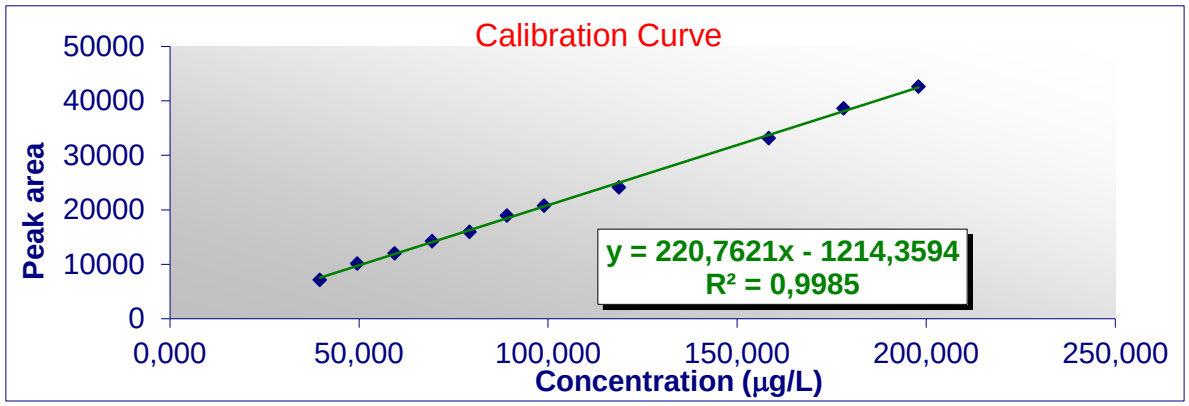


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
8979,3	0,95	37,620	-5,0
11065,3	1,01	47,025	0,7
13151,3	0,99	56,430	-0,7
15237,2	1,08	65,835	7,6
17323,2	1,02	75,240	2,3
19409,2	1,01	84,645	0,9
21495,1	0,97	94,050	-2,6
25667,1	0,97	112,860	-3,4
34011,0	0,97	150,480	-2,9
38182,9	1,03	169,290	2,8
42354,8	1,00	188,100	0,2

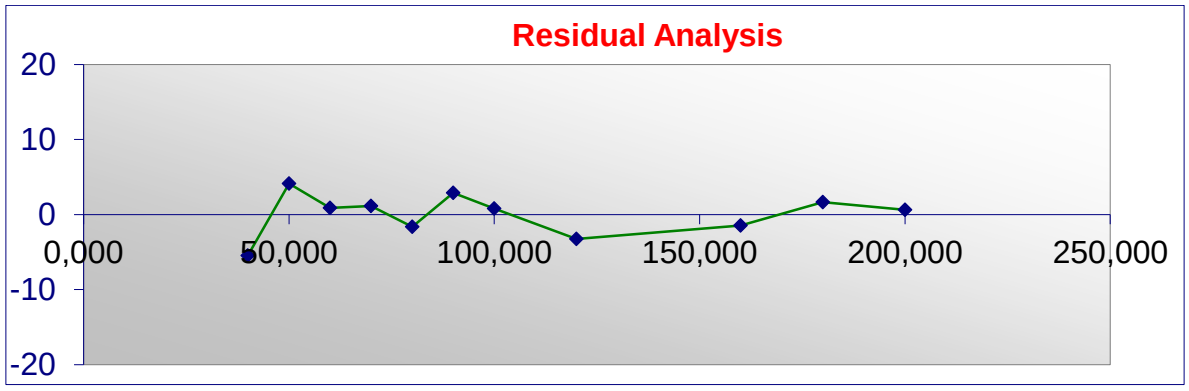


- **Quinoxyfen**

Concentration (µg/L)	Peak area
39,60	7145,97
49,50	10135,85
59,40	12019,45
69,30	14250,63
79,20	16000,43
89,10	18971,38
99,00	20786,79
118,80	24164,38
158,40	33188,78
178,20	38661,83
198,00	42654,20

