NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

SCHOOL OF SCIENCE

DEPARTMENT OF CHEMISTRY

DEPARTMENT POSTGRADUATE PROGRAMME IN “CHEMISTRY”
SPECIALIZATION “ANALYTICAL CHEMISTRY”

MASTER THESIS

Determination of Pesticide Residues in Orange Samples using Chromatographic Techniques coupled with High Resolution Mass Spectrometry

STEFANOS KOKOLAKIS
CHEMIST

ATHENS
JULY 2019

MASTER THESIS

Determination of Pesticide Residues in Orange Samples using Chromatographic Techniques coupled with High Resolution Mass Spectrometry

STEFANOS KOKOLAKIS

Registration Number: 11606

SUPERVISING PROFESSOR:

Nikolaos Thomaidis, Professor, NKUA

THREE-MEMBER EXAMINATION COMITTEE:

Nikolaos Thomaidis, Professor, NKUA

Anastasios Economou, Professor, NKUA

Christos Kokkinos, Assistant Professor, NKUA

DEFENDING DATE 03/07/2019

ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Προσδιορισμός Υπολειμμάτων Φυτοπροστατευτικών Ουσιών σε Δείγματα Πορτοκαλιού με χρήση Χρωματογραφικών Τεχνικών συζευγμένων με Φασματομετρία Μάζας Υψηλης Διακριτικής Ικανότητας

ΣΤΕΦΑΝΟΣ ΚΟΚΟΛΑΚΗΣ

Α.Μ.: 11606

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ:

Νικόλαος Θωμαΐδης**,** Καθηγητής ΕΚΠΑ

ΤΡΙΜΕΛΗΣ ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ

Νικόλαος Θωμαΐδης**,** Καθηγητής, ΕΚΠΑ

Αναστάσιος Οικονόμου, Καθηγητής, ΕΚΠΑ

Χρήστος Κόκκινος, Επίκουρος Καθηγητής, ΕΚΠΑ

ΗΜΕΡΟΜΗΝΙΑ ΕΞΕΤΑΣΗΣ 03/07/2019

ABSTRACT

Pesticides are widely used worldwide due to modern agriculture, to increase agricultural production and improve the quality of agricultural products. Incorrect use of them leads to accumulation of residues in plants and adversely affects the environment and human health. Orange is a citrus fruit with an important nutritional value in human nutrition and a great commercial value in world trade. In this study, development and validation of a multi-residue method for the determination of pesticide residues in fresh oranges was achieved.

More specifically, a method for the determination of pesticides in orange samples was developed and applied. The resulting extracts from the pretreatment were analyzed in systems of reversed phase liquid chromatography coupled to hybrid mass spectrometry analyzer of type quadrupole time of flight (RPLC-QTOF-MS) in positive and negative ionization with an electrospray source (ESI), and gas chromatography coupled to hybrid mass spectrometry analyzer of type quadrupole time of flight (GC-QTOF-MS) in positive ionization with a source of Atmospheric Pressure Chemical Ionization (APCI). The method was validated and its performance characteristics were evaluated, in accordance with the European Commission Directive SANTE / 11813/2017 on linearity, accuracy and precision while the limits of detection and quantification of the method and instrument were determined and matrix effect was estimated.

In addition, a digital library was created which contained the information for scanning of the analytes using the GC-APCI-QTOF method. Moreover, commercial oranges were analysed for pesticide residues. We evaluated if the commercial oranges were in compliance with legislation. Potential distribution of pesticide concentrations in the different parts of oranges such as peel, flesh and juice for was also investigated.

In conclusion, high resolution mass spectrometry can be used in the context of routine pesticide residues analysis. As far as the performance criteria of the method is concerned, the optimum linearity for the most of the analytes was obtained for positive ionization with LC-ESI-QTOF. Concerning presicion, positive ionization with LC-ESI-QTOF and GC-APCI-QTOF proved to be equivalent. It is noteworthy that the LOQs of the method were below European MRLs for validated analytes. In positive ionization with LC-ESI-QTOF signal suppression for most of the analytes was observed, while signal enhancement was observed in GC-APCI-QTOF. In commercial orange samples, seven pesticide residues were detected that were below MRLs. However pesticide residues of Pyriproxyfen were detected on organic samples. Higher concentrations of pesticide residues of Chlorpyrifos were detected in the orange peel.

