João Eduardo Rodrigues Sedas Brinco

Bsc in Chemistry

Determination of pesticides in post-consumer containers by GC/TOFMS

Dissertation submitted in partial fulfillment
of the requirements for the degree of

Master of Science in
Bioorganic Chemistry

Adviser: Marco Diogo Richter Gomes da Silva, Assistant Professor with habilitation, NOVA University of Lisbon

Examination Committee

Chair: Paula Cristina de Sério Branco, Assistant Professor with habilitation, NOVA University of Lisbon

Rapporteur: Maria João Pires de Bastos Cabrita, Associated Professor with habilitation, University of Évora

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[1] https://github.com/joaomlourenco/novathesis [2] http://www.di.fct.unl.pt

handa dāni, bhikkhave, āmantayāmi vo,
vayadhammā sankhārā appamādena sampādethā.

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Abstract

Modern agriculture is entirely dependent upon the use of pesticides. The need for high ammounts of food, and discontinuation of traditional agricultural techniques have made these compounds a staple in food production all over the world. Pesticides are commercially available both in solid form and liquid solutions. In portugal, the volume of pesticides sold in 2018 translates into more than 740 tons of packaging material. Most of these materials are plastic containers, the remainder being mainly cardboard and metal. After use, the containers may still have relatively high ammounts of toxic pesticides, which renders them hazardous.

The triple rinse practice has been proven to reduce the ammount of pesticides in the packaging, in order to allow for them to be recycled, or to other ends. This technique consists in rinsing the empty pesticide container three times with water, and using it for application to crops. In portugal there is an ongoing campaign by Valorfito to encourage the triple rinse, and a way to monitor the concentration of leftover pesticides in the packages is necessary, in order to gauge the addherence to this practice.

This work describes the development of a methodoloy for the determination of 22 pesticides in the empty containers, by gas chromatography coupled to time-of-flight mass-spectrometry (GC/TOFMS). The samples were milled to a fine powder, and extracted with tetrahydrofuran and a mixture of tetrahydrofuran with 1,1,1,3,3,3-hexafluoro-2-propanol, by sonication. Calibrations were performed in the 0.2-2.5 $\mu g/mL$ range, depending on the analyte, and the coefficient of determination (R^2) was above 0.982 for all compounds except acetamiprid. The recoveries obtained ranged from 86-106% except for Captan and Folpet, whose values are lower, presumably because of degradation.

Four collections of pesticide containers were made from 2018-2020, and the results show a much lower quantity of the monitored pesticides in the last sample, from 2020, which is indicative that the triple rinse practice is being adopted.

Keywords: Pesticides, Post-consumer containers, GC/TOFMS.

Resumo

A agricultura moderna é inteiramente dependente do uso de fitofármacos. A necessidade de elevado volume de alimentos, e descontinuação de métodos agrícolas tradicionais, fizeram com que estes compostos se tornassem comuns na produção de comida em todo o mundo. Os fitofármacos são vendidos em formulações sólidas ou líquidas. Em Portugal, o volume de produtos fitofarmacêuticos vendidos em 2018 traduz-se em mais de 740 toneladas de embalagens. A maioria destes materiais são compostos por plástico, sendo o resto essencialmente cartão e metal. Após o uso, as embalagens podem ainda ter quantidades relativamente altas de pesticidas tóxicos, o que as torna perigosas.

Foi provado que o método da tripla lavagem reduz a quantidade de fitofármacos nas embalagens, permitindo que sejam recicladas, ou que tenham outros fins. Este método consiste em lavar as embalagens vazias três vezes com água, e usá-la para aplicação na cultura. Em Portugal, há uma campanha por parte da Valorfito para encorajar os agricultores a aderirem à tripla lavagem; desta forma, é necessária uma metodologia para monitorizar a quantidade de pesticidas nas embalagens usadas.

O presente trabalho descreve o desenvolvimento de uma metodologia analitica para a determinação de 22 fitofármacos nas embalagens vazias, por cromatografia gasosa acoplada a espetrometria de massa tempo-de-voo (GC/TOFMS). As amostras foram trituradas até um pó fino, e extraídas com tetrahidrofurano e uma mistura de tetrahidrofurano com 1,1,1,3,3,3-hexafuoro-2-propanol, usando ultra-sons. As calibrações foram feitas entre 0.2-2.5 $\mu g/mL$, dependendo do analito, e o coeficiente de determinação (R^2) obtido foi acima de 0.982 para todos os compostos, excepto a acetamiprida. As recuperações obtidas foram entre 86-106%, excepto parao captano e folpet, cujos valores foram mais baixos, presumivelmente devido a degradação.

Foram realizadas quatro coletas de embalagens de fitofármacos entre 2018-2020, e os resultados mostram uma quantidade menor da soma de fitofármacos monitorizados na última amostra, de 2020, indicando que a prática da tripla lavagem está a ser adotada.

Palavras-chave: Fitofármacos, embalagens de fitofármacos usadas, GC/TOFMS.

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Glossary

Accuracy	How close a measurement is to a reference value.
Analyte	The chemical compound of interest in any analysis. An analytical process may have one or more target analytes.
Analytical Process	The entire procedure by which a measurement of a certain analyte is made.
Band (chromatography)	The space occupied by a particular compound inside the column. The band becomes a peak when it elutes and is detected.
Eluate	Fluid (liquid or gas), emerging from the end of a chromatographic column.
Glass transition temperature	The temperature at which an amorphous solid changes from brittle and glassy to rubbery and elastic.
Half-life	The time it takes for a quantity to reduce to half of its original value.
Matrix	The components of a sample other than the analyte(s).
Mechanical Strength	Ammount of stress required to break a material.
Median lethal dose (LD_{50})	The amount of a certain substance necessary to kill half of a tested population of a certain species, over a specified duration.
Pesticide Residue	A collection of chemical compounds with toxicological relevance, which may be present in a product as a result of the usage of a particular pesticide. This includes the pesticide itself, degradation products and metabolites.
Precision	A measure of how close results are to one another.

Ruggedness	A measure of the hability of a method to remain unaffected by small changes in variables such as temperature, extraction time, etc.
Selectivity	The extent to which a compound present in a complex matrix can be detected without interference from the other compounds. It is usually used in a qualitative sense.
Sensitivity	The degree to which the signal increases with analyte quantity. Usually expressed as the slope in a calibration curve.
Trace analysis	Analysis of an element that is in small concentration within the matrix

Acronyms

%RSD	Percent Relative Standard Deviation
P_{OW}	Octanol-water partition coefficient
t'_r	Adjusted retention time
t_m	Dead time
t_r	Retention time
ECD	Electron capture detector
EI	Electron ionization
EU	European union
FID	Flame ionization detector
GC	Gas chromatography
GCxGC	Comprehensive two dimensional gas chromatography
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
HPLC	High-pressure liquid chromatography (or high-performance liquid chromatography)
ID	Internal diameter
LD_{50}	Median lethal dose
LoD	Limit of detection
LoQ	Limit of quantitation
MS	Mass spectrometry
POP	Persistent organic pollutant
ppb	Part per billion (1 per 10^9)
ppm	Part per million (1 per 10^6)

QuEChERS	Quick, easy cheap, effective, rugged and safe. A sample preparation method for the analysis of pesticides in food
s	Standard deviation
Tg	Glass transition temperature
THF	Tetrahydrofuran
TIC	Total ion chromatogram (or total ion current)
TOF-MS	Time of flight mass spectrometry
XIC	Extracted ion chromatogram

Chapter 1

Introduction

1.1 Pesticides in our industrial society

The modern world is entirely dependent upon technology. As we move to a more and more specialized society, the degree of inter-connecteness and inter-dependentness an individual experiences is ever increasing. Traditionally, humankind has relied on technology passed down from older generations (knowledge about farming, hunting, building, etc.). Even though individuals by themselves could not accomplish much, a small community comprised of workers with different masteries could produce most, if not all, of the things they needed to survive. However, since there was hardly any mechanical power available, all pre-industrial large civilizations would have had to employ human power in their endeavours. Therefore, "A vast proportion of mankind in the early civilizations were employed in purely mechanical drudgery" [1].

This paradigm changed with the dawn of the industrial age. As traditional labour was replaced by cheap mechanical power, the need for job specialization rose, and individuals became more and more dependent on an increasingly complex net of many different technologies, of which they have no knowledge.

A consequence of the advancement of industrial society is that people become increasingly estranged from the technologies and production processes they need to survive. Take the automobile, for example: nearly all city dwellers nowadays rely on some form of motorized vehicle. However, virtually none have the necessary skills to produce, or even repair them. This fact is inherent to a technological society.

The increased reliance on more complex forms of technology is also a feature of our society. As stated by Theodore Kaczynski: "When a new item of technology is introduced as an option that an individual can accept or not as he chooses, it does not necessarily REMAIN optional. In many cases the new technology changes society in such a way that

people eventually find themselves FORCED to use it" [2].

This is very much the case with pesticides. These compounds, intended to control pests in agricultural production, are widely and almost universally used, in all kinds of crops. Being chemical compounds designed to exert a (usually negative) effect on some biological species or group, pesticides have the potential of creating many problems for the environment and humans alike. However, any dialogue about the dangers of pesticides must be preceded by the assertion that they are invariably necessary to modern society.

The necessity of pesticides (as well as fertilizers) arises from an enormous demand for food. The exponential growth of Human population (itself predicated upon the increased availability of food), and the migration to urban areas, have made intensive farming inevitable and irreplaceable. A stable supply of food is necessary to feed the ever increasing amount of urban dwellers, who by themselves have no capability whatsoever to produce food. Moreover, pesticides protect against several health hazards that can come with pests. Therefore, it is clear that pesticides are very much intertwined and inseparable from 21^{st} century society.

1.1.1 Pesticide toxicity and environmental problems

It is well known that pesticides carry health and environmental hazards. An ideal pesticide should be effective in controlling a target organism, while being safe to other organisms and the environment. However, this is usually not the case. Chemical pesticides have very strict rules regarding their use, and these aim to protect the consumer from potentially toxic amounts of pesticides. Also, farmers themselves have safety guidelines intended to protect them. In developed countries, farmers tend to be aware of the health threat that pesticides represent, and are likely to be cautious when using them [3].

The effect on the environment is not so easily bypassed. Recently, there have been many works published on the toxicity of pesticides in bees [4, 5]. The worldwide decline in the number of bees is a serious ecological and economic problem, especially because of these insects' role in pollination. And although this problem is not caused entirely by pesticides, it is mostly agreed that they have had an important contribution [6].

Although the term "pesticide" may carry a negative connotation, it encompasses a very large number of compounds, and it is important to note that some will be more toxic than others. Potassium salts of fatty acids, for example, are used as insecticides and acaricides. These are some of the lesser toxic pesticides [7].

Government bodies are in charge of regulating the use of pesticides, and may withdraw the usage of a particular pesticide, if it is shown to have a high risk. Recently, the European Union has removed the approval of the commonly used herbicide Diquat, for example [8].

In order to safeguard public health and the environment, it is essential to have a method of characterizing pesticides according to their toxicity, and possible side effects.

The World Health organization has such a method, based on the median lethal dose (LD_{50}). Since there is no data for humans (as human testing is illegal), the values used are LD_{50} of mice, except when another animal is more suitable (due to presenting a smaller tolerance). For mixtures of pesticides, an arithmetic average is calculated, based on each active ingredient's LD_{50} [9].

This method of classification is somewhat anthropocentric, because its objective is to provide an estimate of human toxicity, based on similar animals reported LD_{50}. It provides very little insight into toxicity towards insects, birds, or aquatic life.

Other very common method of gauging a pesticide's toxicity (and indeed any chemical substance), is by its **G**lobally **H**armonized **S**ystem of Classfication and Labelling of Chemicals (GHS) hazard statement. GHS is the standard nomenclature, internationally agreed upon, to label chemicals. The hazard statements are used to label a chemical's possible dangers, whether they be physical, health, or environmental. This method is more general and inclusive than the (LD_{50}), but does not provide specific values (it is by nature qualitative). Diquat, for example, has nine hazard labels, namely: H302, H315, H317, H319, H330, H335, H372, H400 and H410 (a list of relevant hazard statements (for pesticides) and corresponding codes is provided in Appendix B).

1.2 Post-consumer pesticide containers

Pesticides are sold in a variety of different formulations. These are extremely important, because when the farmer dilutes the product to apply it, he must assume that the mixture is homogeneous (there are also other considerations, such as storage stability and hability to penetrate biological surfaces). Pesticide formulations are either liquid or dry [10]. Although there are many different variants (depending on the chemical properties and mode of action), most of these are packaged in plastic containers, although cardboard and metal are also used. In Portugal, plastic accounts for an average 77% of usage in all pesticide containers, 11% for cardboard and 2% for metal, while the remainder is composite of several materials [11–13].

After use, pesticide containers must be disposed of. Since they may still have residues of pesticides, these cannot be treated as regular household wastes. Most countries have an organization charged with the collection and proper disposal of this specific type of agricultural byproduct, and farmers are encouraged to return the empty containers. Figure 1.1 shows the typical appearance of post-consumer pesticide containers.

In 2018, the sum of pesticides sold in Portugal amounted to almost 750 tons in packaging material [13]. Even though this is not a very large quantity from an industrial point of view, it is nonetheless relevant, and therefore, it is essential to have ways of dealing with this waste.

Figure 1.1: Empty pesticide containers in waste management plant

1.2.1 Chemical Composition

Most plastic pesticide containers are made from High density Polyethylene (HDPE) or Polyethylene terephthalate (PET). These materials are both solid, and have a somewhat high plasticity. Other materials might include aluminum foil, and other plastic polymers.

Polymeric materials (namely HDPE and PET) have a relatively high resistance to dissolution by organic solvents at ambient temperature and pressure. However, they can be dissolved at higher temperatures, and are not totally invulnerable to certain organic solvents (depending on the polymer) [14].

Because the pesticide container waste is a mixture in varying percentages of different materials, it is difficult to gauge physical properties such as the glass transition temperature (Tg), density and strength. However, these are very important issues when discussing sample preparation methods for these matrices.

1.2.2 Pesticide container treatment in Portugal

In Portugal, there is a system for collection and disposal of empty pesticide containers entitled "Valorfito", managed by SIGERU, Lda. There are over 1000 return points scattered through the country for the recovery of this type of waste. In each pesticide package is printed the Valorfito logo, along with disposal instructions.

Currently, the used containers are processed, and used mostly for co-incineration. This method of disposal is good for this type of material, because it uses the chemical energy present in the plastic polymers (by combustion), and does not have a problem with organic contaminants (although incomplete combustion may produce very toxic molecules, such as dioxins [15]). However, because co-incineration can be used as a solution in the disposal of many different types of industrial wastes, there may be a

problem of supply and demand. Since co-incineration always needs a main fuel (such as fossil fuels), the quantity of waste that can be burned is somewhat limited, and the supply can easily far outgrow the demand. Therefore, other venues for treatment of wastes must be pursued as well.

In the case of pesticide containers, the most reasonable approach is to make the waste less toxic, so that it can be repurposed in other ways, such as recycling. This is achieved by reducing the amount of leftover pesticide in the empty bottles. Pesticide consumers are encouraged to follow the "triple rinse" technique, which entails washing the empty containers three times with water, and using the rinse. This method has been proven to reduce the leftover pesticide amount in the containers to levels considered non hazardous [16]. It also ensures that farmers get to use all the pesticide they purchased. In Portugal, SIGERU (Valorfito) has been advertising the triple rinse through various outlets. One example can be seen in figure 1.2.

Figure 1.2: A Portuguese pamphlet advertising triple rinse. The title reads: "Triple Rinse" subtitle: "Washing is bringing value into our agriculture". And bellow: "Do as the Prudence Family, wash empty pesticide containers".

Another way of achieving a clean waste is to wash it in a treatment plant. This approach is much less beneficial, not only because it increases the processing cost, but also because the effluent from the rinsing will be contaminated with pesticides.

In order for the waste to be eligible for recycling, it must be determined non-toxic. Therefore, an analytical method must be developed to quantify the pesticides present in the empty containers.

1.2.3 Waste evaluation by EU standards

The method for classifying wastes follows several different and complementary European directives, some of which are noted bellow. The Portuguese environmental agency has compiled a guide summarizing the steps and rules that must be followed [17].

There are four steps required in waste evaluation, as following:

1. **Waste classification:** Firstly, the type of waste must be determined according to the European Residue List (2014/955/EU: Commission Decision of 18 December 2014).

2. **Waste characterization:** This step consists of gathering all possible information about the residue, in order to identify dangerous substances present. If it is not possible to obtain this information, the residue must be characterized as hazardous.

3. **Determination of dangerousness:** Whenever dangerous substances are detected, these should be analysed individually in regards to their level of toxicity and concentration.

4. **Determination of Persistent Organic Pollutants:** Whenever the compounds present in the residue are not dangerous (as defined by regulation nº 1357/2014 of the EU), then they must undergo another evaluation to ascertain the presence of certain persistent organic pollutants (POP's).

The classification depends on the area of activity from which the waste comes (mining, agriculture, etc.), and the specific type of waste. Some entries are considered absolutely non hazardous (*e.g.* animal tissues), while others are considered absolutely hazardous (*e.g.* synthetic hydraulic oils). Wastes that fall into any of these categories do not require any further evaluation, as they have already been labelled appropriately [18].

Empty pesticide containers are a "mirror" entry in the European residue list, meaning they can be given two different codes, depending on the level of dangerous substances present:

02 01 08* Agrochemical waste containing hazardous substances.

02 01 09 Agrochemical waste other than those mentioned in 02 01 08.

The first two digits refer to the chapter, in this case 02: "Wastes from agriculture, horticulture, aquaculture, forestry, hunting and fishing, food preparation and processing". The second two digits refer to the sub-chapter 01: "wastes from agriculture, horticulture, aquacultrue, forestry, hunting and fishing", and the last two digits are the identifiers for a particular entry.