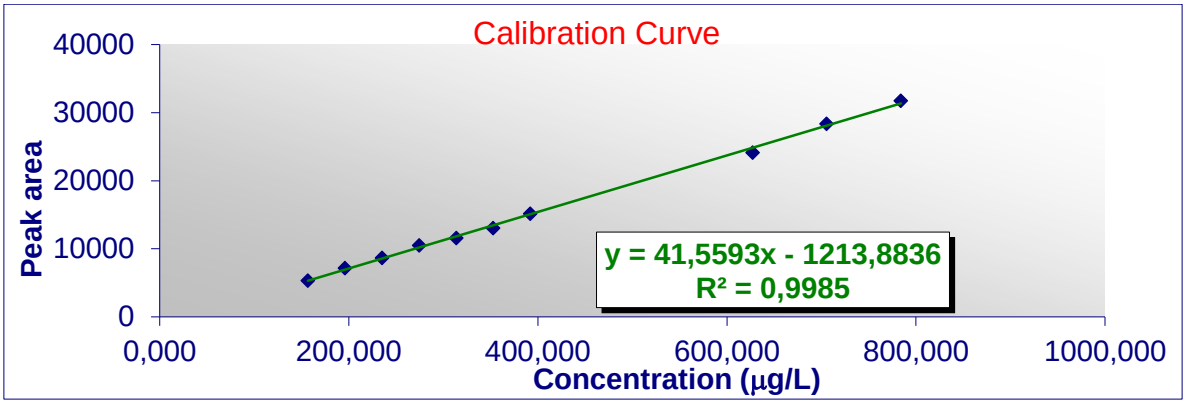


Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
7527,8	0,95	39,600	-5,1
9713,4	1,04	49,500	4,3
11898,9	1,01	59,400	1,0
14084,5	1,01	69,300	1,2
16270,0	0,98	79,200	-1,7
18455,5	1,03	89,100	2,8
20641,1	1,01	99,000	0,7
25012,2	0,97	118,800	-3,4
33754,4	0,98	158,400	-1,7

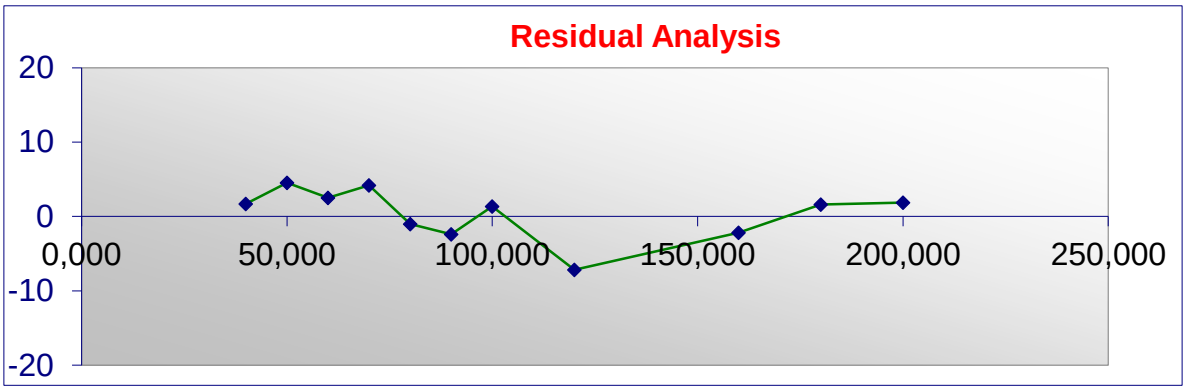


- **Cypermethrines**

Concentration (µg/L)	Peak area
156,80	5308,84
196,00	7152,93
235,19	8674,43
274,39	10506,94
313,59	11588,04
352,79	13009,26
391,99	15153,07
627,18	24143,81
705,58	28371,08
783,98	31748,45
156,80	5308,84



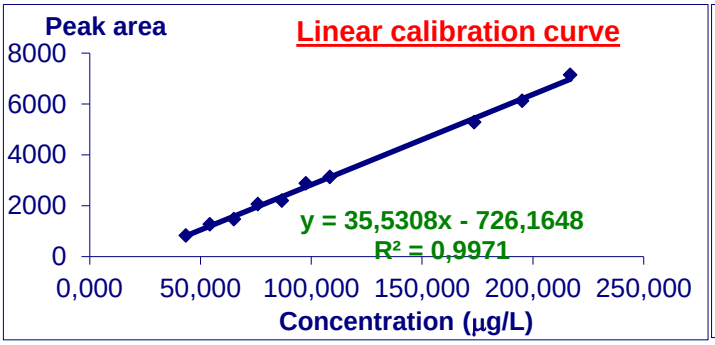
Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
5302,5	1,00	156,796	0,1
6931,5	1,03	195,995	3,2
8560,6	1,01	235,194	1,3
10189,7	1,03	274,393	3,1
11818,8	0,98	313,592	-2,0
13447,9	0,97	352,791	-3,3
15077,0	1,01	391,990	0,5
24851,5	0,97	627,184	-2,8
28109,6	1,01	705,582	0,9
31367,8	1,01	783,980	1,2