SUBJECT AREA: Analytical Chemistry

KEYWORDS: pesticides, orange, liquid chromatography, gas chromatography, validation

ΠΕΡΙΛΗΨΗ

Τα φυτοφάρμακα χρησιμοποιούνται ευρέως στη σύγχρονη γεωργία παγκοσμίως, με σκοπό την αύξηση της αγροτικής παραγωγής και βελτίωση της ποιότητας των γεωργικών προϊόντων. Η μη σωστή χρήση τους όμως οδηγεί στη συσσώρευση υπολειμμάτων αυτών στα φυτά και δημιουργεί δυσμενείς επιπτώσεις στο περιβάλλον και στην ανθρώπινη υγεία. Το πορτοκάλι είναι ένα εσπεριδοειδές φρούτο με σημαντική θρεπτική αξία στη διατροφή του ανθρώπου και μεγάλη εμπορική αξία στο παγκόσμιο εμπόριο. Στην παρούσα εργασία πραγματοποιήθηκε αναπτυξη και επικύρωση μίας πολυϋπολειμματικής μεθόδου για τον προσδιορισμό καταλοίπων φυτοφαρμάκων σε νωπά πορτοκάλια.

Πιο συγκεκριμένα, αναπτύχθηκε και εφαρμόστηκε μέθοδος προσδιορισμού φυτοφαρμάκων σε δείγματα πορτοκαλιών. Τα εκχυλίσματα που προέκυψαν από την προκατεργασία, αναλύθηκαν σε συστήματα υγροχρωματογραφίας αντίστροφης φάσης συζευγμένης με υβριδικό αναλυτή φασματομετρίας μάζας τύπου τετραπόλου χρόνου πτήσης (reversed-phase liquid chromatography quadrupole-time-of-flight mass spectrometry, RPLC-QTOF-MS) σε θετικό και αρνητικό ιοντισμό με πηγή ηλεκτροψεκασμού (electrospray ionization, ESI) και αεριοχρωματογραφίας συζευγμένης με υβριδικό αναλυτή φασματομετρίας μάζας τύπου τετραπόλου χρόνου πτήσης (gas chromatography quadrupole-time-of-flight mass spectrometry (GC-QTOF-MS) σε θετικό ιοντισμό με πηγή χημικού ιοντισμού ατμοσφαιρικής πίεσης (Atmospheric Pressure Chemical Ionization, APCI). Η μέθοδος επικυρώθηκε και αξιολογήθηκαν τα χαρακτηριστικά ποιότητάς της σύμφωνα με την οδηγία της Ευρωπαϊκής Επιτροπής SANTE/11813/2017, ως προς τη γραμμικότητα, ορθότητα και πιστότητα. Προσδιορίσθηκαν τα όρια ανίχνευσης και ποσοτικοποίησης της μεθόδου και του οργάνου καθώς επίσης εκτιμήθηκε το φαινόμενο επίδρασης της μήτρας.

Επιπλέον, δημιουργήθηκε μια ψηφιακή βιβλιοθήκη που περιείχε την πληροφορία για την σάρωση των αναλυτών με την μέθοδο GC-APCI-QTOF.

Ακόμα, εξετάστηκαν πορτοκάλια του εμπορίου για την ύπαρξη υπολειμμάτων φυτοφαρμάκων. Εκτιμήθηκε αν τα πορτοκάλια της αγοράς τηρούσαν την ισχύουσα νομοθεσία. Επίσης διερευνήθηκε η πιθανή κατανομή των συγκεντρώσεων των φυτοφαρμάκων στα διάφορα μέρη του πορτοκαλιού όπως η φλούδα, η σάρκα και ο χυμός.