The asterisk denotes hazardous wastes. Therefore, in order to determine if a waste of this nature is hazardous or not, it is necessary to follow steps 2-4 (above).

The second step is the identification of all relevant substances present. There are three critical points in this step, namely: sampling of the material, extraction, and instrumental analysis. All of these will be explained further.

When the relevant compounds present are found, the next step is to determine the level of dangerousness for each one, in order to calculate the maximum concentration allowed by law. There are fifteen properties of waste which render it hazardous, namely: explosive, flammable, ecotoxic, *etc.* [19]. When any of these properties are attributed to a waste, it is classified as hazardous.

These properties are linked to GHS hazard statements, so that if a compound present in the waste is classified with a certain GHS hazard statement, then it must be accessed in terms of the regulation for that specific property. The assessment may vary. In the case of the explosive property, for example: "When a waste contains one or more substances classified by one of the hazard class and category codes and hazard statement codes shown in Table 1[not displayed here], the waste shall be assessed for HP 1 [= explosive], where appropriate and proportionate, according to test methods." [19].

Other classes have maximum concentration limits, with regards to a certain GHS statement. These can be the sum of all compounds with that specific GHS statement, or assessed individually, for each compound. The full extent of waste classification is very complex, and will not be addressed here. For pesticides, only some of the GHS statements apply, and so the classification is somewhat simpler.

The final step, after determining which (if any) properties apply to the waste, is to find persistent organic pollutants (POP's). These are compounds with a lasting effect on the environment. The list has fifteen entries, and many of them are outlawed pesticides, like DDT. Each of these has a limit of $50 mg/kg$, except for Polychlorinated dibenzodioxins and dibenzofurans, which have a limit of 15 mg/kg [18]. If any of these compounds are found above the allowed threshold, the waste must be processed in a way that they are irreversibly transformed.

1.3 Pesticides and their chemical nature

"Pesticide" is an umbrella term used to describe a large number of compounds which are used to control pests in agriculture. The chemical nature and biological effect of different pesticides is very heterogeneous. The pests themselves can be either animal (vertebrate or invertebrate), vegetal, fungal, microbial, etc. Therefore, pesticides can be grouped by their intended application, as below:

Acaricide Control mites;

Algicide Control algae;

Avicide Control or repel birds;

Bactericide Control bacteria;

Fungicide Control fungi;

Growth regulator Alter the growth or development of a plant or animal;

Herbicide Control weeds;

Insecticide Control insects and related arthropods;

Molluscicide Control snails and slugs.

In a purely analytical sense, the classification according to use is of little importance. The chemical class of each compound is much more relevant.

Because the concept "pesticide" does not originate from a chemical standpoint but a biological one, the molecular structures vary immensely. However, in order to determine the best analytical tool and behaviour of the analyte, the structure is the only thing that matters (along with the matrix on which it is).

Often, a simplification can be made, so as to analyse a molecule by its functional groups. These are what give the compound a biological effect to a certain species. Therefore, compounds with the same functional groups will tend to be used for the same purpose. There is also a way to classify pesticides based on their chemical structure. This classification is much more useful to the analyst, as one might infer that compounds in the same chemical class (with similar structures, or a common functional group) will behave similarly. However, there is no hard and fast rule, or systematic standard to classifying pesticides in this way. Folpet, for example (as shown in figure 1.3), is labeled in the *pesticide property database* [20] as belonging to the phthalimide group, because this moiety is present in the molecule (an imide derived from phthalic anhydride). However, another author has grouped it in the N-Trihalomethylthio class, because it also has this functional group [21].

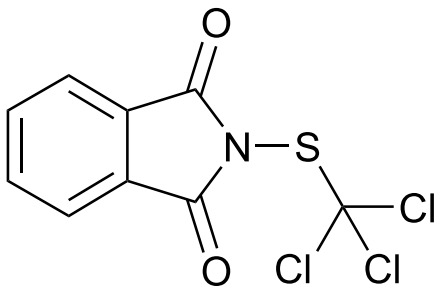

Figure 1.3: Molecular structure of Folpet

Nearly all pesticides used nowadays are organic molecules. However, their chemical properties vary widely. While some are very hydrophobic, being nearly insoluble in water, others are ionic. This further adds to the assertion that although one calls them "pesticides", in a purely analytical sense this term is somewhat deceiving, because these compounds require very different analytical methods in order to be analysed.

1.3.1 Pesticides chosen as analytes

The pesticides analysed in this work were selected based on their usage in Portugal, throughout all seasons and regions. Since waste toxicity classification is based on the sum of all dangerous compounds present, it is only necessary to quantify those that make up the biggest percentage in that waste.

The list of analytes is presented in table 1.1.

Table 1.1: Analytes, intended use and chemical classes [20].

Compound	Use	Chemical Class
Acetamiprid	Insecticide	Neonicotinoid
Bromoxynil Butyrate	Herbicide	Hydroxybenzonitirile
Bromoxynil Octanoate	Herbicide	Hydroxybenzonitirile
Captan	Fungicide/Bactericide	Phthalimide
Chlorpyrifos	Insecticide	Organophosphate
Chlorthalonyl	Fungicide	Chloronitrile
Deltametrin	Insecticide	Pyrethroid
Diflufenican	Herbicide	Carboxamide
Dimetoate	Insecticide/acaricide	Organophosphate
Fluazifop-p-butyl	Herbicide	Aryloxyphenoxypropionate
Folpet	Fungicide	Phthalimide
Indoxacarb	Insecticide	Oxadiazine
Iprodione	Fungicide	Dicarboximide
Lambda-cyalothrin	Insecticide	Pyrethroid
Linuron	Herbicide	Urea
Metiocarb	Isecticide/molluscicide/avicide	Carbamate
Metribuzin	Herbicide	Triazinone
Penconazol	Fungicide	Triazole
s-Metolachlor	Herbicide	Chloroacetamide
Tebuconazol	Fungicide/Plant growth regulator	Triazole
Terbutylazine	Herbicide/Bactericide/Algicide	Triazine
Thiametoxam	Insecticide	Neonicotinoid

As evidenced by the different chemical classes, this group is very heterogeneous in terms of chemical nature. None of the compounds analysed are ionic salts, and most have nitrogen containing functional groups. A complete list of all analytes, selected properties and structure can be found in appendix A.

Pesticide residue analysis involves the determination of all compounds that can arise from the usage of a particular pesticide. This includes metabolites and degradation products that have have significant toxicity to be considered dangerous; such compounds are produced by chemical or biochemical transformation of the original pesticide, and are

unlikely to be present in post-consumer packaging. The exception to this are compounds that decompose easily in contact with water or air, like folpet and captan.

1.4 Chromatography

Chromatography is a term used to describe an assortment of techniques aiming to separate compounds. The basic premise that is common to all chromatographic methods is the use of a mobile phase and a stationary phase. The compounds are separated based on their different interactions with these phases. In partition chromatography, it is the selective partitioning of each compound between mobile and stationary phase that produces the separation. This is one of the most common types of chromatography, and the one dealt with in this work.

1.4.1 Why chromatography

When working with complex samples, separation is almost always a necessity. There are very few methods selective enough to dispel the need to isolate target compounds before detection. Yet, these methods (when existing) might be superior in most respects to a classical "non selective" technique, since they are only useful for a single compound or a very small family. An example of this would be compounds that become coloured in the presence of certain metal ions (also known as chromogenic) [22].

Besides separation (*i.e.* chromatography), there are also methods that involve a simplification of the sample prior to instrumental detection (as is the case for inorganic digestion, prior to quantification of metals by atomic absorption spectroscopy). However, for analysis of organic compounds, some form of chromatography is almost always employed, followed by a suitable detection method.

1.4.2 Theoretical principles

Both GC and HPLC share a theoretical background, in terms of basic chromatographic parameters. What unifies these techniques is the fact that they are both considered "high performance", and are highly dependent on instrumentation, as opposed to other techniques such as classic thin-layer chromatography. More importantly, they both have "on-line" detectors, meaning that compounds are detected as soon as they exit the column. The variation of detector signal along the period of a chromatographic run creates a graphical representation called a chromatogram, from which information can be extracted (figure 1.4).

The chromatogram will depend on the type of detector. Different detectors provide selectivity towards specific types of compounds, and some provide whole spectra per time unit, making the chromatogram three dimensional (Signal intensity vs Time vs wavelength, for example).

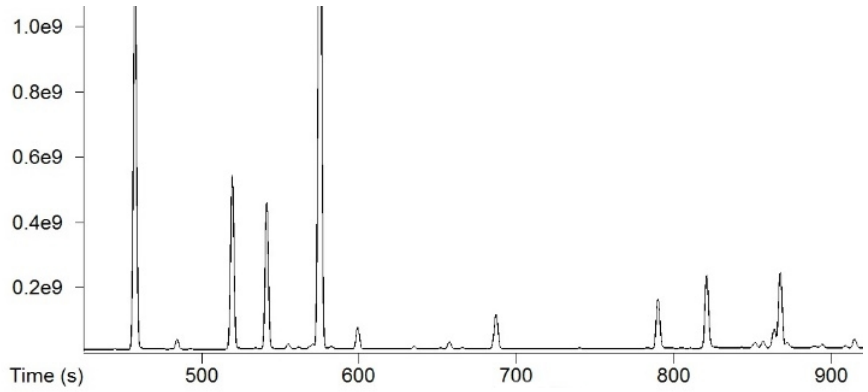

Figure 1.4: Portion of a chromatogram. Signal vs time. Each peak is the result of increased signal caused by an eluting compound (or more, in case of co-elution).

The retention time (t_r) is the time at which a certain compound elutes from the column within a chromatographic run. It is measured between the beginning of the run (the time of sample introduction) and the maximum of the peak. There are two contributions to t_r: the time the compound is being moved with the mobile phase, and the time it is in the stationary phase. A compound only moves through the column while on the mobile phase, therefore the time that the compound spends on the stationary phase can be obtained by subtracting to t_r the time it spent traveling through the column, which is called the dead time (t_m) and is the obtained from an unretained peak (*i.e.* a compound that spent all its time on the mobile phase). This subtraction yields the adjusted retention time (t'_r) [23]. In practical terms, t_r is used, because of its simplicity. But for theoretical purposes the adjusted retention time becomes very important, as does expressing it in terms of eluted volume. Figure 1.5 shows a chromatogram with retention time parameters for a certain peak.

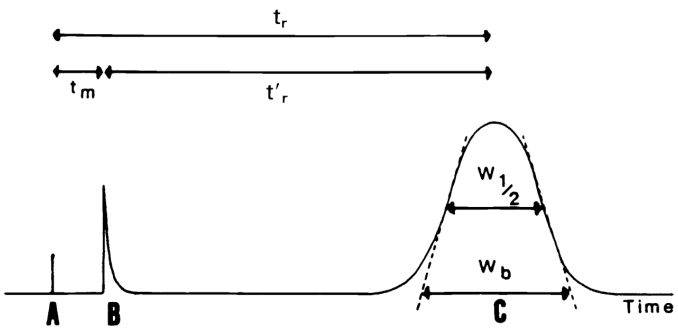

Figure 1.5: Schematic representation of a chromatogram, showing several units. **A** is the start of the chromatographic run, **B** is the peak of an unretained compound and **C** is the peak of interest. $W_{1/2}$ and W_b represent the peak width at half height and base, respectively.[23]

When all chromatographic conditions are kept constant, the same compound will

have a constant t_r. This is the most basic premise of compound identification in GC. However, other compounds may have the same retention time as the analyte. Therefore, in the absence of structural information from the detector, other techniques must be used, such as running the samples on two columns with different selectivity [24].

The baseline refers to the portion of a chromatogram where only eluate is exiting the column [25]. Establishing a stable baseline is very important, because every analyte signal will be measured against it. Usually, the baseline does not correspond to zero detector signal (unless a baseline correction method was applied on the raw chromatogram). Even when no compounds are eluting, there is always signal, caused primarily by impurities in the mobile phase or detached stationary phase material (called column bleeding). Because these are constantly eluting, they do not appear as peaks.

There is another characteristic of the baseline, called noise. This is described as "The random fluctuations occurring in a signal that are inherent in the combination of instrument and method" [26]. It is a well defined phenomenon and, if a stable baseline is achieved, it has a certain amplitude, measured in signal units. Since noise is by its very definition random, interfering peaks from other compounds should not be called "noise", even though chemical noise from column bleeding, mobile phase, *etc.* are a part of it.

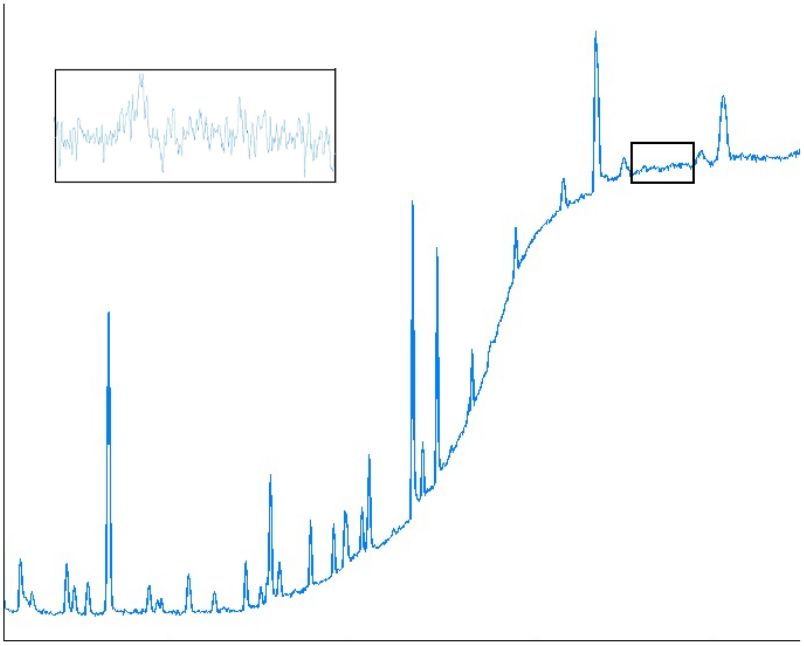

Figure 1.6: GC chromatogram showing increased baseline signal due to detached column material (bleeding) caused by the higher oven temperature, and closeup of baseline signal noise.

One of the most important effects in partition chromatography is the broadening of analyte bands as they travel through the column. In fact, the greatest criticism of the plate theory developed by Martin and Synge [27], is that it fails to properly explain band broadening [23].

The rate theory, developed later and somewhat based on the plate theory, attributes band broadening to three major effects:

- Variation in flow path, due to uneven distribution of stationary phase particles. Only relevant in packed columns;
- Molecular diffusion in the mobile phase. This is a consequence of random molecular motion, and its extent is inversely proportional to mobile phase velocity;
- Resistance to mass transfer in the mobile and stationary phases. This arises when the mobile phase flow is such that equilibrium distribution of analytes between the two phases cannot be achieved. Therefore, this effect increases with higher mobile phase velocities.

Quantitative treatment of these terms is rather complex, but a general knowledge provides much information about the chromatographic process and how to optimize separation. It is important to note that these terms are only applicable to band broadening inside the column, and effects in sample introduction are also extremely important, especially in capillary gas chromatography.

Theoretically, if there was no broadening of the compound bands within the column, then it would be possible to separate anything, as long as sufficient time and column length was provided, and the compounds had different partitions between stationary and mobile phase, no matter how small.

If the time axis on the chromatogram is fixed to a certain length, then separation power within that time will increase as peaks become more narrow. Furthermore, sharp peaks are taller (for the same mass), having higher signal to noise ratios.

The chromatographic peak should ideally have the shape of a Gaussian function. This reflects a normal distribution of analyte molecules along the band. There are three main deviations from the ideal shape [23]:

Tailing peak Usually caused by heterogeneous interaction of the analyte with silanol groups (or others), causing sorbed molecules to migrate slower. As a result, the peak has a sharp front, but diffuse tail.

Fronting peak Most often the cause is column overload: there is too much analyte for the stationary phase, so a portion cannot partition effectively, and will migrate faster causing a diffuse front and a sharp tail.

Chemisorption Analyte molecules become chemically bonded to certain sites, and are released very slowly. Causes peaks with a very large tail, or even missing altogether.

Figure 1.7 shows the ideal peak shape, as well as unwanted deviations.

For quantitative purposes, having a good peak shape is crucial. In order to integrate a peak and obtain a signal to mass relation, it is very important that the base width is

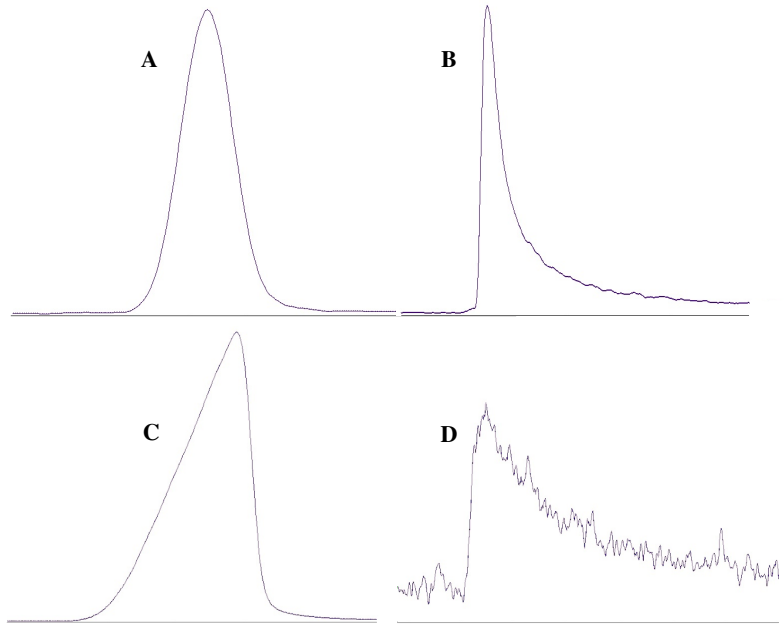

Figure 1.7: Most common peak shapes. **A**, ideal, gaussian-like peak shape; **B**, tailing peak; **C**, fronting peak caused by column overload; **D**, Strong chemisorption giving rise to a very small peak.

the same within a calibration range. Poor peak shapes tend to have widths that vary with concentration (because the phenomena that create unideal peaks are concentration dependent). Furthermore, since unideal peaks have diffuse fronts or tails, it is very difficult to assess where peak integration should start or end, respectively.