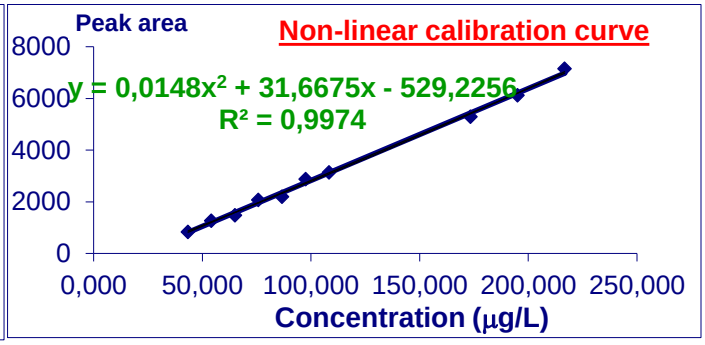

Residual Analysis
20
10
0
-10
-20
0,000
50,000
100,000
150,000
200,000
250,000

8.3. Annex 3 – Mandel Test

- **Aclonifen**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
43,36	835,54	815	872
54,20	1266,65	1200	1231
65,04	1476,20	1585	1593
75,88	2066,19	1970	1959
86,72	2198,58	2355	2328
97,56	2876,95	2740	2701
108,41	3132,62	3126	3078
173,45	5287,26	5437	5409
195,13	6124,26	6207	6214
216,81	7146,79	6977	7032





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 2,600E+05

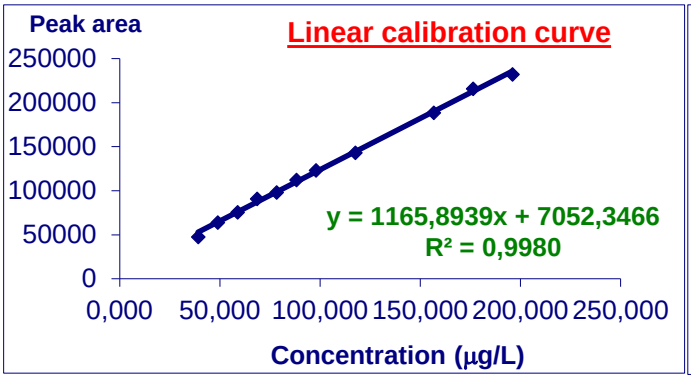
$$VT = DS^2 / S^2_{y/x(2^o)}$$

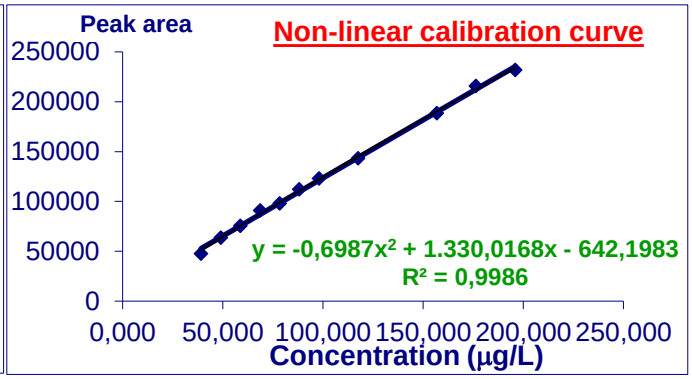
$VT =$ 5,274E+00

$F_{(1,6)95\%} =$ 5,32

- **Biphenyl**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
39,20	47641,36	52759	50425
49,00	63949,99	64186	62856
58,80	75586,69	75612	75153
68,61	91013,96	87039	87315
78,41	98134,86	98465	99344
88,21	112179,32	109892	111238
98,01	123137,80	121319	122998
117,61	143306,13	144172	146115
156,81	188691,02	189878	190739
176,41	215924,18	212732	212246
196,02	232073,46	235585	233216