Συμπερασματικά, η φασματομετρία μάζας υψηλής διακριτικής ικανότητας μπορεί να χρησιμοποιηθεί στο πλαίσιο της ρουτίνας της ανάλυσης των φυτοφαρμάκων. Όσον αφορά τα κριτήρια απόδοσης της μεθόδου, η βέλτιστη γραμμικότητα για τους περισσότερους αναλύτες ελήφθη για θετικό ιονισμό με LC-ESI-QTOF. Όσον αφορά την πιστότητα, ο θετικός ιονισμός με LC-ESI-QTOF και GC-APCI-QTOF αποδείχθηκε ισοδύναμος. Αξίζει να σημειωθεί ότι τα όρια ποσοτικοποίησης της μεθόδου ήταν κάτω από τα ευρωπαϊκά ανώτατα όρια καταλοίπων για τους επικυρωμένους αναλύτες. Σε θετικό ιοντισμό με LC-ESI-QTOF για τους περισσότερους αναλύτες παρατηρήθηκε καταστολή του σήματος, ενώ με GC-APCI-QTOF παρατηρήθηκε ενίσχυση του σήματος. Στα δείγματα πορτοκαλιών του εμπορίου ανιχνεύθηκαν επτά κατάλοιπα φυτοφαρμάκων που ήταν κάτω από τα ανώτατα όρια καταλοίπων. Ωστόσο, κατάλοιπα φυτοφαρμάκου Pyriproxyfen ανιχνεύθηκαν σε οργανικά δείγματα. Μεγαλύτερες συγκεντρώσεις υπολειμμάτων φυτοφαρμάκων Chlorpyrifos ανιχνεύθηκαν στην φλούδα πορτοκαλιού.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Αναλυτική Χημεία Τροφίμων

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: φυτοφάρμακα, πορτοκάλι, υγροχρωματογραφία, αεριοχρωματογραφία, φασματομετρία μαζών, επικύρωση

CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

PREFACE

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

First of all, I would like to thank my supervisor, Dr. Nikolaos Thomaidis, for giving me the opportunity to become a member of his research group as well as for the cooperation regarding this master thesis, the valuable professional and personal advice. I would also like to thank the other two members of the examination committee, Professor Anastasios Economou and Assistant Professor Christos Kokkinos , for their insightful comments and remarks.

Furthermore, I am really thankful to PhD candidate Dimitrios Damalas for his contribution in the experimental setup and constant guidance, assistance and cooperation but also for the piece of knowledge I acquired. Moreover, I am obligated to thank the master student Apostolos Karagiannidis for the great cooperation during the experimental procedure.

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

Finally, I would like to thanks my parents and my friends for encouragement and support throughout my whole life and the wonderful memories I have these two years.

THEORETICAL PART

CHAPTER 1
PESTICIDES

1.1 Introduction

According to Food and Agriculture Organization (FAO), “Pesticide” means any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport [1]. ‘Residues’ means one or more pesticides present in or on plants or plant products, edible animal products, drinking water or elsewhere in the environment and resulting from the use of a plant protection product, including their metabolites, breakdown or reaction products. ‘Metabolite’ means any metabolite or a degradation product of a pesticide, safener or synergist, formed either in organisms or in the environment [2].

Chemical pesticides have been a boon to equatorial, developing nations in their efforts to eradicate insect-borne, endemic diseases, to produce adequate food and to protect forests, plantations and fibre (wood, cotton, clothing, etc.). Many developing countries are in transitional phases with migration of the agricultural workforce to urban centres in search of better-paying jobs, leaving fewer people responsible for raising traditional foods for themselves and for the new, industrialized workforce. To attain goals as more agricultural production, there has been increased reliance on chemical pesticides [3].

The use of pesticides, especially synthetic ones, despite the benefits of improving agricultural productivity, protection of crop losses, vector disease control (e.g. malaria), poses risks to non-target organisms, including humans but also has an impact on environment by surface water contamination, ground water contamination, soil contamination, and air contamination [4][5].

Therefore, strict legislation and other measures concerning the protection of public health have been promoted by national and international authorities. Setting rules for the approval of active substances used as plant protection products, the establishment of MRLs in food commodities, and the careful monitoring of pesticide residues in foodstuffs and environment are the main objectives of legislation [6].

1.2 History of Pesticide Use

The practice of agriculture first began about ten thousand years ago in the Fertile Crescent of Mesopotamia (part of present day Iraq, Turkey, Syria and Jordan) where edible seeds were initially gathered by a population of hunter/gatherers. Cultivation of wheat, barley, peas, lentils, chickpeas, bitter vetch and flax then followed as the population became more settled and farming became the way of life. Similarly, in China rice and millet were domesticated, whilst about 7,500 years ago rice and sorghum were farmed in the Sahel region of Africa. Local crops were domesticated independently in West Africa and possibly in New Guinea and Ethiopia. Three regions of the Americas independently domesticated corn, squashes, potato and sunflowers.