Another very important consideration in peak shape is the number of data points, *i.e.* the number of signal values per peak. Considering that chromatograms are always formed by discrete values rather than a continuous function, the number of data points will determine the quality of a peak's shape, as can be seen in figure 1.8.

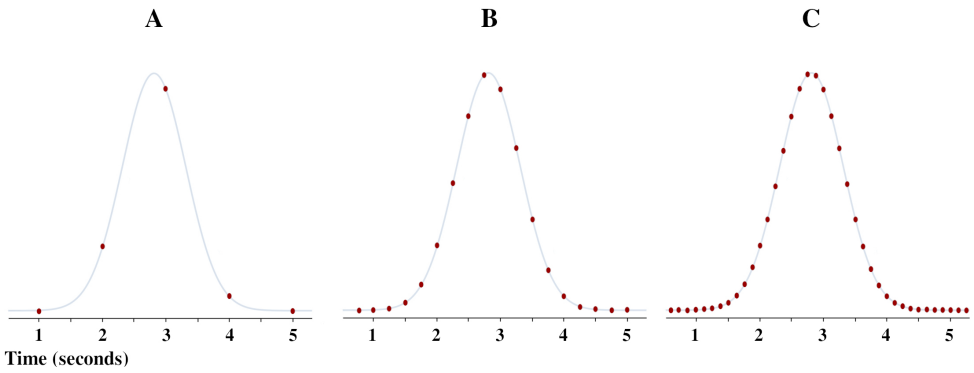

Figure 1.8: Influence of scan speed on peak appearance. **A**: 1 scan per second; **B**: 5 scan per second; **C**: 9 scan per second. The blue line represents the real peak shape, which the computer needs to guess in order to perform integration.

Finally, for trace analysis, it is essential to have a method with high sensitivity. In practical terms, this is defined as the slope in a calibration curve: the higher the slope, the bigger the signal response as analyte mass increases. The sensitivity of a particular analyte results from the analytical method and the chemical properties of the analyte itself. However, by increasing the selectivity of a method to a particular analyte, the sensitivity will also increase. Theoretically, if all interferences contributing to the analyte signal can be eliminated, then only electronic fluctuations (noise) and the analyte itself would be contributing to the recorded signal. In such a situation, it would be possible to detect nearly any amount of analyte, no matter how small, so long as it reached the detector. Therefore, the purpose of highly selective methods is to reduce the amount of interferences that contribute to the signal.

1.4.3 Gas-liquid chromatography

Gas chromatography uses a gas as the mobile phase, also called the carrier gas. Traditional GC employed metal columns packed with a liquid or solid stationary phase. The advent of open tubular capillary columns has revolutionized the practice of gas chromatography, since these have a much higher separation power, require smaller gas flow, and are more suitable for coupling with mass spectrometry. Nowadays, packed GC columns are relegated to a few applications, while capillary GC is the dominant technique.

By far, the most common type of column used is wall coated open tubular (WCOT). These columns are made from fused silica (SiO_2), protected with a polymer on the outside (usually polyimide); the inner wall is coated with a thin layer (0.1-8 μm) of liquid stationary phase [28]. The open interior of the column allows for a much smaller resistance to carrier gas flow, enabling the use of much longer columns, resulting in increased separation power and speed. Column inner diameters range from 0.1 mm to 0.52 mm for most applications. A thinner column has a higher separation per length unit, but can handle a smaller amount of sample. Thus, a compromise must be met between chromatographic resolution and sample quantity needed for detection.

The operation of a GC is quite straightforward: There is a sample introduction device (commonly called injector), an oven where the column is, and a detector. Modern instruments are fitted with electronic pressure controllers, to regulate the gas flow in the column.

Sample introduction is often the most crucial step in GC analysis. The analytes must be transferred to the capillary column, either by volatilization or direct introduction. This step is where compound degradation and activity are most likely to happen, resulting in poor peak shape and reproducibility.

The split/splitless injector is the most common in use today. The sample is volatilized in a hot glass liner, and transfered to the column by carrier gas. It can be operated in splitless mode, whereby the whole gas cloud is forced into the column over a period of time (usually 0.5-2 min), or in split mode, in which only part of the vaporized sample is

transferred to the column.

Problems in split/splitless injection arise for several reasons:

- The solvent vapour cloud may be too large for the liner volume, causing backflash and contaminating the system, compromising reproducibility. This is also one of the reasons why water is not recommended as a solvent in GC analysis;
- Thermal degradation of analytes;
- Sorption of analyte to the liner wall, usually caused by exposed silanol groups or metal residue, leading to poor peak shape or missing peaks.

Solvent backflash happens because the amount of sample injected is too large. However, injecting smaller amounts might compromise analyte detectability in trace analysis. Also, larger inlet liners tend to increase sample transfer time. One solution to this is the pulsed pressure splitless technique, which consists of increasing injector pressure during the spitless time, thus constricting the vapour cloud.

Problems related to chemical activity are more pronounced for compounds like pesticides, because these generally have strong electronic charge polarization, due to functional groups such as amines, halogens, *etc.* In this type of analysis, it is very important to ensure a chemically inert sample inlet, as well as optimize the injection variables.

Separation in Gas-liquid chromatography is very temperature dependent. The analytical column is inside an oven, allowing temperature programming, which in turn enables the analysis of compounds with very different vapour pressures in the same run. Column temperature affects everything from separation to peak shape and width, as it will influence the partitioning of compounds between mobile and stationary phase. Oven programming is probably the simplest and easiest variable to optimize in GC analysis, while also being one of the most important.

There are several detectors that can be used with GC. In pesticide analysis, there are three main ones: flame ionization detector (FID), electron capture detector (ECD) and mass-selective detection (mass spectrometer, MS).

The ECD measures the capture of electrons by eluting compounds to create a signal. It is selective towards compounds with electronegative groups (like most pesticides), and can reach some of the lowest limits of detection for highly halogenated analytes [29]. The ECD uses the radioactive isotope ^{63}Ni as the source of electrons, so there are several legal constraints involved in its purchase and maintenance.

The FID is one of the most widespread detectors in use today. It is extremely cheap, easy to use and rugged. The principle of operation is quite simple: a hydrogen flame ignites the eluting compounds, leading to formation of electrically charged species that are detected by an electrode. The FID can detect nearly all organic molecules, making it extremely versatile. On the other hand, the lack of optimization potential and selectivity make it unsuitable for trace analysis, except when it is coupled with a pre-concentration

step or multi-dimensional methods, like comprehensive two dimensional gas chromatography (GCxGC).

The advent of mass selective detectors has revolutionized the practice of GC. These detectors are unique because they provide whole spectra per time unit rather than a unidimensional signal value. The large amount of information collected enables the selective extraction of data, resulting both in structural information and chromatogram deconvolution. Mass spectrometry is a very complex technique, and will be explained in section 1.5.

Capillary gas chromatography provides several advantages over HPLC, or similar techniques:

- Its operation is cheap and environmentally friendly, because it uses non-toxic gas as mobile phase;
- Open tubular columns have inherently higher chromatographic resolution power than packed columns;
- Mass spectrometry coupling is comparably easy (section 1.5.2).

There are also some drawbacks, stemming from the fact that GC needs the analyte to be volatile and thermo-stable, and that capillary columns can only handle a limited amount of sample.

Only about 20% of known organic molecules can be analysed by GC with ought prior treatment [30]. This means that although very powerful, this technique is relatively limited in its scope of application. The analysis of proteins, sugars, or any ionic substance cannot be performed by GC, except in the special cases where a prior chemical modification can make them GC amenable.

The small sample capacity of even megabore (0.52 mm internal diameter) columns, makes capillay GC a somewhat unpractical technique to use in preparative work *i.e.* separation of an analyte as a purification step, so that other analyses can be performed. However, because the mobile phase is a gas, it is possible to obtain a pure compound more easily than with HPLC, where complete solvent removal may be difficult.

1.5 Mass Spectrometry

Mass spectrometry is an analytical technique used to obtain information from molecules or atoms, based on their ions' mass to charge ratios (m/z). It works by ionizing a certain compound, and measuring its m/z. The result is a mass spectrum, from which structural (and sometimes quantitative) data can be obtained.

1.5.1 Theoretical principles

Mass spectrometry involves three main stages: ionization of some organic or inorganic compound by a suitable method, separation of the resulting ions by their mass to charge ratio, and detection, based both on m/z and intensity. The ion path must be kept in a high vacuum, to prevent collisions. Therefore, sample introduction is of major importance, as organic compounds quickly vaporize when exposed to such a low pressure (it is also the reason why non-volatile analytes can only be analysed using very specific kinds of ionization methods) [31]. Figure 1.9 shows the basic outline of any mass spectrometer.

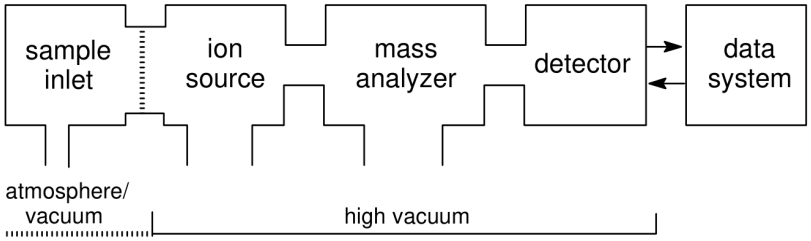

Figure 1.9: Schematic representation of a mass spectrometer. There are several different sample inlets, ion sources, mass analysers and combinations thereof, making a very large number of commercial instruments available. Taken from [32].

It is important to define m/z properly. Taking the international system units (SI) for mass and charge, one would get kilogram per coulomb; this would be very difficult to use in practice. For mass spectrometry, m/z is measured in a scale of atomic mass divided by elementary charge [32]. It may be assumed that the elementary charge is equal to 1, however this is not always the case, especially with some forms of ionization. If the elementary charge of the ion is one, then m/z is equal to the unified atomic mass unit (or Dalton).

Figure 1.10 shows a mass spectrum. The intensity is typically shown as a percentage (or per mille) relative to the largest peak, also called the base peak. The representation of the mass spectrum as a bar graph is standard, which is a useful type of data simplification. The mass spectrometer records continuous peaks, and afterwards the m/z of each peak is determined from its centroid. A mass spectrometer with a capacity to resolve more peaks in a given m/z range has a higher mass resolution, and consequently can give more accurate m/z readings (not to be confused with chromatographic resolution).

Electron ionization (EI) is the most common type of ionization employed in the analysis of small organic molecules. It involves a stream of highly energetic electrons, produced by a heated filament, that interact with the sample. The kinetic energy of these electrons is adjustable, but is almost always set at 70 electron volts (eV), in order to provide a reproducible and comparable spectrum [33].

The energetic electrons collide with a neutral, transferring energy to it. If this energy exceeds the ionization energy of the molecule, then an electron from the neutral is ejected, forming a radical ion, as in equation 1.1.

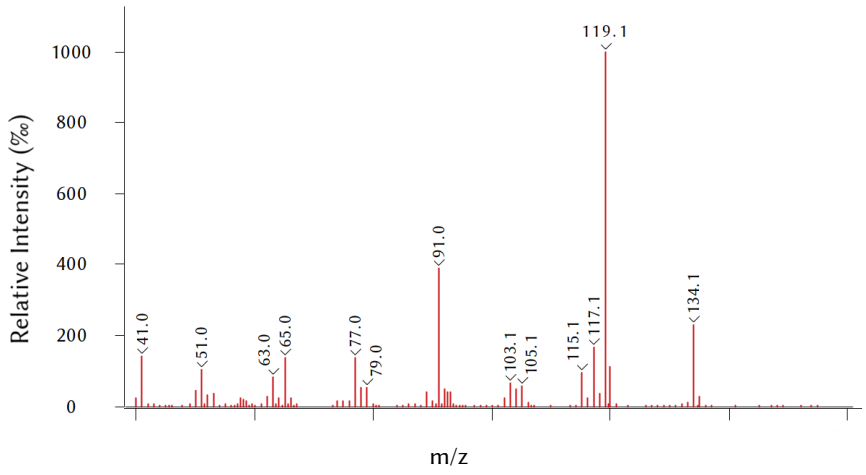

Figure 1.10: EI mass spectrum of a cymene isomer, molecular mass: 134 Da.

$$M + e^- \longrightarrow M^{+\bullet} + 2e^- \tag{1.1}$$

If the energy is not high enough, then the neutral will simply become electronically excited. Also, there is the possibility of multiple electron ejections from the neutral, but EI predominately creates singly charged ions [31].

The excess energy impaired on the newly formed ion results in higher vibrational states. The ion also has weaker bonds when compared to its neutral counterpart. If the excess energy is enough, then bond dissociation will occur. The pattern of fragmentation is dependent on an ion's potential energy surface, and so will be characteristic of that specific ion (and of the neutral preceding it). An ion created by a 70 eV electron will most often dissociate once although, if the energy is enough, can dissociate twice or even three times [31]. A mass spectrum is the statistical result of many millions of structurally equivalent neutrals being ionized with different internal energies and the m/z of their charged fragments. Thus, a mass spectrum is characteristic of a compound and will always be the same for that compound, if experimental conditions are also maintained.

After ionization, the fragments are accelerated in the ionization chamber, by an electrically charged plate and a grounded plate. The charged plate has a polarity so that it repels the ions of interest (negative or positive). There is a slit in the grounded plate, so that an ion beam is created, imparting a certain kinetic energy on the ions. Ideally, all ions should have similar kinetic energies, because these have an effect on the separation (especially for the time-of-flight analyser). The acceleration can also happen on two consecutive stages, as a way to improve the beam shape and reduce kinetic energy spread [34]. Figure 1.11 shows a diagram of a very primitive ion source. Modern mass spectrometers use more than one stage acceleration, and lenses for ion focussing.

In the mass analyser, ions are separated by their m/z values. There are many different types of analysers, but almost all of them are used to separate the ions in time or space, so that they can be detected by some form of electron multiplier, which simply produces

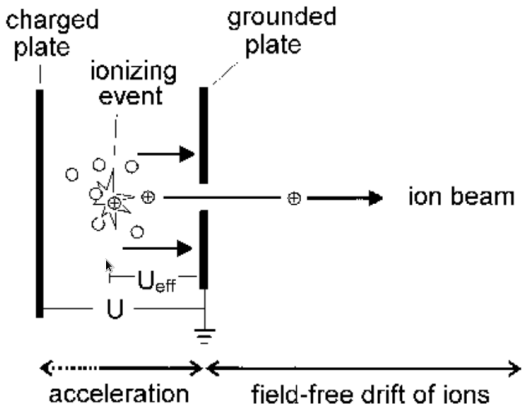

Figure 1.11: Diagram of a primitive ion source. U is the electric potential between the charged and ground plate (voltage). U_{eff} is the effective accelerating voltage, dependent on the ion's position. Reprinted from [31].

an amplified "on" signal whenever an ion impacts. The exception to this are the orbitrap and Ion cyclotron mass analysers, which record an induced current caused by orbitally trapped ions [35, 36].

For hyphenation to GC and HPLC, linear quadrupole and triple quadrupole (QqQ) instruments are the most used. These are cheap and very versatile, being able to operate both in full scan mode, for acquisition of whole spectra (within an m/z range), or in selected ion monitoring (SIM), a technique which increases selectivity at the expense of structural information. The triple quadrupole can further be used in selected reaction monitoring (SRM), an incredibly selective technique, able to achieve some of the lowest detection limits, even when compared to high resolution instruments [37].

There are, however, several drawbacks in these mass spectrometers, namely:

- Most commercially available instruments have low mass resolution, providing only nominal masses (after conversion from m/z);
- They are by nature scanning instruments, meaning they act as a sort of filter that only lets one m/z pass at a time. This results in most of the ions being lost.

Another consequence of scanning instruments is spectral skewing. This phenomenon happens because ions are recorded sequentially, according to their m/z, therefore if the concentration of an analyte arriving at the detector changes during the scan, the spectrum will be skewed. This problem is especially salient in GC, where peaks are extremely sharp. Spectral skewing does not affect significantly the comparison with spectra libraries (section 1.5.3), but is a problem in automatic data processing of chromatograms with the intent of obtaining pure spectra from partially co-eluting compounds [38].

The time-of-flight mass analyser (TOF) bypasses most of these problems. This analyser works by measuring the time charged particles with a given kinetic energy take to travel a certain length of space (the flight tube). The velocity of each ion traveling through the

tube will be dependent on their mass and charge. Whence, the m/z ratio can be measured by the velocity of each ion [31].