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 2,328E+07

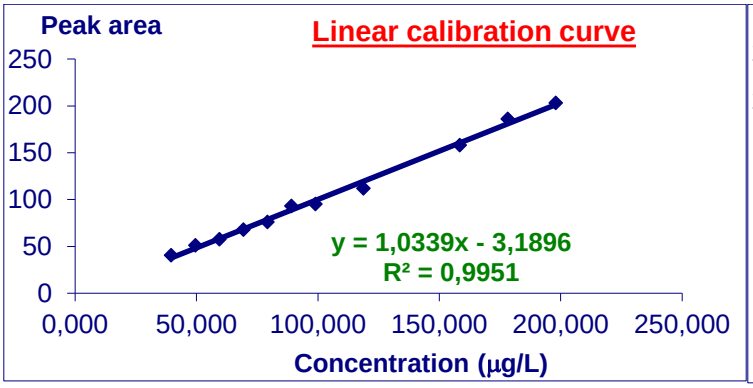
$$VT = DS^2 / S^2_{y/x(2^o)}$$

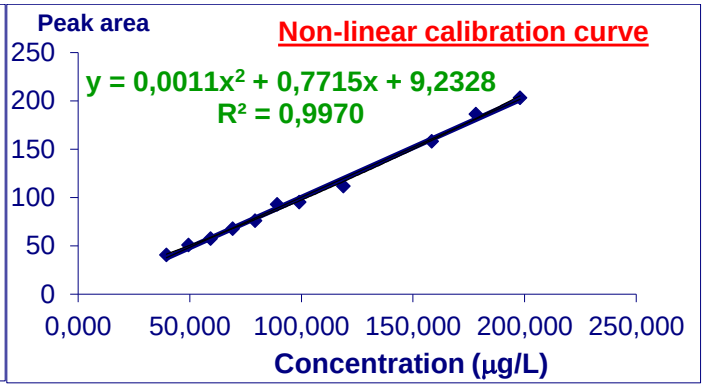
$VT =$ 3,57

$F_{(1,6)95\%} =$ 5,32

- **Chlorpyrifos**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
39,60	40,71	38	42
49,50	51,13	48	50
59,40	57,81	58	59
69,29	68,05	68	68
79,19	76,04	79	77
89,09	93,18	89	87
98,99	95,29	99	96
118,79	112,00	120	116
158,39	158,25	161	159
178,19	186,17	181	182
197,99	203,26	202	205





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 6,055E+01

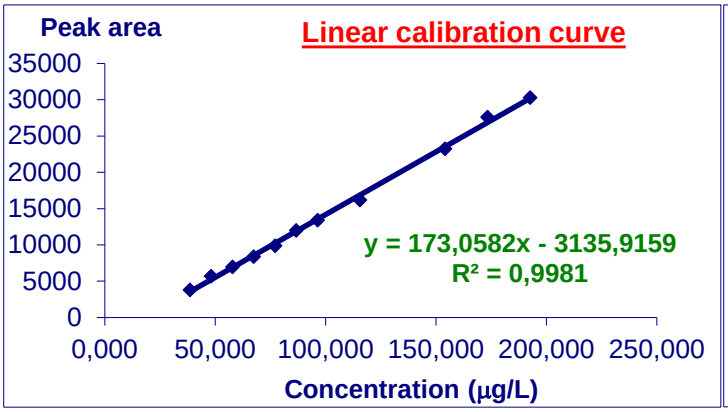
$$VT = DS^2 / S^2_{y/x(2^o)}$$

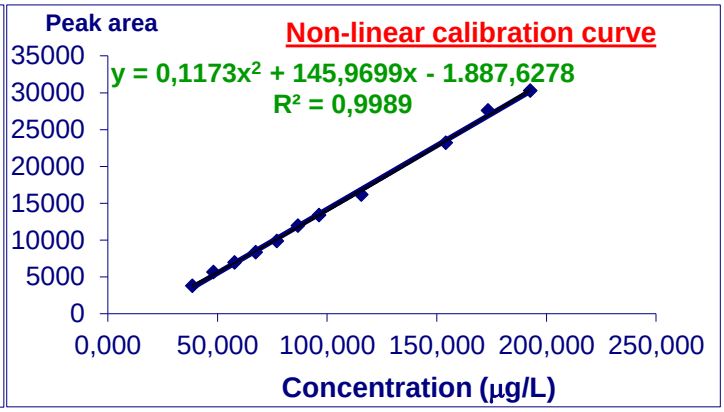
$VT =$ 5,293E+00

$F_{(1,6)95\%} =$ 5,32

- **Cybutryne**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
38,53	3771,33	3533	3911
48,17	5668,53	5200	5415
57,80	6938,39	6867	6941
67,43	8356,89	8534	8489
77,07	9881,45	10201	10059
86,70	11964,92	11868	11650
96,33	13403,05	13535	13263
115,60	16177,52	16870	16554
154,13	23207,01	23538	23398
173,40	27579,51	26873	26951
192,67	30277,27	30207	30590