It is clear that the farmed crops would suffer from pests and diseases causing a large loss in yield with the ever present possibility of famine for the population. There was thus a great incentive to find ways of overcoming the problems caused by pests and diseases. The first recorded use of insecticides is about 4500 years ago by Sumerians who used sulphur compounds to control insects and mites, whilst about 3200 years ago the Chinese were using mercury and arsenical compounds for controlling body lice. Writings from ancient Greece and Rome show that religion, folk magic and the use of what may be termed chemical methods were tried for the control of plant diseases, weeds, insects and animal pests. As there was no chemical industry, any products used had to be either of plant or animal derivation or, if of mineral nature, easily obtainable or available. Thus, for example, smokes are recorded as being used against mildew and blights. The principle was to burn some material such as straw, chaff, hedge clippings, crabs, fish, dung, ox or other animal horn to windward so that the smoke, preferably malodorous, would spread throughout the orchard, crop or vineyard. It was generally held that such smoke would dispel the blight or mildew. Smokes were also used against insects, as were various plant extracts such as bitter lupin or wild cucumber. Tar was also used on tree trunks to trap crawling insects. Weeds were controlled mainly by hand weeding but various "chemical" methods are also described such as the use of salt or sea water. Pyrethrum, which is derived from the dried flowers of Chrysanthemum cinerariaefolium "Pyrethrum daisies", has been used as an insecticide for over 2000 years. Persians used the powder to protect stored grain and later, Crusaders brought information back to Europe that dried round daisies controlled head lice. Many inorganic chemicals have been used since ancient times as pesticides, indeed Bordeaux Mixture, based on copper sulphate and lime, is still used against various fungal diseases.

Up until the 1940s inorganic substances, such as sodium chlorate and sulphuric acid, or organic chemicals derived from natural sources were still widely used in pest control. However, some pesticides were by-products of coal gas production or other industrial processes. Thus early organics such as nitrophenols, chlorophenols, creosote, naphthalene and petroleum oils were used for fungal and insect pests, whilst ammonium sulphate and sodium arsenate were used as herbicides. The drawback for many of these products was their high rates of application, lack of selectivity and phytotoxicity. The growth in synthetic pesticides accelerated in the 1940s with the discovery of the effects of DDT, BHC, aldrin, dieldrin, endrin, chlordane, parathion, captan and 2,4-D. These products were effective and inexpensive with DDT being the most popular, because of its broad-spectrum activity. DDT was widely used, appeared to have low toxicity to mammals, and reduced insect-born diseases, like malaria, yellow fever and typhus; consequently, in 1949. However, in 1946 resistance to DDT by house flies was reported and, because of its widespread use, there were reports of harm to non-target plants and animals and problems with residues.

Throughout most of the 1950s, consumers and most policy makers were not overly concerned about the potential health risks in using pesticides. Food was cheaper because of the new chemical formulations and with the new pesticides there were no documented cases of people dying or being seriously hurt by their "normal" use. There were some cases of harm from misuse of the chemicals. But the new pesticides seemed rather safe, especially compared to the forms of arsenic that had killed people in the 1920s and 1930s. However, problems could arise through the indiscriminate use and in 1962 these were highlighted by Rachel Carson in her book Silent Spring. This brought home the problems that could be associated with indiscriminate use of pesticides and paved the way for safer and more environmentally friendly products.

Research into pesticides continued and the 1970s and 1980s saw the introduction of the world’s greatest selling herbicide, glyphosate, the low use rate sulfonylurea and imidazolinone (imi) herbicides, as well as dinitroanilines and the aryloxyphenoxypropionate (fop) and cyclohexanediones (dim) families. For insecticides there was the synthesis of a 3rd generation of pyrethroids, the introduction of avermectins, benzoylureas and B_t (Bacillus thuringiensis) as a spray treatment. This period also saw the introduction of the triazole, morpholine, imidazole, pyrimidine and dicarboxamide families of fungicides. As many of the agrochemicals introduced at this time had a single mode of action, thus making them more selective, problems with resistance occurred and management strategies were introduced to combat this negative effect.

In the 1990s research activities concentrated on finding new members of existing families which have greater selectivity and better environmental and toxicological profiles. In addition new families of agrochemicals have been introduced to the market such as the triazolopyrimidine, triketone and isoxazole herbicides, the strobilurin and azolone fungicides and chloronicotinyl, spinosyn, fiprole and diacylhydrazine insectides. Many of the new agrochemicals can be used at grams rather than the kilograms per hectare.