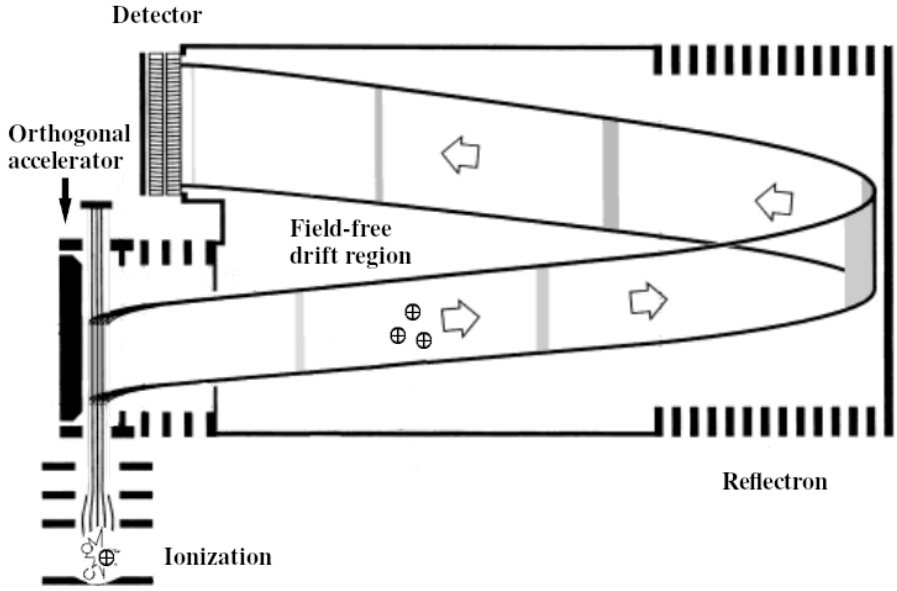

Figure 1.12: Simplified diagram of a TOF instrument with electron ionization. The orthogonal accelerator extracts ions from the continuous beam. Adapted from [39].

The TOF analyser requires a pulsed ion beam, because ions entering the tube must do so at the same time. For a continuous beam ionization method (like electron ionization), pulses must be extracted orthogonally from the original beam. This is an adaptation to original TOF-MS which enables the analyser to be paired with traditional ionization methods [40]. Each pulse will become a mass spectrum, with the ions arriving at the detector separated in time. Figure 1.12 shows a diagram of an EI-TOF instrument.

Since separation in the TOF analyser is dependent only on the kinetic energy of the ions and their mass, the initial spread of energy between particles with the same m/z is the main cause of low resolution in TOF-MS instruments. Consequently, a way of refocussing ions of different kinetic energies in time was developed. This technology is called a reflectron, because it reflects ions like a mirror, correcting the differences in energy. The reflectron also increases the flight path, because reflected ions have to travel a longer distance to the detector, but this effect is not as important as the kinetic energy correction.

TOF-MS instruments present several advantages to scanning mass analysers like the linear quadrupole [41]:

- Much higher acquisition speed. This means that a TOF-MS can produce more spectra per second, yielding better chromatograms when coupled to chromatographic techniques. This is especially important in fast GC applications and GCxGC;

- They can have higher mass resolution, when compared to the traditional linear quadrupole;
- Acquisition of complete spectra with improved ion transmission *i.e.* from each ion pulse most particles are detected, whereas for scanning instruments most are lost in the process. This improves instrument sensitivity while acquiring full spectra;
- There is no spectral skewing in chromatographic applications. This enables computerized data processing capable of obtaining pure mass spectra from co-eluting compounds;
- Extended m/z range with ought significant loss in scanning frequency.

There are also some disadvantages to TOF-MS, namely its higher price and, most importantly, the fact that triple quadrupole remains superior in terms of target analysis, because of its extremely high selectivity, yielding very low detection limits only equaled (and possibly surpassed) by high resolution MS or hybrid quadrupole-TOF at a much higher price point. It is noteworthy, however, that the selective methods used in QqQ are at the expense of structural information so that, if a full scan method can equal its performance in terms of detection limits while maintaining sufficient selectivity, it will always be preferable.

Mass spectrometry cannot on its own distinguish enantiomers, since these have the same mass and identical spectra. There are several methods developed to introduce chiral recognition in mass spectrometry [42, 43]. However, when coupled to GC or HPLC, a separation of diasteriomers will often happen, leading to resolved peaks in the chromatogram. It is not possible to identify separated diasteriomers in terms of stereochemistry from the mass spectrum alone, because they will be similar (*i.e.* the mass spectra will be the same for all diasteriomers). A separation of enantiomers can be achieved by using chiral chromatographic stationary phase.

1.5.2 Hyphenation with GC

Mass spectrometry has asserted itself as the most useful (in general) detector for high performance chromatography.

The biggest problem to overcome when coupling these two techniques is the necessity of high vacuum by the mass spectrometer. For HPLC this presents a serious problem, because the volume of even just 0.1 $mL.min^{-1}$ of liquid is very large when vaporized. This is why LC/MS instruments developed much later than GC/MS, as a consequence of new atmospheric pressure ionization methods [44]. These do not fragment the molecule to the extent of EI, so they are not the most suited for structural identification, although they can be used with tandem techniques such as QqQ and high resolution instruments, with improved results.

For GC, the use of capillary columns enables a direct coupling between the chromatograph and the ion source, because modern vacuum pumps are perfectly capable of handling the carrier gas outlet. The most common ionization source for GC coupling is EI, although others can be used [45].

The carrier gas of choice for GC/MS is helium. Hydrogen can also be used, but presents several problems such as gas-phase reactivity within the ion source.

1.5.3 Structural analysis and quantitation

Interpretation of EI mass spectra can provide a lot of structural information, even possible structures for unknowns, especially when the molecular ion is present [46].

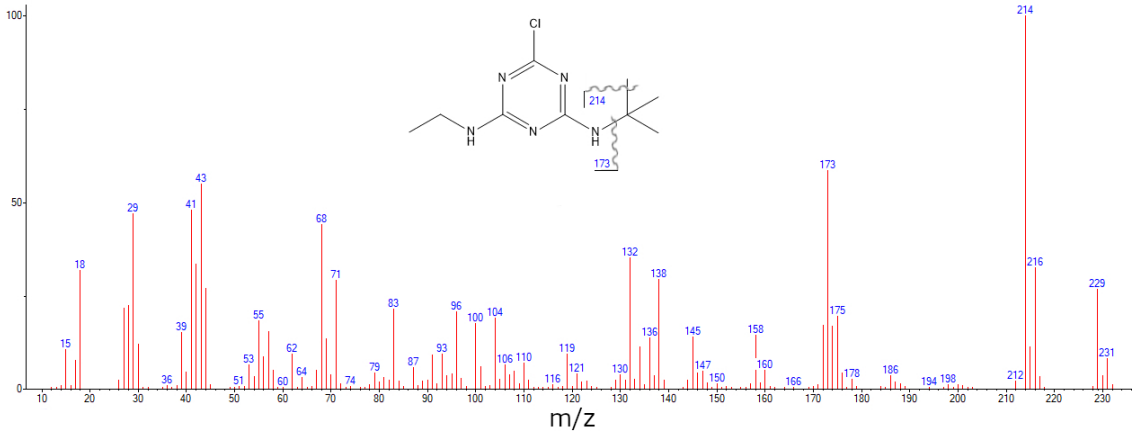

Figure 1.13: EI mass spectrum of terbutylazine with proposed fragmentation for the bigger peaks. Spectrum from NIST mainlib.

For quantitative work structural elucidation is usually not necessary, because both the structure and respective mass spectrum are known. However, it can be very important in method development: often there are unknown compounds interfering with the analysis that need to be identified.

Nowadays, the most common approach to tentatively identify unknown peaks is to compare the spectrum with a computer database. As said previously, EI spectra taken at standard conditions are very reproducible and repeatable, therefore it is possible to run an algorithm that compares the unknown spectrum with thousands in a database, and find the best match. The development of this process has been made possible by modern computers, and in recent years it has superseded manual spectra analysis. There are many instances, however, where the older method must still be employed.

The most comprehensive databases of EI spectra are provided by the United State's National Institute of Standards and Technology (NIST), and Wiley [47]. These may be bundled and used together.

For true identification of a compound, spectral comparison is never accepted alone. It is often combined with the Kovat's retention index, which provides a value conceptually analogous to a retention time (but adimentional) that can be compared with those already

published for a certain compound. Even so, true identification of a compound in a sample must by achieved by using a pure standard for that compound, whose structure has been identified by nuclear magnetic resonance, melting point and other techniques (usually a combination thereof).

In quantitative analysis, mass spectrometry offers several advantages in terms of performance when compared to any other detector, either in GC or HPLC:

- Can be used in highly selective methods, or the information acquired can be filtered to remove interferences and obtain a very "clean" analyte signal;
- With proper method development, MS can detect nearly any compound that is chromatographed;
- Isotopically labeled standards for internal standardization produce very precise results, namely for quantitative purposes.

When detecting full spectra in an m/z range, it is possible to extract a chromatogram of a single m/z value, or a sum of selected m/z's. In essence, we can plot m/z's characteristic of an analyte versus time, and obtain a reduced chromatogram, with highly improved selectivity. It is important to note that this is done in post-processing, with no data loss. The effect of this data manipulation can be seen in picture 1.14.

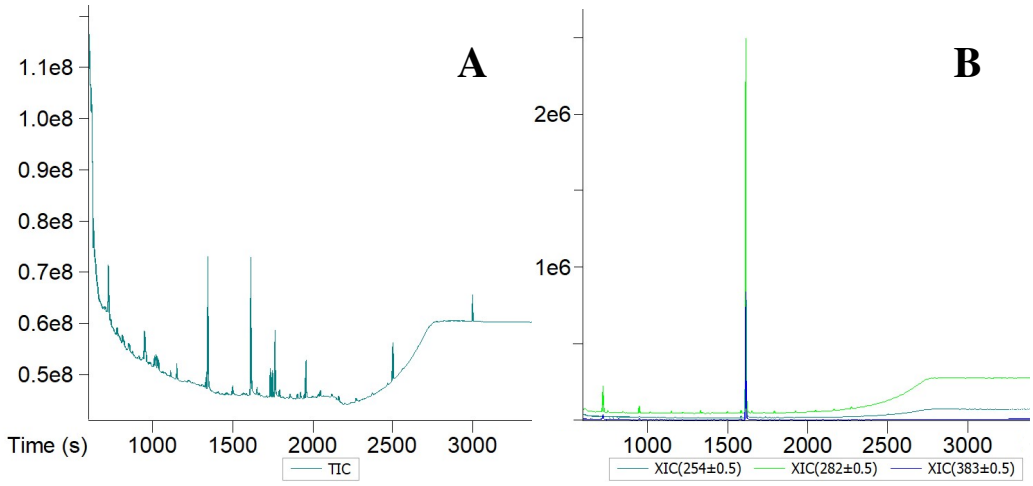

Figure 1.14: Different data displays for the same sample. **A**, Total ion chromatogram *i.e.* the sum of all m/z detected in each mass spectrum; **B**, overlapped extracted ion chromatograms of three m/z characteristic of fluazifop-p-butyl (254, 282 and 383). 1 μL injection in GC/TOFMS of several pesticides at 1 mg/L.

The classic calibration method of using solutions with the analyte at known concentrations can yield high relative standard deviations in GC/MS. This is because of error in injection, even when using autosamplers, and small changes in the mass spectrometer [48]. Internal standardization is a well known method which allows for the correction

of GC errors by using another compound which should be as chemically close to the analyte as possible, called the internal standard (IS). The same amount of this compound is added to all samples, and a relative value is calculated between the analyte and IS signals. In GC/MS, it is possible to use isotopically labeled standards, which are nearly identical to the analyte, but have certain atoms of isotopes uncommon (or absent) in nature. These are usually deuterium (^2H) or carbon-13 (^{13}C). The downside to this method is that isotopically labeled standards are very expensive.

1.6 Approaches to pesticide analysis

As stated previously, "pesticides" are a very heterogeneous group of compounds, with different physical and chemical properties. Obviously, any analysis method will be entirely dependent upon the analyte's properties. Furthermore, there are many different types of matrix in the realm of pesticide analysis: soil, water, food, *etc.* Because of this, the extraction method and instrumental analysis must be developed and optimized for each intended application. In multi-residue methods (determination of several pesticide residues from the same analysis), only one type of extraction is used, but there may be a need to use more than one form of instrumental analysis [49–51]. This holds true for organic compounds, but not necessarily when some of the analytes are inorganic salts, which have totally different chemical properties, and whence may require a different extraction method.

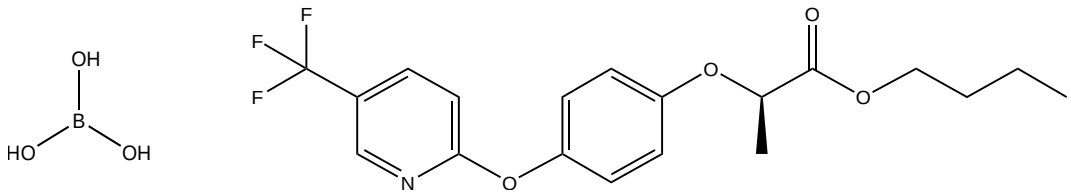

Figure 1.15: Boric acid (left) and fluazifop-p-butyl (right) are both pesticides, but have very different physio-chemical properties

Pesticide analysis roughly follows four steps:

- Collection of a sample representative of the whole object (soil, food, packages, *etc.*);
- Extraction of the analytes from the sample material, and possible clean-up;
- Instrumental analysis;
- Data treatment.

The sampling process and data treatment involve statistical considerations, and will be discussed in section 1.7.

1.6.1 Extraction procedures

Chromatographic analysis requires a dissolved sample. For GC, solvents that expand less upon vaporization are preferable.

There are two important factors in the extraction of analytes from any material: recovery and presence of contaminants. Recovery is the measure of extraction efficiency for a particular analyte. It is expressed by the percentage of analyte the method can extract from the material, and will be described in section 1.7.2. The presence of matrix contaminants can be a problem, especially when these co-elute with the analytes. Normal extraction methods are generally not targeted on a particular analyte and will extract any matrix compound with characteristics similar to it. In order to bypass this problem, the extraction method can be made more selective, even targeted, as is the case with molecularly imprinted polymers [52]. Another approach is to use more selective detection methods, so that the analyte signal can be separated from that of the matrix components.

For pesticide determination in food, QuEChERS method is the most used. The extraction is performed with acetonitrile, using magnesium sulfate and sodium chloride, followed by centrifugation and clean-up via dispersive solid phase extraction (d-SPE) [53]. This method is easy and yields very good extraction recoveries. It was developed for food, so it excels in samples with high water content, but chemically heterogeneous.

When considering a solid polymeric material such as pesticide containers, there are several extraction approaches, although they all follow the same general path: homogenization (milling) to increase surface area and improve repeatability, followed by extraction with an organic solvent or mixture, using a variety of possible methods, followed by filtration (if necessary) and analysis.

The use of heat and/or pressure is not recommended for multi-pesticide analysis, because some will degrade. Therefore, agitation and mixing should be used instead.

The table below summarizes some methods for extraction of pesticides in solid polymeric materials.

Table 1.2: Short overview of three selected methods for extraction of pesticides from solid, polymeric materials

Objective	Analytes	Extraction Method	Analysis	Results	Advantages and Disadvantages	Ref.
Determination of leftover pesticide amounts in postconsumer agrochemical packages	18 pesticides, including dimethoate and terbutylazine.	Reduction to small pieces, extraction with 250 mL of dichloromethane-acetone-methanol (50-25-25) in ultrasound bath. This process was repeated two more times. Then samples were milled and 1g was extracted with 10 mL of solvent in ultrasound.	Evaporation of solvent under nitrogen, redisolution and analysis by UPLC/MS/MS.	High standard deviation, possibly due to heterogeneous pesticide distribution within the package. Some pesticides had low recoveries even after 3 extractions from the same material.	Subsequent extractions permitted very high recoveries. The extracts were analysed separately to determine the efficiency of each one, and it is possible to reduce the procedure to just one extraction with pieces and one with milled material. However, the extraction uses an enormous amount of solvent (over 250 mL per sample).	[54]
Quantification of pesticide residues on plastic mulch and soil from Chinese farmlands	35 pesticides, including tebuconazole, acetamiprid, thiacloprid, indoxacarb, cyhalothrin and thiametoxam.	Based on QuEChERS, but using ultrasound for acetonitrile extraction, and no water partitioning before d-SPE	Evaporation of solvent and redisolution in acetone for GC/MS and methanol for LC/MS/MS	Recoveries between around 70-106% and Relative standard deviation between 1-20%.	The method yields acceptable recoveries for most pesticides, but QuEChERS is expensive and time consuming, and is tailored for materials with high water content.	[55]
Determination of pesticides residues in post-consumer packages	51 pesticides were tested, but the method was developed with the intent of being extended to other pesticides	Cryogenic milling to bellow 0.5 mm. Extraction with Tetrahydrofuran (THF) and a mixture of THF and hexafluoroisopropanol in ultrasound bath.	Dilution with mobile phase and analysis by LC/MS/MS.	Lowest fortification level successfully tested was 10 mg/kg (10 ppm).	Uses a very small amount of solvent and only 0.1g of material, which can lead to imprecise results, if the material is not very homogeneously milled. This method is compatible with both HPLC and GC with ought solvent shift.	[56]

Because the QuEChERS method is so widespread and well accepted, it may be logical to adapt it into this type of material. However, in Anastassiades *et. al.* original paper describing the development of this methodology [53] it is cleat that the main concerns were the water content of food products, and co-extracted contaminants such as lipids. The water content in post-consumer pesticide containers is more unpredictable, and generally lower as well. For relatively dry material, it is not necessary to employ centrifugation. In order to remove unwanted water from the extract, it is simpler to use a small amount of magnesium sulfate, and then filter. In GC applications, it is important to have a sample as free from metals as possible to prevent inlet deposit and subsequent activity, so acetone should not be used in conjunction with magnesium sulfate, because it dissolves about 13 mg/mL of this salt [21].

Solvent choice is very important not only for extraction efficiency, but also because many compounds degrade easily in solution. It has been reported by Maštovská and Lehotay [21] that captan and folpet entirely degraded in certain MeCN lots at room temperature, while being stable in other lots, even after 5 days (initial concentration of 0.5 $\mu g/mL$, full degradation after 24 hours). This may be caused by residual water, for example. Acidification of the solvent with 0.1% (vol./vol.) acetic acid reduced degradation of all labile pesticides tested, while none of the others was affected negatively.