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 6,127E+05

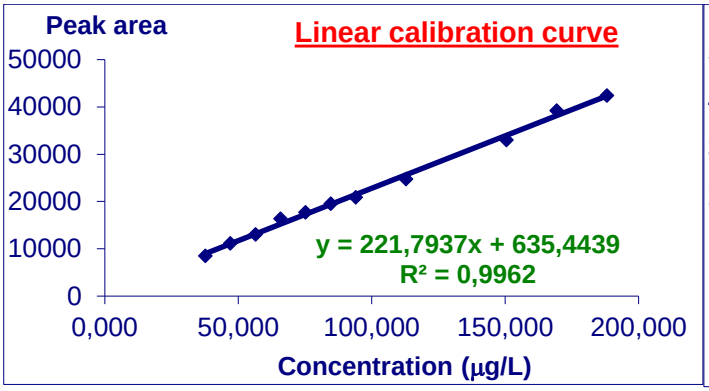
$$VT = DS^2 / S^2_{y/x(2^o)}$$

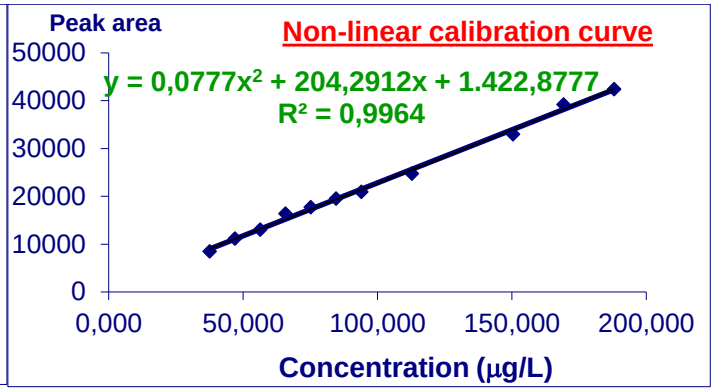
VT= 5,309E+00

$F_{(1,6)95\%} =$ 5,32

- **Dicofol**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
37,62	8534,33	8979	9218
47,03	11146,87	11065	11201
56,43	13057,04	13151	13198
65,84	16397,44	15237	15209
75,24	17720,95	17323	17234
84,65	19575,35	19409	19272
94,05	20933,63	21495	21324
112,86	24787,05	25667	25469
150,48	33032,08	34011	33924
169,29	39234,35	38183	38234
188,10	42457,36	42355	42599





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 2,438E+05

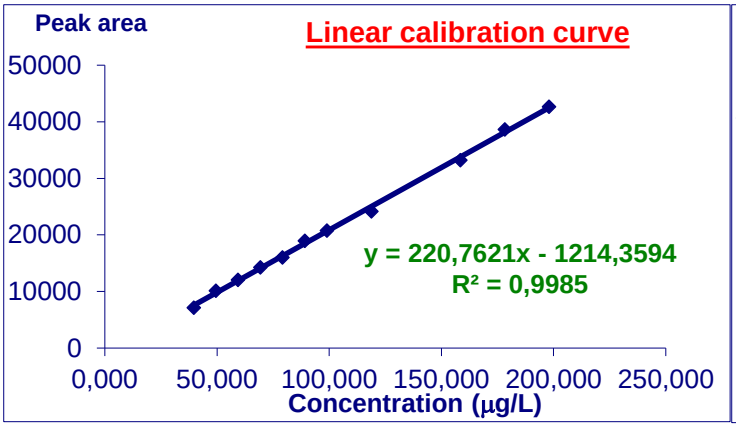
$$VT = DS^2 / S^2_{y/x(2^o)}$$

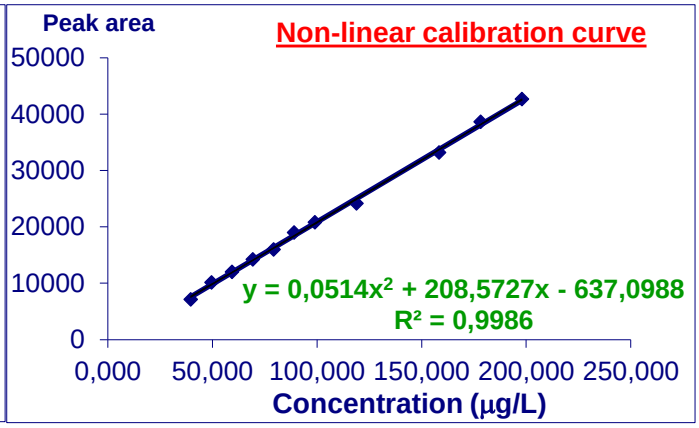
VT= 4,180E-01

$F_{(1,6)95\%} =$ 5,32

- **Quinoxyfen**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
39,60	7145,97	7528	7703
49,50	10135,85	9713	9813
59,40	12019,45	11899	11933
69,30	14250,63	14084	14064
79,20	16000,43	16270	16204
89,10	18971,38	18456	18355
99,00	20786,79	20641	20515
118,80	24164,38	25012	24867
158,40	33188,78	33754	33690
178,20	38661,83	38125	38163
198,00	42654,20	42497	42675