Products for biological control of insects, which have been known for many years and applied in practice, include biopreparations, in which a toxic protein produced by soil bacteria Bacillus thuringiensis, is the active substance as a poison of natural origin. Unlike typical insecticides of synthetic origin, the death of an insect occurs in this case over a longer time, even after seven days. It should be mentioned that introduction of a gene causing synthesis of the above described protein to plants proved that one of the drawbacks of the genetic engineering methods is the acquisition of resistance by insects, just as in case of chemical plant protection.

New insecticide and fungicide chemistry has allowed better resistance management and improved selectivity This period also saw the refinement of mature products in terms of use patterns with the introduction of newer and more user-friendly and environmentally safe formulations. Integrated pest management systems, which use all available pest control techniques in order to discourage the development of pest populations and reduce the use of pesticides and other interventions to levels that are economically justified, have also contributed to reducing pesticide use [7][8].

Many of today’s pesticides are designed after “natural” pesticides. For example, “pyrethroid” insecticides are modeled after “pyrethrins,” which are natural, plant-derived poisons that have been used as insecticides for hundreds of years. “Insect growth regulators” (IGRs) mimic hormones that affect insect growth, but they have little effect on non-target animals. These products and similar ones using bacteria, viruses, or other natural pest control agents are called “biorational” pesticides [8].

Today the pest management toolbox has expanded to include use of genetically engineered crops designed to produce their own insecticides or exhibit resistance to broad spectrum herbicide products or pests. These include herbicide tolerant crops like soybeans, corn, canola and cotton and varieties of corn and cotton resistant to corn borer and bollworm respectively. In addition the use of Integrated Pest Management (IPM) systems which discourage the development of pest populations and reduce the use of agrochemicals have also become more widespread. These changes have altered the nature of pest control and have the potential to reduce and/or change the nature of agrochemicals used [9].

1.3 Classification of Pesticides

Pesticides can be classified into different categories depending on their characteristics such as chemical class, target action, biochemical mode of action or toxicity.

1.3.1. Based on their Action on Target Organisms

There are many different forms of pesticides, each is meant to be effective against specific pests. The term ‘-cide’ comes from the Latin word ‘to kill’. The target can be an animal, a plant or a germ. According to EPA, there is a wide range of types of pesticides can be seen at the table 1.1, below [10].

Table 1.1 : Categorization of Pesticides based on their Action on Target Organisms

Pesticide	Action
Algicides	kill algae in lakes, canals, swimming pools, water tanks and other sites
Antifoulants	kill or repel organisms that attach to underwater surfaces, such as barnacles that cling to boat bottoms
Antimicrobials	kill microorganisms such as bacteria and viruses

Attractants	lure pests to a trap or bait, for example, attract an insect or rodent into a trap
Biopesticides	are derived from natural materials such as animals, plants, bacteria and certain minerals
Biocides	kill microorganisms
Defoliants	cause leaves or foliage to drop from a plant, usually to facilitate harvest
Desiccants	promote drying of living tissues, such as unwanted plant tops
Disinfectants and sanitizers	kill or inactivate disease-producing microorganisms on inanimate objects
Fungicides	kill fungi (including blights, mildews, molds, and rusts)
Fumigants	produce gas or vapor intended to destroy pests, for example in buildings or soil
Herbicides	kill weeds and other plants that grow where they are not wanted
Insect growth regulators	disrupt the molting, maturing from pupal stage to adult, or other life processes of insects.
Insecticides	kill insects and other arthropods
Miticides (or Acaricides)	kill mites that feed on plants and animals
Microbial pesticides	are microorganisms that kill, inhibit, or out-compete pests, including insects or other microorganism pests
Molluscicides	kill snails and slugs
Nematicides	kill nematodes (microscopic, worm-like organisms that feed on plant roots)
Ovicides	kill eggs of insects and mites
Pheromones	disrupt the mating behavior of insects
Plant growth regulators	lter the expected growth, flowering or reproduction rate of plants (does not include fertilizers)
Plant Incorporated Protectants	are substances that plants produce from genetic material that has been added to the plant
Repellents	repel pests, including insects (such as mosquitoes) and birds
Rodenticides	control mice and other rodents

1.3.2. Based on their Chemical Group

Pesticide compounds depending on their chemical structure can be classified as:

Inorganic: compounds of copper, zinc, mercury, arsenic, fluoride salts or salts of sulphur.