1.6.2 Instrumental analysis

Nearly all pesticides can be analysed by some form of HPLC, if a suitable method is developed. However, if the analysis can also be carried out satisfactorily by GC, then this will usually be preferable, because of the reasons enumerated in section 1.4.3. Especially when analysing many different compounds, the higher separation power of GC is favourable, because in order to separate more compounds in HPLC it would be necessary to increase run time and as a consequence increase the amount of solvent used and cost, assuming they could even be separated. The performance of both techniques in pesticide analysis has been compared for many commercial pesticides, and it is clear that neither one nor the other is ideal for all compounds [49, 50].

In terms of detection, generally multi-residue methods tend do employ tandem MS, because this yields the best selectivity and lowest limits of detection. Even though high-resolution MS can give better results, the increased cost prevent it from being used in routine analysis [57].

1.6.3 Difficulties

Solid materials need to be homogenized into a fine powder before extraction to ensure good repeatability, especially when a small amount of sample is used. However, even though plastic polymers are solid, they are not entirely rigid, and exhibit some plasticity at ambient temperature. This makes the milling process much more difficult.

Furthermore, some analytes are not stable in solution, and some are photosensitive, which entails extra care in extraction and storage of extracts and standard solutions.

1.7 Statistics and method validation

1.7.1 Sampling

Sampling is the act of reducing the amount of material under study to allow analytical procedures. As instruments and methods become more sophisticated, they require less amounts of sample, which makes sampling errors more problematic. An ideal sample should be representative of the lot from which it was taken, but this is usually not the case. Sample homogeneity and particle size distribution in the original material are critical factors in sampling. A very heterogeneous lot with regard to the analyte in study will provide high sample deviation.

Sample deviation can be calculated by sampling a lot randomly several times (which may itself be a problem, as any human choice tends not to be random) and measuring the property under study in each one. In depth statistical treatment for sampling methods has been developed and reviewed [58], but is seldom used. For empty containers, industrial milling of the whole lot is an excellent way to ensure homogeneity. Taking a sample from unprocessed, physically intact material is not possible, because any method employed would significantly deviate from randomness.

1.7.2 Overall method performance

Method validation entails judging the fitness of that method for a particular purpose. In general, quantitative methods require a more thorough validation than qualitative ones. The parameters studied to establish fitness for purpose in a proposed quantitative methodology are, according to [59]:

Selectivity The measured signal should be due to analyte only. Method selectivity is a product of extraction selectivity, chromatographic resolution and detection selectivity. In order to test method selectivity, possible co-elutants must be investigated.

Precision The closeness between different measures under repeatability conditions must be assessed for different concentrations.

Accuracy It is measured using reference standards whose concentration was determined in a certified lab. However these are not usually obtainable, so it is common to spike the material with a known quantity of analyte and determine the percentage of that quantity detected by following the whole analytical procedure. This is usually called recovery; both extraction and instrumental bias contribute to the final value. In order for this method to yield true results, it must be assumed that spiking solutions have small deviations from the true value.

Working range and linearity These are functions of instrumental performance for a certain analyte. If a suitable linearity is established within a concentration range (by regression analysis), then the working range is bound by the limit of quantitation and the highest concentration value in the calibration.

Ruggedness The same sample should be analysed in different conditions, by changing the variables slightly (ambient temperature, extraction time, *etc.*).

Limit of Detection and Quantitation Values in the concentration domain which allow quantitative knowledge of the method performance. There are several ways to calculate them, but they all reflect the level bellow which a result is not acceptable, within a certain confidence level.

The precision can be easily assessed by the relative standard deviation (RSD) of the results. The same sample measured several times will yield a spread whose standard deviation can be calculated. This is caused by random errors, and any result must take into account the standard deviation, and number of measurements taken.

In terms of accuracy, the method bias is estimated by the recovery percentage, given by the formula:

$$R(\%) = \frac{\overline{x}' - \overline{x}}{x_{spike}} \times 100 \tag{1.2}$$

$\overline{x}'$ is the mean value obtained for the spiked sample, $\overline{x}$ is the mean value for the non-spiked sample and x_{spike} is the amount spiked. If a blank sample is spiked instead of a real one, then the $\overline{x}$ term becomes zero (assuming that a blank sample corresponds to zero signal), and the calculation is simpler. In some cases, a blank sample will not have the same characteristics as a real one, as it may lack many other matrix components, so its use might not give reliable results. On the other hand, using a sample which already has a substantial amount of analyte and increasing that quantity further might put the concentration above what is regularly found on real samples, and the extraction method may not behave the same way.

In presenting the final result of a measurement, bias should be removed to the extent that it is known, and a confidence interval should be given. There are two main ways of presenting a result [60]:

$$\overline{x} \pm s \tag{1.3}$$

$$\overline{x} \pm \frac{t_{n-1} \cdot s}{\sqrt{n}} \tag{1.4}$$

Where $\overline{x}$ is the arithmetic mean, s is the standard deviation, n is the number of measurements taken, which must always be expressed, and t_{n-1} is the t-distribution value for $n-1$ degrees of freedom, in the desired confidence interval. Both equations are interchangeable.

Limit of detection and quantitation (LOD and LOQ, respectively) are somewhat dubious terms because there is no standardized calculation method in use, despite efforts to do so [61]. There are several methods for determining the limit of detection. The usual method involves measuring the signal for a blank sample and obtaining its standard deviation. However, in chromatography it is very difficult to obtain a signal for a blank sample, as there is (ideally) no peak, and only noise.

Both LOD and LOQ need to be presented in concentration or mass domain, and for that some sort of calibration is necessary, to convert from the signal domain. Because the LOD is lower than the smallest working concentration, it is necessary to use samples spiked with very small amounts of analyte, in order to calculate it. Although this is the most accurate way to calculate LOD and LOQ, it is both time consuming and yields results that may be far from the working concentrations.

Another way to calculate these values is by using the calibration curve itself, as described by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [62]:

$$LOD = \frac{3.3\sigma}{S} \tag{1.5}$$

$$LOQ = \frac{10\sigma}{S} \tag{1.6}$$

Where σ is the population standard deviation, which can be estimated from the residual standard deviation of the regression line, and S is the slope. This method yields the instrumental LOD and LOQ, because it does not take into account the extraction procedure and contaminants.

1.8 Intended purpose

The purpose of the present work was to develop a methodology for the quantification of the main hazardous substances found in the empty pesticide containers, that can be analysed by GC/MS, to be implemented by Valorfito in its own laboratory, thus providing a proper evaluation of this waste according to EU regulations. The method should ideally be quick, somewhat cheap, easy to perform, and give reliable results.

CHAPTER 2

EXPERIMENTAL

2.1 Material, chemicals and instrumentation

2.1.1 Extraction

For milling the sample, a Retzsh ZM1 ultra centrifugal mill with 1 and 0.25 *mm* ring sieves was used. A Bandelin Sonorex Super ultrasonic bath and an RSLAB-6PRO vortex mixer were used for the extraction. HPLC grade THF was purchased from Carlo Erba.1,1,1,3,3,3-hexafluoro-2-propanol was purchased from Sigma-Aldrich. All pesticide standards were of analytical grade, also purchased from Sigma-aldrich.

2.1.2 Analytical instrumentation

A LECO Pegasus BT GC/TOFMS was used, comprised of an Agilent 7890B GC with a split/splitless injector, and a LECO Time-of-flight mass spectrometer. Injections were performed by a CTC-Analytics L-PAL3 autosampler fitted with a 10 μL syringe. The GC column was an Agilent HP-5MS Ultra-inert (L= 30m, ID= 0.25mm, d_f= 0.25μm). A Restek topaz single taper liner, with 4mm ID was used.

2.2 Sampling

Samples were taken from the waste processing facilities, which changed from 2018 to 2020. The first facility (**A**) had an industrial milling machine which produced very roughly milled material, and samples were taken by hand from big-bags, with most being still semi intact containers. The second facility (**B**) mixed the pesticide container with other wastes, such as paper bags and cloth rags, and then milled them. The third facility (**C**) produced a very homogeneous milled material with sizes of around 0.5-2 *mm*.

For both of these, samples were taken from the milled material. Figure 2.1 shows the type of materials collected. Two collections were made from facility **A**, in April and July 2018, entitled $\mathbf{1}^{st}$ and $\mathbf{2}^{nd}$, respectively, one from facility **B** in October 2019, and another from facility **C** in February 2020 entitled $\mathbf{3}^{rd}$ and $\mathbf{4}^{th}$, respectively. From each collection, two samples were made, from material taken at different times in the milling process, or from different bags. In the $\mathbf{3}^{rd}$ collection, one sample was taken from pesticide packages only, which had been previously milled, and another was taken from the milled material which was mixed with paper and cloth.

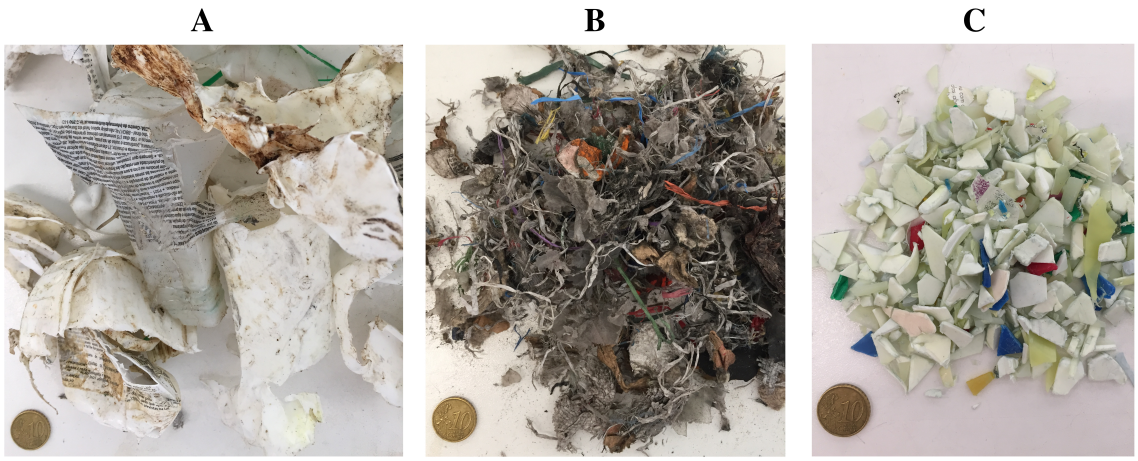

Figure 2.1: Used pesticide container samples taken from facility **A**, **B** (mixed with paper and cloth), and **C**. A 0.1€ coin is used for scale.

2.3 Extraction

The method was adapted from [56].

Samples were cut to pieces of approximately 1 *cm* and milled firstly with the 1 *mm* sieve, and then with the 0.25 *mm*. The resulting material was sieved with a 0.425 *mm* hand sieve, and stored in the freezer until extraction.

0.150 ± 0.001 *g* of each sample was measured into culture tubes with Teflon lined caps. 2 *mL* of THF was added, the mixture was vortexed for 5 seconds, and extracted in ultrasound bath for 15 minutes. Afterwards, the solvent was removed with a pasteur pipette, taking care not to remove most of the plastic, and 2 *mL* of 5%(v/v) 1,1,1,3,3,3-hexafluoro-2-propanol in THF was added to the culture tubes, vortexed and sonicated in the same way. The extracts were combined and filtered using a 0.45 *µm* PTFE syringe filter into a 5 *mL* vial, after which 0.5-1 *mL* of THF was passed through the syringe and filter into that same vial until the 5 *mL* mark was met. The samples were stored in the freezer until analysis.

2.4 Standard solutions and spiked materials

Stock solutions were prepared by weighing $5 \pm 0.1 mg$ of each analyte and adding 20 mL of solvent. Captan was weighed under a nitrogen atmosphere, and chlorthalonyl solutions were protected from light.

Standard solutions for GC were prepared in THF. The compounds were separated into two groups: α and β, because they exhibited different sensitivities. The compounds with the highest signal to injected concentration ratio were put into group α, and calibrated between 0.2-1.4 $\mu g/mL$. The others were put into group β, and calibrated between 0.7-2.5 $\mu g/mL$.

The spiked material for recovery experiments was made using a HDPE pesticide container taken from the production line before being filled, so that it had no pesticides. This was milled as explained in section 2.3, 2 g were weighed, then 1.6 mL of each stock solution was added, and the solvent was evaporated under a nitrogen current, so that the sample had 200 mg/kg of each analyte.

2.5 Instrumental analysis

2.5.1 GC/TOFMS conditions

Injections were performed in pulsed splitless mode at 260º C, with a 25 $psig$ pressure pulse for 0.2 minutes. The splitless time was set to 1 minute (including the pulse time), and afterwords a 100 mL/min split until 3 minutes, and then 20 mL/min until the end of the run. The septum purge flow was set to 3 mL/min, and the column was set to a constant flow of 1.2 mL/min. The injected volume was 1.2 μL. The oven program was as follows: 80º C for 1 minute, then an increment of 15º C/min. until 130º C, then an increment of 3º C/min. until 200º C, followed by an increment of 8º C/min. until 300º C, held for 5 minutes. The MS transfer line was set to 300º C, and the MS acquired 10 spectra per second from 40-550 Da at 70 eV, with an acquisition delay of 10 minutes.

2.5.2 Sample quantification

Each sample was injected into the GC/TOFMS with no prior dilution. Based on each analyte's signal versus the signal in the calibration curve, the dilution factor required for every sample was calculated, often more than one per sample. The dilutions were performed by adding the internal standard and THF. Afterwards, each dilution was injected.

Chapter 3

Results

3.1 Method development

3.1.1 Extraction procedure

Milling the sample to a fine powder is of major importance when the extracted material has a small mass. Three different milling methods were tested: Ball impact cryo milling, ultra-centrifugal milling with the sample at around 0º C and ultra-centrifugal milling with the sample cryogenically frozen with liquid nitrogen.

A Retzsh CryoMill was tested, whose principle of operation is to cool the sample using liquid nitrogen inside a small chamber with ceramic balls, and then using a very vigorous motion to break the sample via impact from the balls. A total of 9 cycles were performed per sample, each with 15 minutes, so that the sample was re-cooled at the beginning of each cycle. Before milling, the samples had been cut to pieces of approximately 1 *cm*. This method proved very inefficient for this material, due to several reasons: the sample chamber only allowed a very small amount, so it would be difficult to ensure representativity of the lot; each sample took several hours to mill, both because of the milling itself, and also because it took a long time to get the sample chamber back to ambient temperature so that it could be opened; the milling yielded a very heterogeneous material.

A Retzsh ZM1 ultra-centrifugal mill was used in two experiments: with a sample close to 0º C, and frozen in liquid nitrogen. In both cases the sample was processed as described in section 2.3. The experiment using liquid nitrogen proved unsuccessful, because the cold sample condensed a lot of atmospheric water. Ideally, a centrifugal mill with a cryogenic accessory should have been used, as described in [56], because that would ensure that the analytes remained stable throughout the process, and would also have improved milling, by taking the plastic material bellow its glass transition temperature,

making it brittle.

For the extraction solvent, several possibilities were considered. In order to avoid an unnecessary solvent shift step (drying the extracted sample under nitrogen and re-dissolving in another solvent), the solvent employed in the extraction should be compatible with both GC and Reverse-phase HPLC, in case the method was to be applied to HPLC as well. With that goal in mind, dicholoromethane and other solvents immiscible with water could not be used. Also, acetone was ruled out because it has a high UV cutoff, which can interfere in diode array detection. There are two widely used solvents that can fulfill these requirements: Acetonitrile (MeCN) and tetrahydrofuran (THF). Acetonitrile is the most amenable to RP-HPLC, but can induce degradation of compounds [21]. Furthermore, it has a higher boiling point, which helps prevent concentration changes due to solvent evaporation, but is also worse for GC/TOFMS, because it is more difficult to vaporize and has a larger gas volume (for the same amount of liquid). THF, on the other hand, is less polar while still having hydrogen bonding interactions, which may allow it to extract a higher range of compounds. Jones and Gordon [56] have used it in conjunction with 1,1,1,3,3,3-hexafluoro-2-propanol, presumably because this compound has high hydrogen bonding properties, making it ideal for dissolving compounds with lone electron pair functional groups, like pesticides. THF is usually sold with a stabilizer (commonly butylated hydroxytoluene, BHT), because it is prone to degradation into other compounds. BHT absorbs in the UV range, so it is a problem for UV detection if HPLC is necessary.

It was found by GC/TOFMS that THF from older bottles (HPLC grade, Carlo Erba) produced chromatograms with many peaks of high signal intensity, mostly eluting before 140° C (using the optimized run, as described in section 2.5.1). In order to test whether this was due to solubilization of volatile compounds from the lab atmosphere, cross contamination (*i.e.* from dirty pipettes, *etc.*) or from the solvent itself, 20 mL of THF from a new bottle were transferred to a clear sample vial with a Teflon lined cap, and placed under sunlight for three days, outside the lab, while another 20 mL were placed in an identical vial, exposed to the laboratory atmosphere (with ought a cap), and a third was placed with cap, sheltered from light (control). The chromatographic analysis was carried using a temperature ramp starting at 80° C for 3 minutes, then an increment of 3° C per minute until 150° C, and finally an increment of 15° C per minute until 300° C, and an MS solvent delay of 360 seconds.

The sample placed under sunlight exhibited a much higher amount of semi volatile compounds (eluting under 100° C), while the other two gave identical results, as can be seen in figure 3.1. The biggest peak was tentatively identified by comparison with the NIST MS database as octahydro-bifuran: two tetrahydrofuran rings united by a σ bond, probably a mixture of structural isomers. Although these compounds do not present a direct problem in GC analysis, because they elute earlier than any analyte, their presence might compromise method reproducibility between different lots of THF, because they are likely to change the pH of the solvent, and modify solubility properties. Furthermore,

it is possible that some degradation products contain π bonds, most likely ketones, which could interfere in HPLC-DAD detection, if it is to be implemented. It was also found that non-stabilized, HPLC grade THF purchased from Honeywell was very similar to Carlo Erba when first open, and degraded in the same way, over time.