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 1,310E+05

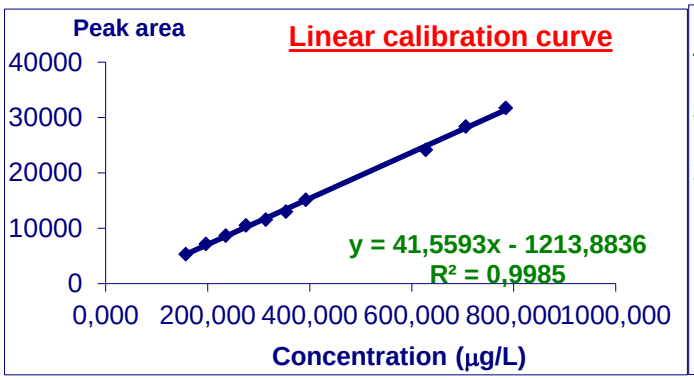
$$VT = DS^2 / S^2_{y/x(2^o)}$$

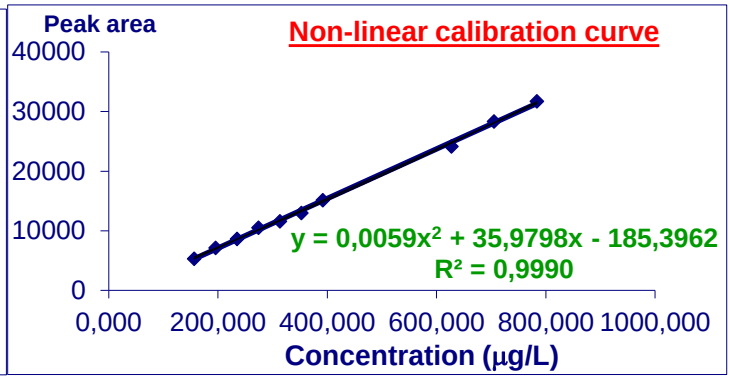
$VT =$ 0,538

$F_{(1,6)95\%} =$ 5,32

- **Cypermethrines**

Concentration (mg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
156,80	5308,84	5302	5601
196,00	7152,93	6932	7093
235,19	8674,43	8561	8603
274,39	10506,94	10190	10131
313,59	11588,04	11819	11678
352,79	13009,26	13448	13242
391,99	15153,07	15077	14825
627,18	24143,81	24851	24701
705,58	28371,08	28110	28139
783,98	31748,45	31368	31648





$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

$DS^2 =$ 3,480E+05

$$VT = DS^2 / S^2_{y/x(2^o)}$$

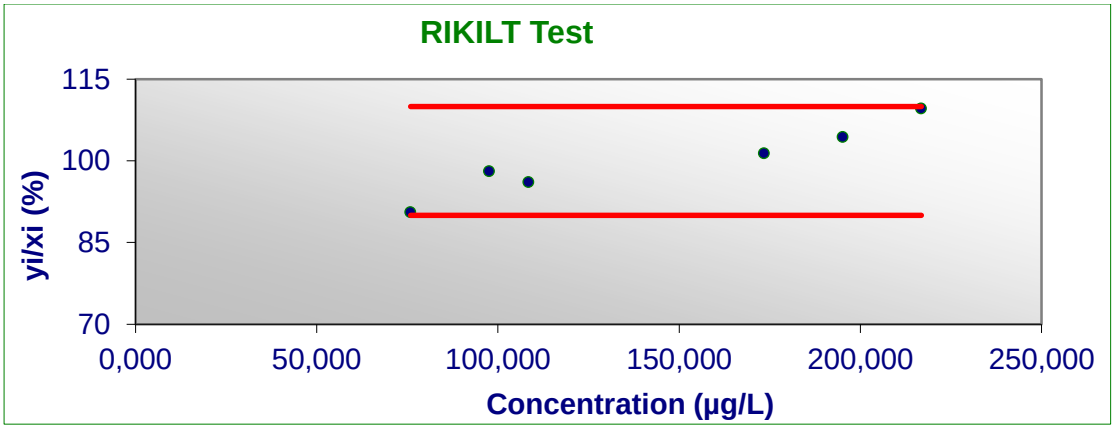
$VT =$ 3,122

$F_{(1,6)95\%} =$ 5,59

8.4. Annex 4 – Rikilt Test

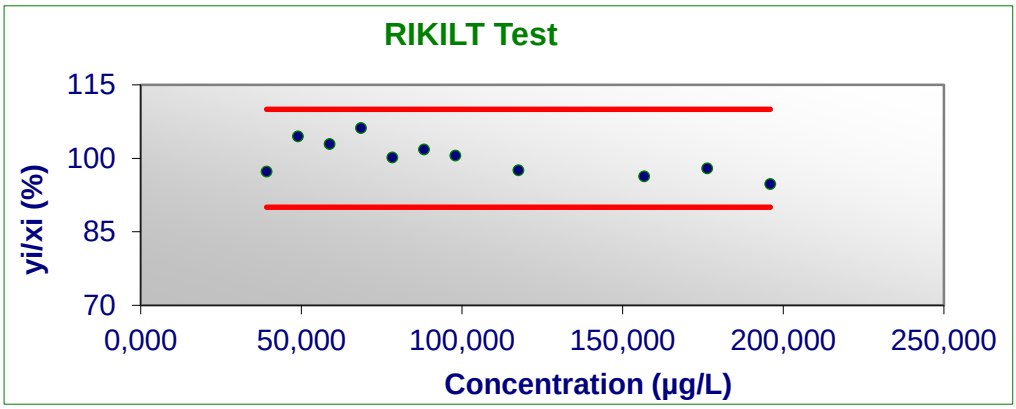
- **Aclonifen**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
75,88	2066,19	27,2	91	110	90
97,56	2876,95	29,5	98	110	90
108,41	3132,62	28,9	96	110	90
173,45	5287,26	30,5	101	110	90
195,13	6124,26	31,4	104	110	90
216,81	7146,79	33,0	110	110	90
	Average	**30,1**			



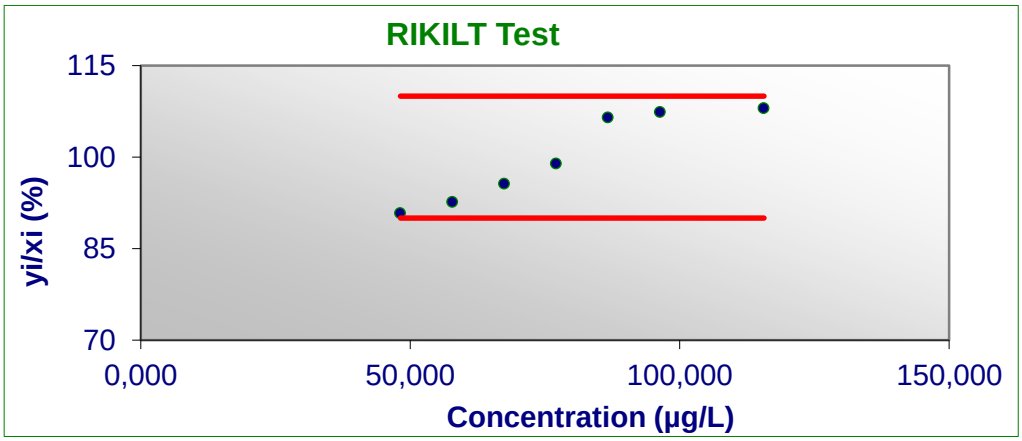
- **Biphenyl**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
39,20	47641,36	1215,2	97	110	90
49,00	63949,99	1305,0	104	110	90
58,80	75586,69	1285,4	103	110	90
68,61	91013,96	1326,6	106	110	90
78,41	98134,86	1251,6	100	110	90
88,21	112179,32	1271,8	102	110	90
98,01	123137,80	1256,4	101	110	90
117,61	143306,13	1218,5	98	110	90
156,81	188691,02	1203,3	96	110	90
176,41	215924,18	1224,0	98	110	90
196,02	232073,46	1184,0	95	110	90
	Average	**1249,3**			



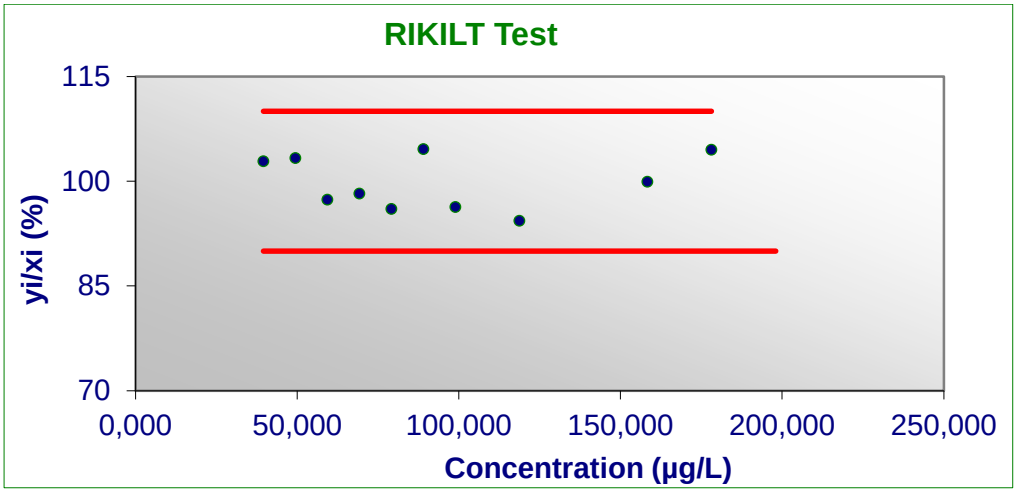
- **Cybutryne**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
48,167	5668,52773	117,7	91	110	90
57,8004	6938,386582	120,0	93	110	90
67,4338	8356,892614	123,9	96	110	90
77,0672	9881,445175	128,2	99	110	90
86,7006	11964,92341	138,0	107	110	90
96,334	13403,0523	139,1	107	110	90
115,6008	16177,52371	139,9	108	110	90
	Average	**129,6**			