It is likely that the electromagnetic radiation simply accelerated the process which happens spontaneously, even in dark THF bottles, catalysed by trace amounts of dissolved atmospheric water or oxygen.

Even though THF is subject to degradation, it still appears to be the most suitable solvent for this work. Therefore, to mitigate this problem, smaller THF bottles should be bought, sheltered from sunlight, and not kept open for a long time, so as to ensure they do not have time to degrade significantly.

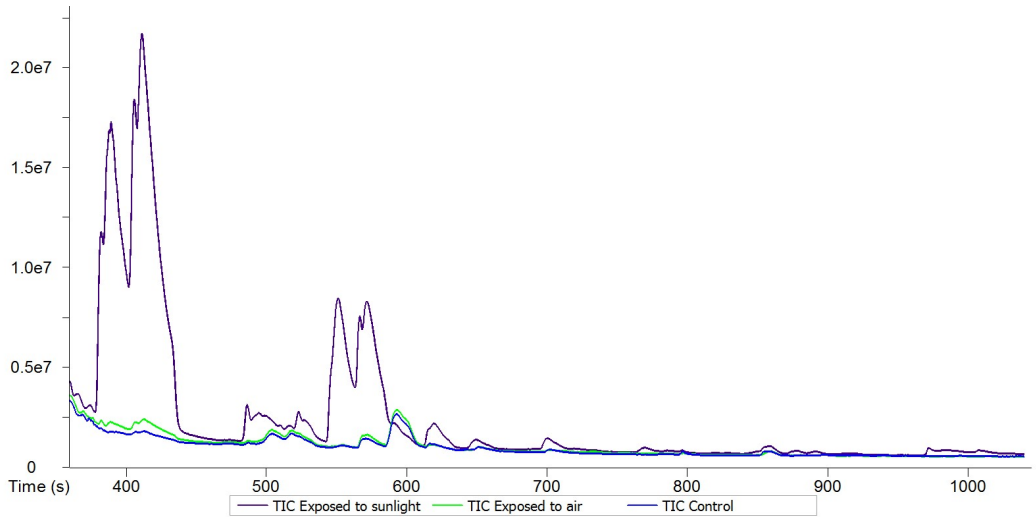

Figure 3.1: Overlapped chromatograms from THF left in different conditions for three days, *i.e.:* sealed in a dark vial, exposed to the laboratory atmosphere in a dark vial, and under direct sunlight in a sealed vial.

Ultrasound extraction is used commonly for plastic materials [54–56], since it improves the efficiency by using mechanical waves to impart kinetic energy onto the sample. Microwave extraction might also be used, but the electromagnetic radiation and higher temperature can increase the rate of degradation for some analytes.

The amount of sample mass to volume of extraction solvent was also tested. While having more sample mass for the same amount of solvent can increase detection limits, it also reduces extraction efficiency because of saturation. Therefore, it is important to have enough solvent to ensure a good solubilization of all analytes. In liquid-liquid partitioning, increasing the amount of extractions (while maintaining the total amount of solvent fixed) is known to increase extraction efficiency. Eras *et. al.* [54] have found this to be the case in the extraction from polymeric material as well. Therefore, for a total extracted volume of $4mL$, two extractions of $2mL$ would give a better result than only one of $4mL$. The first material used for the extraction tests was milled as described in 2.1.1, but with ought using the hand sieve. The spiked material was extracted using three

different masses: 0.1, 0.15 and 0.2 g of sample. 2 times $2mL$ of extraction solvent was used, because after extraction it is necessary to dilute the extract to an exact volume before applying any internal standard, or performing any other dilutions, in order to know the exact volume to mass ratio in the extraction. Therefore, 2 times $2mL$ allowed a further cleaning of the extraction vial and syringe with 0.5-1 mL of THF, to fill a $5mL$ volumetric flask. Thus, an extraction method somewhat similar to liquid-liquid partitioning, using sonication, was used.

The first extraction tests gave very irreproducible results, with relative standard deviations (%RSD) of extraction recovery in the order of 30-40%. The results were attributed to the heterogeneous size distribution of the particles, and therefore all subsequent samples were hand sieved after milling. It should be noted that if a better mill is used, capable of producing a very homogeneous particle size distribution, this step will not be necessary.

The subsequent tests were performed by spiking the milled and sieved plastic with 200 mg/kg of each analyte, and extracting each mass (0.1, 0.15 and $0.2mg$) three times, then diluting to an appropriate concentration and adding the internal standard. The spiked concentration is relatively high compared to what might be expected in the real samples (for legal purposes, the sum of all analytes should not be over $1000mg/kg$, or 0.1%), but it is expected that the real samples have many more co-extracted compounds, which also have an influence in the extraction efficiency. The distribution ratio (Kd) between solvent and polymer for a certain compound is described as the concentration of a compound in the solvent divided by the concentration in the polymer. That is, assuming that the Kd remains constant for any concentration. This, however, is most likely not the case, because of several reasons, namely that there are many different co-extractants, some of which are not even detected by the methods employed, and may interfere with the analytes, and also because the polymeric material does not behave in the same way as a liquid, and a portion of the compounds may be absorbed inside the polymer [54]. Furthermore, if the sum of organic compounds in the sample is very high, saturation of the solvent can occur, reducing the extraction percentage. Therefore, if the Kd changes significantly with concentration, different spike levels should have been tested.

The results for selected pesticides can be seen in figure 3.2. The full results for every analyte are listed in appendix C.

The results displayed are representative of almost every analyte, with 0.15 g yielding the best results. 0.1 g extractions have a higher standard deviation, because the smaller mass makes the extraction more prone to errors. Using 0.2 g will reduce method detection and quantification limits, but also makes the extraction prone to solvent saturation, especially in matrices with many compounds, which will compromise reproducibility. When considering the best performance, a low standard deviation might be more important than a high recovery, because as long as the recovery value is stable, the quantitation results can be adjusted to account for this *i.e.* a method with a constant systematic error that is accounted for and can be mitigated is better than one with a random deviation in values. Folpet and Captan have recoveries bellow 80%. This behaviour is expected,

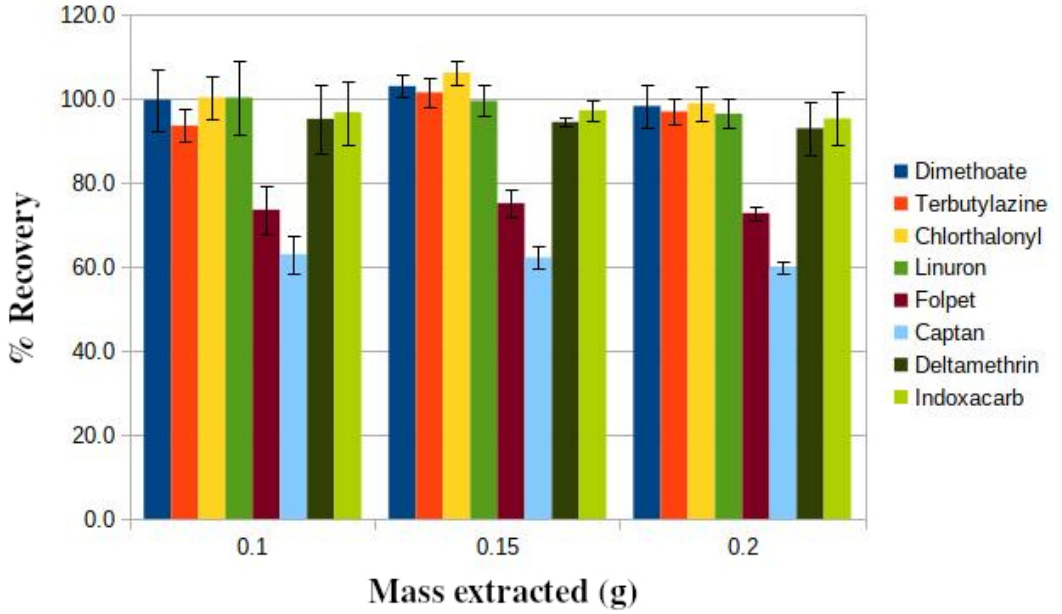

Figure 3.2: Recovery experiment results for selected pesticides, using three different masses. n = 6.

especially because these compounds are prone to degradation into their hydrolysis products: phthalimide and tetrahydrophtalimide, respectively. Even though the recovery standard deviations may seem low, this is most likely because every spiked blank sample was treated exactly the same. However, for real samples who may be exposed to the atmosphere or at ambient temperatures for different times, it cannot be asumed that the recoveries will always be the same (because the rate of degradation is difficult to predict). The ideal solution for this would be to use isotopically labeled captan and folpet as surrogate standards after milling the samples (to monitor the degradation from this point forward) and quantify phthalimide and tetrahydrophtalimide as well. In order to mitigate hydrolysis, the extraction solvent should be dried with molecular sieves or a similar method. Given the results and adaptability to different matrix concentrations, the ideal quantity of material to be extracted is 0.15 g.

3.1.2 GC/TOFMS

Four internal standards were tested for usage in GC. Two were linear alkanes and two were pesticides: metalaxyl and oxadixyl.

The alkanes, Heneicosane and tritriacontane ($C_{21}H_{44}$ and $C_{33}H_{68}$, respectively), were tested because they are some of the most stable and reproducible compounds in GC analysis. This is due to their lack of functional groups and homogeneous electron distribution. However, especially because of these characteristics, they are very bad at mimicking most pesticides, and therefore are not suited as internal standards. The results for selected analytes are shown in table 3.1. The approval for oxadixyl has been revoked by the EU

in 2018 [63], therefore it is expected that this compound will not be found in any sample. However, because of possible illegal use, its presence must still be assessed. For all quantified samples in the present work, neither oxadixly or metalaxyl were found.

Table 3.1: Selected analytes and coefficient of determination for calibration obtained with four different internal standards. The concentration values used were the same as for the quantitation experiments. The experiment was carried with an older inlet liner and column.

Analyte	R^2				
	Heneicosane	Tritriacontane	Oxadixyl	Metalaxil	No IS
Captan	0.976	0.974	0.983	0.975	0.975
Chlorpyrifos	0.993	0.989	0.996	0.993	0.983
Deltametrin	0.967	0.966	0.985	0.975	0.937
Folpet	0.977	0.975	0.984	0.977	0.975
Indoxacarb	0.965	0.963	0.982	0.973	0.941
Iprodione	0.901	0.896	0.940	0.920	0.860
λ–Cyhalothrin	0.992	0.986	0.996	0.991	0.951
Average	0.967	0.964	0.981	0.972	0.946

Several GC variables were optimized, namely the injection parameters and oven program. For greater method sensitivity, splitless injection must be used, even though this might increase degradation when compared to split injection. A pulsed pressure method was tested, so that the increased carrier pressure in the inlet would allow for a higher volume of sample to be injected with ought risk of backflash and carryover. This also allowed for a better transference of the sample onto the column, and ensured that the solvent re-condensed when entering the column, in order to focus early eluting peaks.

The oven program was optimized in regards to resolution and time efficiency. The initial oven temperature was set to 80º C because this allowed for a proper re-condensation of the compounds at the base of the column, and consequent focussing. Afterwards, a very sharp temperature increase to 130º C helps reduce the analysis time with ought much loss in resolution or peak shape, because even early eluting compounds will only start moving through the column at around this temperature. Afterwards, the ramp slows down considerably, because it was found that not only many analytes, but many matrix compounds tend to elute at this 130-200º C range. Afterwards, the temperature gradient is increased, to reduce analysis time. There are four pairs of co-eluting compounds in the final oven program: Methiocarb and Linuron; Captan and Penconazol; Acetamiprid and Iprodione; Deltamethrin and Indoxacarb. It was found that all of these could be separated if an adequate temperature program was used, but at significant cost of analysis time and throughput. Furthermore, by taking pure spectra of each compound, it was found that each compound has at least 3 m/z's of high relative intensity that the other compound does not have (with the exception of Linuron, explained below). An example can be seen in figure 3.3. Therefore, even if the chromatographic peaks completely eclipse, it is

possible to separate them by their m/z's. Except for deltamethrin and indoxacarb (who have the exact same retention time), the other pairs are partially resolved.

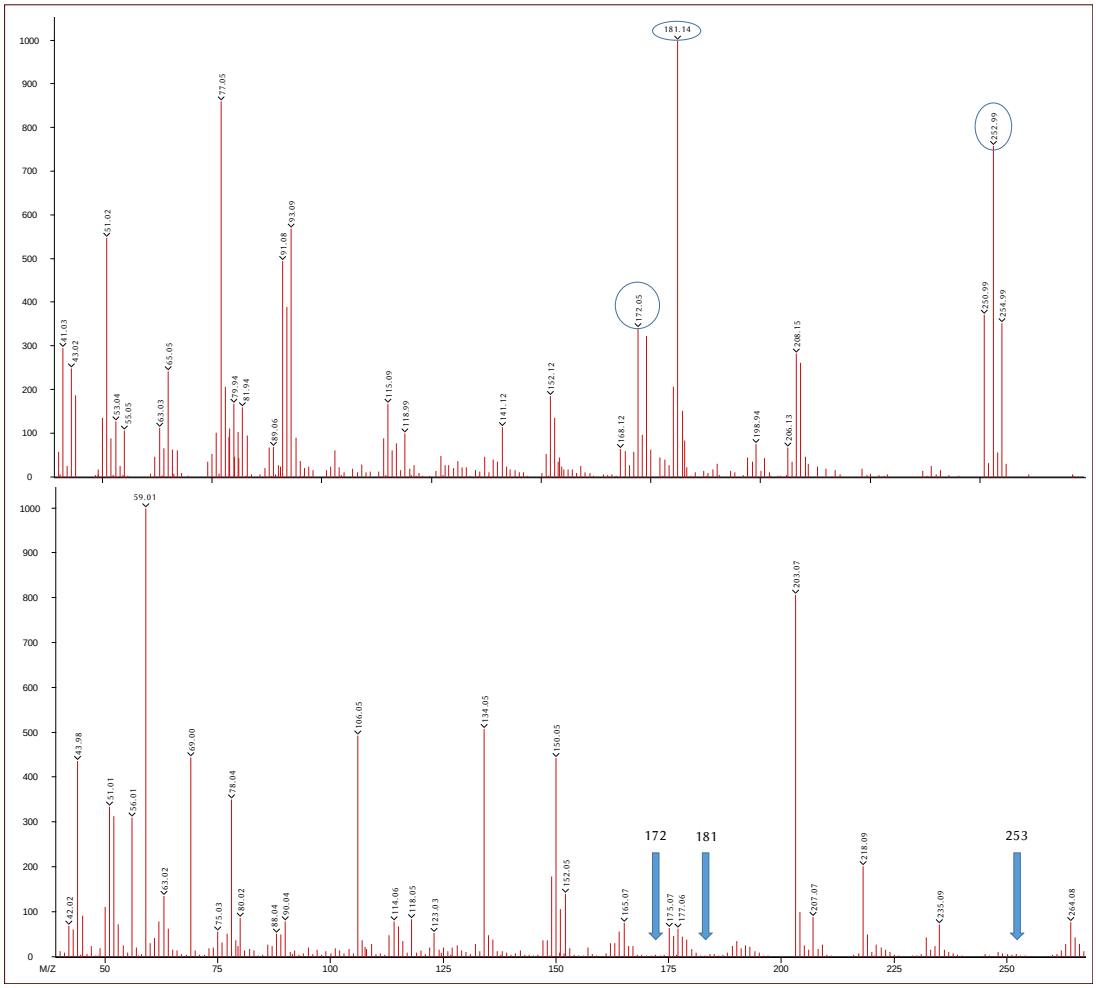

Figure 3.3: Mass spectra section of Deltamethrin (above) and Indoxacarb (bellow). The selected quantification and qualification ions for deltamethrin are highlighted.

For the other analytes, quantification and qualification m/z's were selected based on their relative intensity (in order to improve detection limits), and on the likelihood of co-elutions. For most analytes, it was found that smaller m/z's (bellow 70, roughly) generally tended to be more prone to co-elutants and interferences in real samples, because it is more likely for a co-elutant to share a smaller structural motif with the analyte than a bigger one. For these cases, namely Linuron iprodione and indoxacarb, a higher m/z was chosen for quantification. In the case of linuron, especially, $m/z = 61$ has more than doubled the intensity of the second highest mass peak, which is $m/z = 46$. However, both of these m/z's were found to have many interferences (especially from methiocarb, because the two compounds partially co-elute, as explained earlier), whose effects can be seen in figure 3.4. Therefore, $m/z = 187$ was chosen for quantification, as it can be integrated more easily and reproducibly. Because methiocarb and linuron are partially resolved, $m/z = 61$ and 46 can still be used as qualification ions.

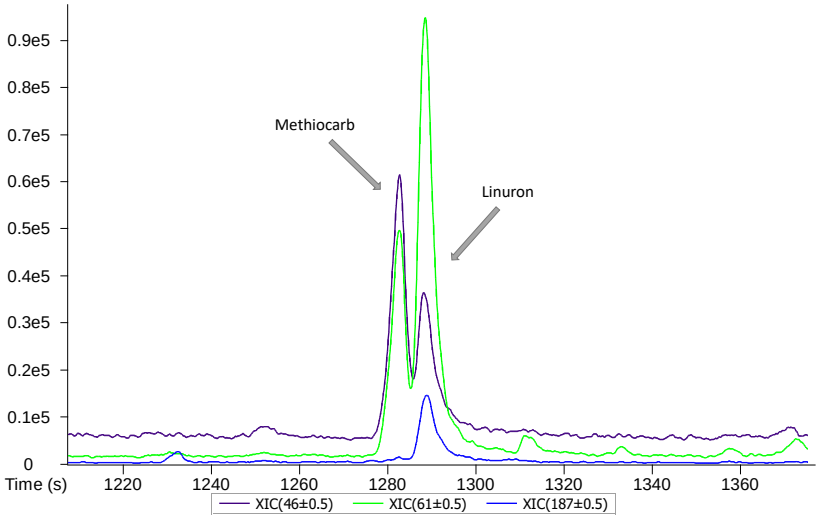

Figure 3.4: Chromatogram from real sample, with qualification and quantification ions for Linuron. The $m/z = 61$ and 46 of methiocarb have an intensity lower than 5% of the base peak, but it is in much higher concentration than linuron.

Acetamiprid was found to suffer from strong chemical activity, as can be seen in figure 3.5. Previously, it had been possible to calibrate and quantify this compound in the 0.2-1400 $\mu g/mL$ range (injected concentration), but at the time these experiments were conducted, the peaks obtained were very small and even absent for the lower concentrations. The sum of $m/z = 126$ and 152 (the quantifier and one of the qualifiers) did not yield acceptable results either. The inlet liner was changed to a new, high grade deactivated one and standard solutions were made fresh, but still the problem persisted, leading to the conclusion that this problem is most likely chemisorption caused by the analytical column.

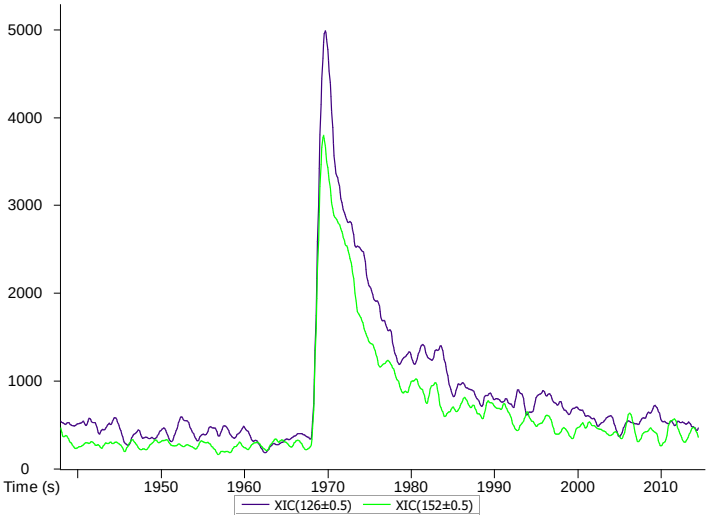

Figure 3.5: Acetamiprid peak for a concentration of 1.4 $\mu g/mL$. The signal to noise ratio of the quantification ion ($m/z = 126$) is around 11.

3.2 Method Performance

The following table shows the relevant merit parameters for all analytes studied. Because it was not possible to obtain a satisfiable signal for Acetamiprid, it was not quantified.

Table 3.2: Analyes, and their respective merit parameters. Retention indexes calculated according to Kovat's formula for temperature program. Range α = 0.2-1.4 $\mu g/mL$, β = 0.7-2.5 $\mu g/mL$. Calibration curve calculated by dividing signal and concentration of analyte for those of the internal standard, oxadixyl, at 0.5 $\mu g/mL$. There were 11 concentrations per range, each was injected three times, and no obtained value was retracted from any of the analytes *i.e.* no outliers were removed.

Name	Retention index	Calibration curve			Recovery	LoD	LoQ	Quantifier	Qualifier
		R2	Range	Slope	(% ± s)	(mg/kg)	(mg/kg)	*m/z*	*m/z*
Bromoxynil Butirate	1906	0.9923	α	5.01	94 ± 3	3.9	11.8	71	88; 277
Bromoxynil Octanoate	2332	0.9969	α	1.72	92 ± 4	2.5	7.5	127	67; 88
Captan	2053	0.9969	α	2.52	62 ± 3	2.5	7.5	79	77; 149
Chlorpyrifos	1986	0.9922	α	1.45	95 ± 5	3.9	11.9	97	197; 199
Chlorthalonyl	1813	0.9919	α	1.73	106 ± 3	4	12.1	266	264; 268
Deltamethrin	3068	0.9879	β	0.35	94 ± 1	7	21.3	181	253; 172
Diflufenican	2399	0.9960	α	1.94	97 ± 4	2.8	8.5	266	394; 101
Dimethoate	1725	0.9962	α	1.70	103 ± 3	2.7	8.3	87	93; 125
Fluazifop-p-Butyl	2247	0.9950	α	0.84	89 ± 3	3.2	9.6	282	254; 383
Folpet	2066	0.9958	α	0.80	75 ± 3	2.9	8.7	104	130; 260
Indoxacarb	3068	0.9889	β	0.65	97 ± 3	6.8	20.5	203	59; 150
Iprodione	2445	0.9914	β	0.27	99 ± 3	5.9	17.9	314	58; 316
Linuron	1946	0.9853	β	0.67	99 ± 4	7.8	23.5	187	61; 46
Methiocarb	1943	0.9873	β	1.31	97 ± 3	7.2	21.9	168	153; 109
Metribuzin	1874	0.9966	α	0.83	90 ± 3	2.6	7.8	198	103; 144
Penconazole	2050	0.9919	α	2.59	94 ± 4	4	12.2	159	161; 248
S-Metolachlor	1973	0.9955	α	3.38	93 ± 4	3	9	162	238; 240
Tebuconazol	2369	0.9965	α	0.96	93 ± 4	2.6	8	125	250;127
Terbutylazine	1778	0.9824	α	4.25	101 ± 3	5.9	18	214	173; 68
Thiametoxam	2015	0.9921	α	0.40	86 ± 2	3.9	11.9	132	182; 212
λ–Cyhalothrin	2612	0.9895	β	1.12	100 ± 4	6.6	19.9	181	197; 208

The Limits of detection and quantification obtained by this method will usually be sufficient for classifying it as hazardous or not, since the legal threshold is 1000 mg/kg for the most hazardous. However, if there are a lot of compounds present in the waste, a lower limit might be necessary.

The calibration yielded acceptable results for all analytes, in the concentration range used. The fact that no outliers were taken for any compound, demonstrates the high precision of the instrumental method. Since the concentration range chosen is quite small, it was difficult to dilute the real samples in order to fit in the calibration curve with ought having to extrapolate any values. Because of this, a higher range was tested, but linearity was not achieved. It might have been useful to make two separate calibration curves at different concentration ranges, in order to facilitate the dilution and quantification of real samples.

Figure 3.6 shows a chromatogram with all analytes.

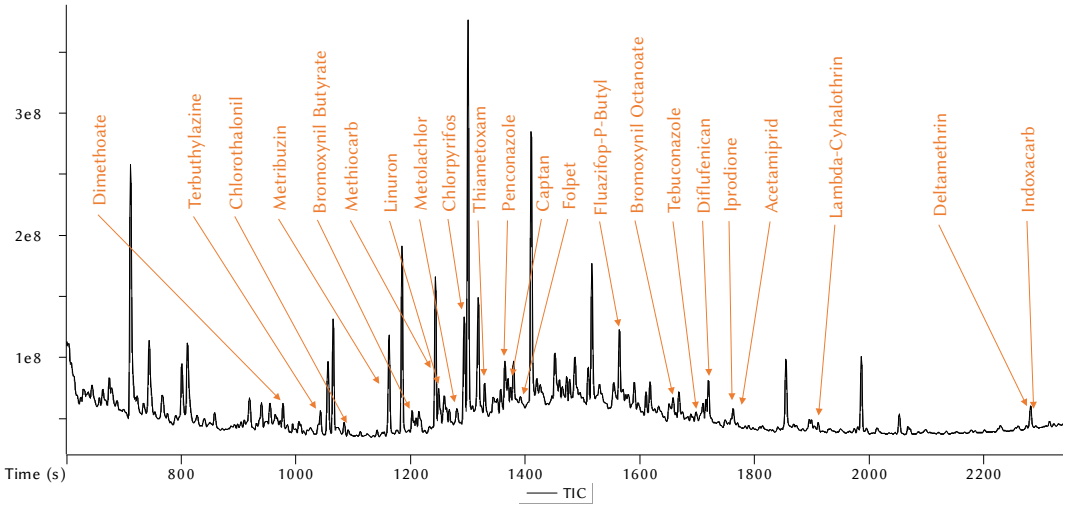

Figure 3.6: Total ion current chromatogram of a real sample, spiked with every analyte. The concentrations range from 10-250 $\mu g/mL$

3.3 Quantification results

The results for all samples are shown in table 3.3. Only four compounds were not detected in any of the samples; bromoxynil butirate, bromoxynil octanoate, captan and iprodione. It is possible that the bromoxynil esters were originally present, but hydrolysed into bromoxynil. The same might have happened for captan. Consequently, it is important to monitor bromoxynil, phtalimide and tetrahydrophtalimide, because these compounds will also contribute to the overall toxicity of the material. When calculating the sum of all compounds, the LoD values were used when the compound was not detected, and LoQ

when it was bellow this value. This approach will yield an estimation by excess of the real value, which is preferable to its opposite (for legal purposes), as can be seen in table 3.3.

The standard deviations presented only reflect the instrumental portion of the method, because they were calculated using different injections of the same solution. The real standard deviation (per collection *i.e.* $\mathbf{1}^{st}$, $\mathbf{2}^{nd}$, etc.) is much higher, as can be seen in the disparity between sample *a* and *b* for most collections. This is a sampling problem, stemming from the fact that human bias was introduced in the collection of the sample and processing. The only solution to this is to mill the whole lot better before collecting samples. Furthermore, in order to obtain a better estimation of each lot, more samples should be made.

Using the LoD and LoQ calculated from the calibration curve increases the sum value considerably, whereas using an LoD and LoQ calculated directly from the signal to noise ratio of each compound (=3 for LoD and =10 for LoQ) would certainly give much lower values for these merit parameters. However, if these were used, the LoQ would have been far bellow the calibration curve range, and although a compound might be detectable at lower concentrations, there is no guarantee that its signal response will be precise (low standard deviation), especially because phenomena such as chemisorption vary considerably with concentration. Therefore, the LoQ obtained by the signal to noise method might not equate to the real limit of quantification.

Table 3.3: Quantification results for all four collections. Each sample (*a* and *b*) was extracted once, diluted to an appropriate concentration, and injected three times (n=3). The values are presented as $\overline{x} \pm s$, *mg/kg*. ND = not detected. For the sum, the LoD was used whenever the analyte was not detected, and LoQ whenever it was detected, but bellow this value (<LoQ).

	1^{st}		2^{nd}		3^{rd}		4^{th}	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Bromoxynil Butirate	ND	ND	ND	ND	ND	ND	ND	ND
Bromoxynil Octanoate	ND	ND	ND	ND	ND	ND	ND	ND
Captan	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	17.4 ± 0.4	14.6 ± 0.1	14.8 ± 0.1	17.6 ± 0.5	156 ± 2	<LoQ	<LoQ	<LoQ
Chlorthalonyl	ND	ND	ND	ND	ND	ND	<LoQ	<LoQ
Deltametrin	ND	ND	31 ± 2	26.7 ± 0.6	42 ± 1	<LoQ	ND	ND
Diflufenican	41 ± 2	75 ± 1	280 ± 3	30 ± 1	83 ± 3	126 ± 6	17.7 ± 0.8	12.1 ± 0.2
Dimetoate	ND	ND	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	ND
Fluazifop-p-butyl	19.8 ± 0.5	28.6 ± 0.5	14.6 ± 0.5	35 ± 1	110 ± 1	<LoQ	ND	ND
Folpet	ND	ND	ND	ND	<LoQ	11 ± 0.1	ND	ND
Indoxacarb	39.5 ± 0.5	28.8 ± 0.6	406 ± 6	317 ± 6	205 ± 8	<LoQ	<LoQ	<LoQ
Iprodione	ND	ND	ND	ND	ND	ND	ND	ND
Linuron	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
Methiocarb	289 ± 5	1173 ± 16	137 ± 2	169 ± 7	1143 ± 32	2080 ± 75	<LoQ	<LoQ
Metribuzin	114 ± 3	104 ± 2	234 ± 6	89 ± 2	736 ± 17	19.6 ± 0.2	18.2 ± 0.9	26.1 ± 0.1
Penconazol	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
s-Metolachlor	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
Tebuconazol	ND	ND	ND	ND	<LoQ	8.6 ± 0.1	<LoQ	<LoQ
Terbutylazine	ND	ND	ND	ND	ND	<LoQ	<LoQ	<LoQ
Thiametoxam	14.5 ± 0.7	18.3 ± 0.7	68.8 ± 0.5	45.8 ± 0.5	60.2 ± 0.6	<LoQ	ND	ND
Lambda-cyalothrin	<LoQ	<LoQ	43 ± 1	36.5 ± 0.8	43.4 ± 0.2	<LoQ	<LoQ	<LoQ
Sum *mg/kg*	639.7	1546.8	1312.4	849.8	2673.0	2430.1	233.0	229.7

The decline in pesticide concentrations from the last collection suggests that the triple rinse campaign by Valorfito has been successful, and that the later materials (4^{th} collection) might be classified as non hazardous. However, the analytes quantified do not represent all that can be found in the residue. There are several compounds that cannot be chromatographed by GC. There were also compounds not monitored that were tentatively identified in the sample, by comparison with the NIST MS database, and Kovat's retention indexes (maximum deviation allowed = 30). The ones with higher detected signal were: Tetraconazole, Metazachlor, Oxyfluorfen, Pyraclostrobin and Cypermethrin.

Chapter 4

Conclusion

In this work a methodology for the analysis of pesticides in post-consumer packaging was developed. The method yielded acceptable results, although the sampling method was not satisfactory for an accurate characterization of the material.

The extraction method proved to have adequate repeatability; The most critical step was the milling, and using an ultra centrifugal mill gave the best results, although if possible a cryogenic method should be employed.

The use of GC/TOFMS permitted a more complete analysis of the samples (to the extent that GC is capable), especially the identification of other pesticides present, in addition to the quantification of analytes. Chlorthalonyl, Folpet and Captan are prone to degradation, and should be handled with extra care. Because of this, they are also less likely to be found in the samples, because a large percentage can degradate in the process of industrial collection, storage, processing and milling. Thus, their degradation products should be monitored.

The results obtained are indicative of the success of Valorfito's campaign to implement the triple rinse. However, in order to fully characterize the post-consumer containers, other high usage pesticides should be monitored as well, many of which, like abamectin and spinosade, cannot be analysed by GC. Therefore, the method should be adapted to HPLC, and the ability to quantify all analytes (including the ones in this present work) by LC/MS/MS should be investigated, because that would reduce the cost and time involved in using two different chromatographic techniques.

Finally, the method developed should be fully validated using proper methodologies as described by EU regulations.

Chapter 5

Further Work

Due to the necessity of further characterization of the pesticide containers, a chromatographic methodology is being developed to analyse 10 more high usage pesticides by HPLC-DAD, namely: Abamectin, Bentazon, Bromoxynil, Chlorantraniliprole, Fenpyroximate, Mesotrione, Penoxsulam, Spinosade, Triclopyr and Tiaclopride. Three of these compounds can be chromatographed by GC (Bromoxynil, Bentazon and Triclopyr), but present several difficulties, and exhibit very poor peak shapes, unless an ultra-inert chromatographic path is guaranteed. Acetamiprid will also be adapted to HPLC-DAD, if it proves to have sufficient absorption in the UV range, and amenability to the technique. At the moment, a chromatographic run has been optimized, using a Phenomenex Kinetex Polar $C_{18}(100 \times 4.6mm \times 1.8\mu m)$, and all compounds (except acetamiprid) can be resolved and have been calibrated in the range of 0-5-5 $\mu g/mL$. The biggest problem in coupling reversed phase HPLC and capillary GC/MS is that the solvent used for extraction is not necessarily ideal for both techniques. It might be necessary to employ a solvent shift (by drying the sample under nitrogen and re-disolving with an appropriate solvent) for HPLC, use another extraction solvent (although this might cause problems for GC/TOFMS), or employ a more sensitive HPLC technique, such as LC/MS/MS, so that the extract can be diluted with mobile phase. This method is being developed and is almost complete.

The final method should be validated according to EU regulations, in order to be used for routine analysis.

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Appendix A

Pesticides analysed and selected properties

Hazard statements according to [64], and remaining data according to the *Pesticide Properties database* [20]. Logarithm of octanol-water partition coefficient (LOG P_{OW}) calculated for 20° C, pH=7.