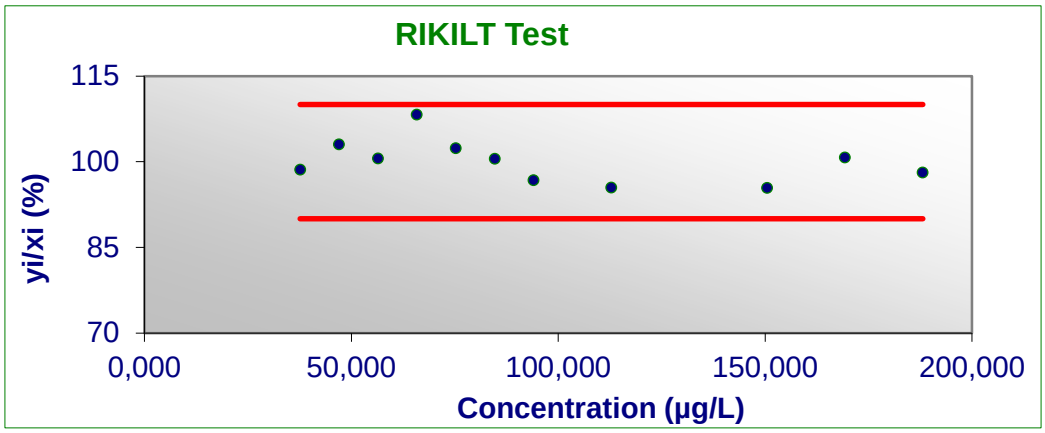
- **Chlorpyrifos**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
39,597	40,71225916	1,028	103	110	90
49,49625	51,12516646	1,033	103	110	90
59,3955	57,80550264	0,973	97	110	90
69,29475	68,04850868	0,982	98	110	90
79,194	76,0398866	0,960	96	110	90
89,09325	93,18040099	1,046	105	110	90
98,9925	95,28618712	0,963	96	110	90
118,791	111,9996299	0,943	94	110	90
158,388	158,2546551	0,999	100	110	90
178,1865	186,1676424	1,045	104	110	90
197,985	203,2611143	1,027	103	110	90
	Average	**1,000**			



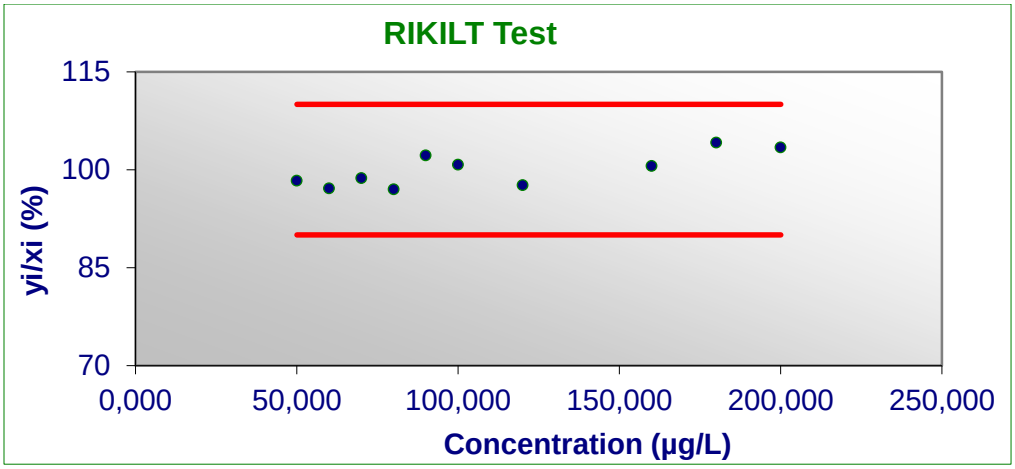
- **Dicofol**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
37,62	8534,33	226,9	99	110	90
47,025	11146,87	237,0	103	110	90
56,43	13057,04	231,4	101	110	90
65,835	16397,44	249,1	108	110	90
75,24	17720,95	235,5	102	110	90
84,645	19575,35	231,3	101	110	90
94,05	20933,63	222,6	97	110	90
112,86	24787,05	219,6	95	110	90
150,48	33032,08	219,5	95	110	90
169,29	39234,35	231,8	101	110	90
188,1	42457,36	225,7	98	110	90
	Average	**230,0**			



- **Quinoxyfen**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
49,50	10135,85	204,8	100	110	90
59,40	12019,45	202,3	99	110	90
69,30	14250,63	205,6	100	110	90
79,20	16000,43	202,0	99	110	90
89,10	18971,38	212,9	104	110	90
99,00	20786,79	210,0	103	110	90
118,80	24164,38	203,4	99	110	90
158,40	33188,78	209,5	102	110	90
178,20	38661,83	217,0	106	110	90
198,00	42654,20	215,4	105	110	90
	Average	**204,8**			



- **Cypermethrines**

Concentration (mg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
156,80	5308,84	33,9	90	110	90
196,00	7152,93	36,5	97	110	90
235,19	8674,43	36,9	98	110	90
274,39	10506,94	38,3	102	110	90
313,59	11588,04	37,0	98	110	90
352,79	13009,26	36,9	98	110	90
391,99	15153,07	38,7	102	110	90
627,18	24143,81	38,5	102	110	90
705,58	28371,08	40,2	107	110	90
783,98	31748,45	40,5	107	110	90
	Average	**37,7**