Acetamiprid

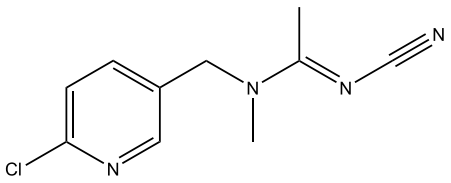

Figure A.1: Structure of Acetamiprid

Molar Mass	226.67 $g.mol^{-1}$
Isomerism	π bond isomerism. Can exist as E- and Z-
Solubility in water	2950 $mg.L^{-1}$
Log (P_{OW})	0.8
Hazard statements	H302, H412
Chemical family	Neonicotionid
Use	Insecticide

Bromoxynil butanoate

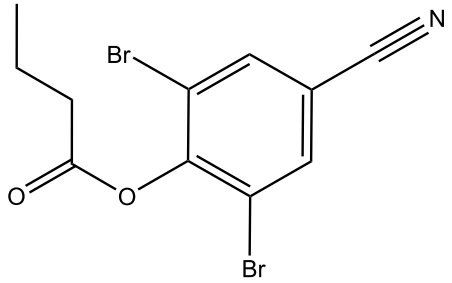

Figure A.2: Structure of Bromoxynil butanoate

Molar Mass	347 $g.mol^{-1}$
Isomerism	–
Solubility in water	No data
Log (P_{OW})	3.86
Hazard statements	Not present in database
Chemical family	Benzonitrile
Use	Herbicide

Bromoxynil octanoate

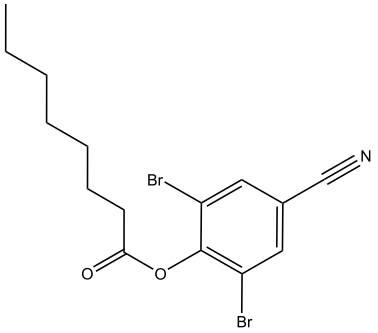

Figure A.3: Structure of Bromoxynil octanoate

Molar Mass	403.1 $g.mol^{-1}$
Isomerism	–
Solubility in water	0.05 $mg.L^{-1}$
Log (P_{OW})	6.2
Hazard statements	H302, H317, H331, H400, H410, H361d
Chemical family	Benzonitrile
Use	Herbicide

Captan

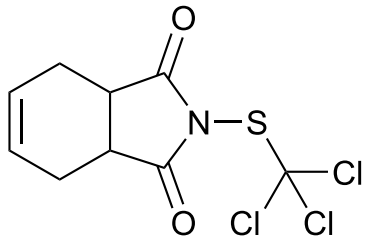

Figure A.4: Structure of Captan

Molar Mass	300.61 $g.mol^{-1}$
Isomerism	Chiral
Solubility in water	5.2 $mg.L^{-1}$
Log (P_{OW})	2.5
Hazard statements	H317, H318, H331, H351, H400
Chemical family	Phtalimide
Use	Fungicide, bactericide

Chlorothalonil

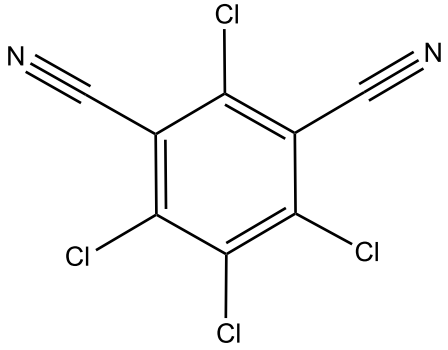

Figure A.5: Structure of Chlorothalonil

Molar Mass	265.91 $g.mol^{-1}$
Isomerism	–
Solubility in water	0.81 $mg.L^{-1}$
Log (P_{OW})	2.94
Hazard statements	H317, H318, H330, H335, H351, H400, H410
Chemical family	Chloronitrile
Use	Fungicide

Chlorpyrifos

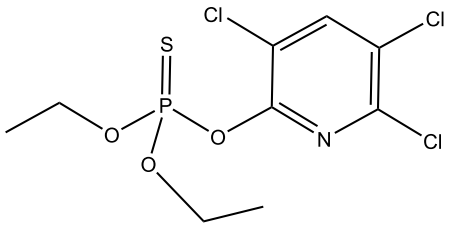

Figure A.6: Structure of Chlorpyrifos

Molar Mass	350.58 $g.mol^{-1}$
Isomerism	–
Solubility in water	1.05 $mg.L^{-1}$
Log (P_{OW})	4.7
Hazard statements	H301, H400, H410
Chemical family	Organophosphate
Use	Insecticide

Deltamethrin

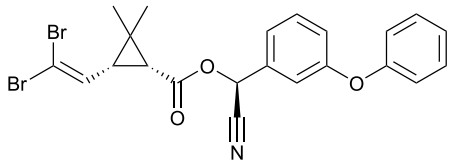

Figure A.7: Structure of Deltamethrin

Molar Mass	505.2 $g.mol^{-1}$
Isomerism	Chiral. Commercial products can have a mixture of diastereomers
Solubility in water	0.0002 $mg.L^{-1}$
Log (P_{OW})	4.6
Hazard statements	H301, H331, H400, H410
Chemical family	Pyrethroid
Use	Insecticide

Diflufenican

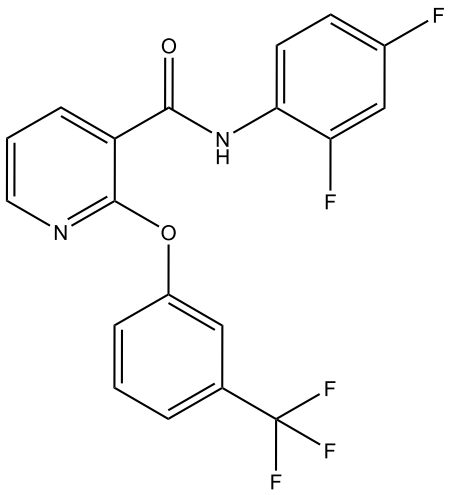

Figure A.8: Structure of Diflufenican

Molar Mass	394.29 $g.mol^{-1}$
Isomerism	–
Solubility in water	0.05 $mg.L^{-1}$
Log (P_{OW})	4.2
Hazard statements	H412
Chemical family	Carboxamide
Use	Herbicide

Dimethoate

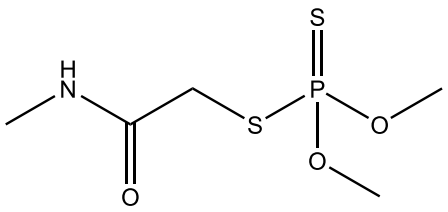

Figure A.9: Structure of Dimethoate

Molar Mass	229.26 $g.mol^{-1}$
Isomerism	–
Solubility in water	25900 $mg.L^{-1}$
Log (P_{OW})	0.75
Hazard statements	H302, H312
Chemical family	Organophosphate
Use	Insecticide, acaricide

Fluazifop-p-butyl

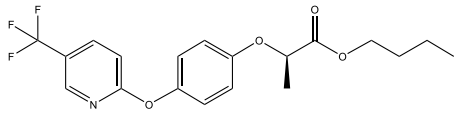

Figure A.10: Structure of Fluazifop-p-butyl

Molar Mass	383.36 $g.mol^{-1}$
Isomerism	Chiral. Two enantiomers
Solubility in water	0.93 $mg.L^{-1}$
Log (P_{OW})	4.5
Hazard statements	H400, H410, H361d
Chemical family	Aryloxyphenoxypropionate
Use	Herbicide

Folpet

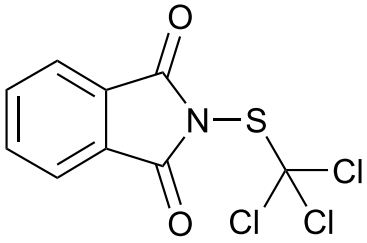

Figure A.11: Structure of Folpet

Molar Mass	296.56 $g.mol^{-1}$
Isomerism	–
Solubility in water	0.8 $mg.L^{-1}$
Log (P_{OW})	3.02
Hazard statements	H317, H319, H332, H351, H400
Chemical family	Phtalimide
Use	Fungicide

Indoxacarb

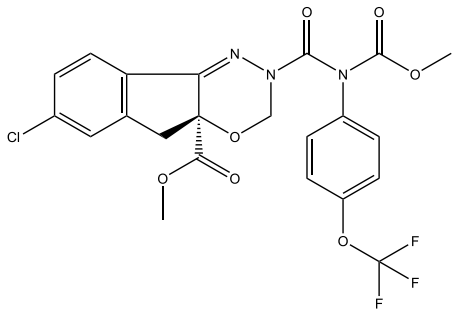

Figure A.12: Structure of Indoxacarb

Molar Mass	527.83 $g.mol^{-1}$
Isomerism	Chiral. Two enantiomers
Solubility in water	0.2 $mg.L^{-1}$
Log (P_{OW})	4.65
Hazard statements	H301, H317, H332, H372, H400, H410
Chemical family	Oxadiazine
Use	Insecticide

Iprodione

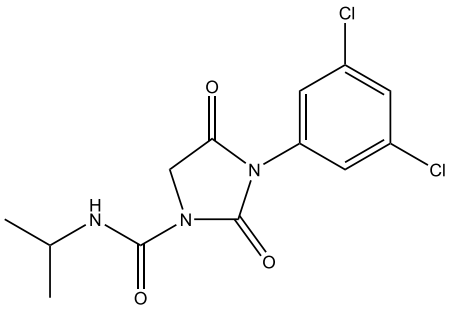

Figure A.13: Structure of Iprodione

Molar Mass	330.17 $g.mol^{-1}$
Isomerism	Commercial substance can have a structural isomer
Solubility in water	6.8 $mg.L^{-1}$
Log (P_{OW})	3
Hazard statements	H351, H400, H410
Chemical family	Dicarboximide
Use	Fungicide

λ-Cyhalothrin

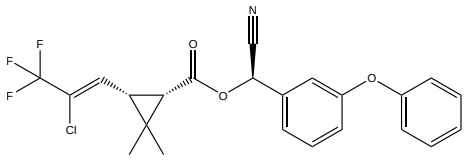

Figure A.14: Structure of one enantiomer of λ-Cyhalothrin. The compound is a 1:1 mixture of this enantiomer and its mirror image

Molar Mass	449.85 $g.mol^{-1}$
Isomerism	Chiral. A mixture of enantiomers
Solubility in water	0.005 $mg.L^{-1}$
Log (P_{OW})	5.5
Hazard statements	H301, H312, H330, H400, H410
Chemical family	Pyrethroid
Use	Insecticide

Linuron

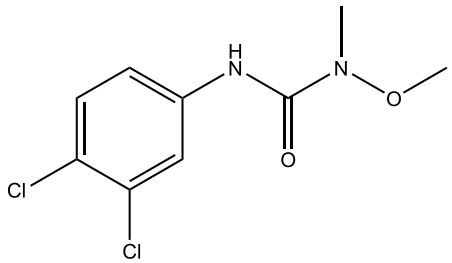

Figure A.15: Structure of Linuron

Molar Mass	249.09 $g.mol^{-1}$
Isomerism	–
Solubility in water	63.8 $mg.L^{-1}$
Log (P_{OW})	3
Hazard statements	H302, H351, H373, H400, H410, H360Df
Chemical family	Urea
Use	Herbicide

Methiocarb

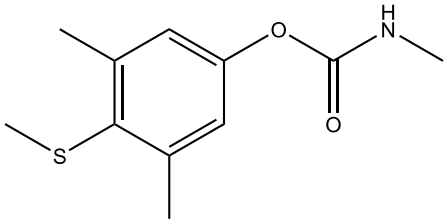

Figure A.16: Structure of Methiocarb

Molar Mass	225.31 $g.mol^{-1}$
Isomerism	–
Solubility in water	27 $mg.L^{-1}$
Log (P_{OW})	3.18
Hazard statements	H301, H400, H410
Chemical family	Carbamate
Use	Insecticide, molluscicide, bird repellent

Metribuzin

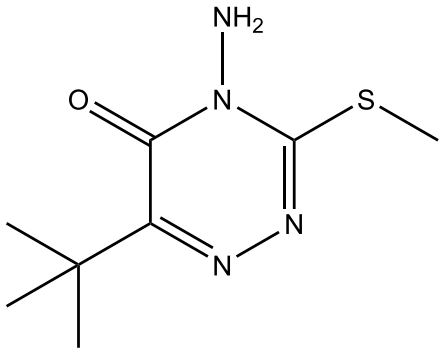

Figure A.17: Structure of Metribuzin

Molar Mass	214.29 $g.mol^{-1}$
Isomerism	–
Solubility in water	1165 $mg.L^{-1}$
Log (P_{OW})	1.65
Hazard statements	H302, H400, H410
Chemical family	Triazinone
Use	Herbicide

Penconazol

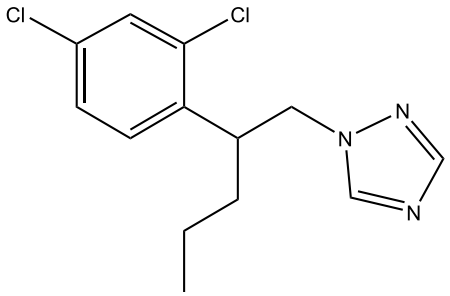

Figure A.18: Structure of Penconazol

Molar Mass	284.18 $g.mol^{-1}$
Isomerism	Chiral. A mixture of enantiomers
Solubility in water	73 $mg.L^{-1}$
Log (P_{OW})	3.72
Hazard statements	H302, H400, H410, H361d
Chemical family	Triazole
Use	Fungicide

S-metolachlor

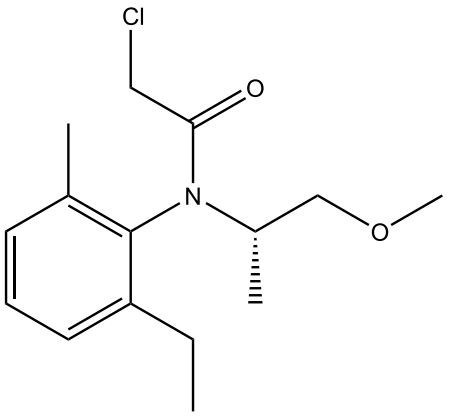

Figure A.19: Structure of S-metolachlor

Molar Mass	283.79 $g.mol^{-1}$
Isomerism	Chiral. S- to R- ratio is about 88:12
Solubility in water	480 $mg.L^{-1}$
Log (P_{OW})	3.05
Hazard statements	H317, H400, H410
Chemical family	Chloroacetamide
Use	Herbicide

Tebuconazol

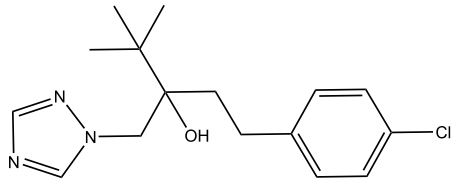

Figure A.20: Structure of Tebuconazol

Molar Mass	307.82 $g.mol^{-1}$
Isomerism	Chiral. A mixture of enantiomers
Solubility in water	36 $mg.L^{-1}$
Log (P_{OW})	3.7
Hazard statements	H302, H400, H410, H361d
Chemical family	Triazole
Use	Fungicide, plant growth regulator

Terbutylazine

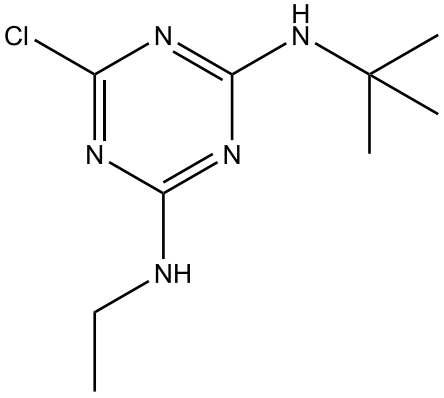

Figure A.21: Structure of Terbutylazine

Molar Mass	229.71 $g.mol^{-1}$
Isomerism	–
Solubility in water	6.6 $mg.L^{-1}$
Log (P_{OW})	3.4
Hazard statements	H315, H319, H335
Chemical family	Triazine
Use	Herbicide, microbiocide, algicide

Thiamethoxam

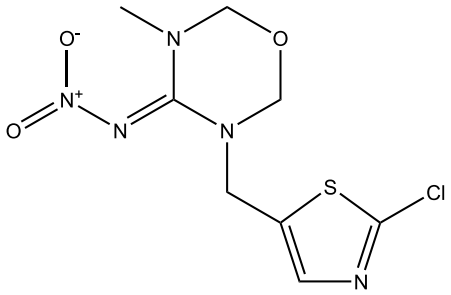

Figure A.22: Structure of Thiamethoxam

Molar Mass	291.71 $g.mol^{-1}$
Isomerism	π bond isomerism. Commercial product is a mixture of both isomers
Solubility in water	4100 $mg.L^{-1}$
Log (P_{OW})	-0.13
Hazard statements	H302, H400, H410
Chemical family	Neonicotinoid
Use	Insecticide

Appendix B

List of hazard statements and corresponding codes

H300 Fatal if swallowed
H301 Toxic if swallowed
H302 Harmful if swallowed
H312 Harmful in contact with skin
H317 May cause an allergic skin reaction
H318 Causes serious eye damage
H319 Causes serious eye irritation
H330 Fatal if inhaled
H331 Toxic if inhaled
H332 Harmful if inhaled
H335 May cause respiratory irritation
H351 Suspected of causing cancer
H360 May damage fertility or the unborn child
H361 Suspected of damaging fertility or the unborn child
H372 Causes damage to organs through prolonged or repeated exposure
H400 Very toxic to aquatic life
H410 Very toxic to aquatic life with long lasting effects
H412 Harmful to aquatic life with long lasting effects

Appendix C

Extraction mass results for all analytes

Table C.1: Recovery values for extractions performed using three different masses of milled material: 0.1; 0.15 and 0.2 grams. Each mass was extracted in triplicate, and each extract was injected twice (n=6). Values in %Recovery ± s.

Extracted mass	0.1	0.15	0.2
Bromoxynil Butirate	93 ± 5	94 ± 3	91 ± 2
Bromoxynil Octanoate	89 ± 10	92 ± 4	87 ± 4
Captan	63 ± 4	62 ± 3	60 ± 2
Chlorpyrifos	92 ± 6	95 ± 5	91 ± 2
Chlorthalonyl	100± 5	106± 3	99 ± 4
Deltamethrin	95 ± 8	94 ± 1	93 ± 6
Diflufenican	91 ± 7	97 ± 4	92 ± 4
Dimethoate	100± 7	103± 3	98 ± 5
Fluazifop-p-Butyl	87 ± 9	89 ± 3	83 ± 3
Folpet	74 ± 6	75 ± 3	73 ± 2
Indoxacarb	97 ± 8	97 ± 3	95 ± 6
Iprodione	93 ± 11	99 ± 3	92 ± 6
Linuron	100± 9	99 ± 4	96 ± 3
Methiocarb	97 ± 13	97 ± 3	91 ± 5
Metribuzin	87 ± 6	90 ± 3	85 ± 3
Penconazole	91 ± 5	94 ± 4	91 ± 2
S-Metolachlor	90 ± 6	93 ± 4	90 ± 2
Tebuconazol	90 ± 11	93 ± 4	87 ± 4
Terbutylazine	94 ± 4	101± 3	97 ± 3
Thiametoxam	85 ± 4	86 ± 2	83 ± 2
λ–Cyhalothrin	99 ± 7	100± 4	99 ± 8