Organic: Amides, Azoles, Benzimidazoles, Benzoic Acids, Benzoylureas, Carbamates, Chloroacetanilides, Dithiocarbamates, Morpholines, Nitriles, Nitroanilines, Organochlorines, Organophosphorus, Phenoxy Acids, Pyrethroids, Pyridines, Pyridazines, Pyridazinones, Phenylureas, Sulfonylureas, Triazines [11].

1.3.3. Based on their biochemical Mode of Action (MoA)

As reported by IRAC (Insecticide Resistance Action Committee), the MoA Classification scheme is based on the best available evidence for the target-sites or MoA of currently available insecticides and acaricides (currently excludes nematicides).

The MoA Classification scheme (Table 1.2) is principally based on the target site, and secondarily on novelty of the chemistry, differential action on the same target site and/or susceptibility to metabolic resistance mechanisms. A compound acting through a target site that is different from all others will be placed in a separate group. Likewise, compounds acting at different sites in the same target are also placed in different groups. Following a similar logic, compounds sharing a common target site, but representing very different types of are placed in different subgroups because they can have distinctly different metabolic profiles minimizing the chances for metabolic cross-resistance [12].

Table 1.2 : Modes of action (based on IRAC MoA classification) for current insecticide groups.

IRAC group	Primary site of action/MoA*	Chemical subgroup/exemplifying active ingredient
Nerve and Muscle Targets		
1	AChE inhibitor	1A carbamates
		1B organophosphates
2	GGCC antagonist	2A cyclodienes
		2B fiproles
3	VGSC modulator	3A pyrethroids and pyrethrins
		3B DDT and analogs
4	nAChR agonist	4A neonicotinoids
		4B nicotine
		4C sulfoximines
		4D butenolides
5	nAChR allosteric	Spinosyns
6	CC activators	Avermectins and milbemycins

9	Modulators of chordotonal organs	9B pymetrozine
		9C flonicamid
14	nAChR blocker	Nereistoxin analogs
19	OA-R agonist	Formamidines
22	VGSC blocker	22A oxadiazines
		22B semicarbazones
28	Ry-R allosteric	Diamides
Growth and Development Targets		
7	JH-R agonist	7A juvenoids
		7B fenoxycarb
		7C pyriproxyfen
10	MGI	10A clofentezine
		10B oxazoles
15	CSI	Benzoylureas
16	CSI	Buprofezin
17	CSI	Cyromazine
18	EcR agonist	Diacylhydrazines
23	Acetyl CoA carboxylase	Tetronic/tetramic acids
Respiration targets		
12	ATP synthase	12A diafenthiuron
		12B organotin miticides
		12C propargite
		12D tetradifon
13	Ox-Ph uncouplers	Chlorfenapyr
20	MET III inhibitors	20A hydramethylnon
		20B acequinocyl
		20C fluacrypyrim
21	MET I Inhibitors	21A MET I inhibitors
		21B rotenone
24	MET IV inhibitors	24A phosphine
		24B cyanide
25	MET II inhibitors	β-ketonitrile derivatives
Midgut targets		
11	Midgut membr.	11A Bacillus thuringienis
		11B Bacillus sphaericus
Miscellaneous		
8	Miscellaneous non-specific (multi-site) inhibitors	8A alkyl halides
		8B chloropicrin
		8C sulfuryl fluoride
		8D borates
		8E tartar emetic

**Abbreviations: AChE, acetylcholiesterase; GGCC, GABA gated chloride channel; CC, chloride channel; VGSC, voltage gated sodium channel; nAChR, nicotinic acetylcholine receptor; OA-R, octopamine receptor; Ry-R, ryanodine receptor; JH-R, juvenile hormone receptor; EcR, ecdysone receptor; CSI, chitin synthesis inhibitor; MET, mitochondrial electron transport; Ox-Ph, oxidative phosphorylation*

1.3.4. Based on their hazzard

At 2009, Classification the WHO (World Health Organization) Hazard Classes have been aligned in an appropriate way with the GHS (Globally Harmonized System of Classification and Labelling of Chemicals) Acute Toxicity Hazard Categories for acute oral or dermal toxicity as the starting point for allocating pesticides to a WHO Hazard Class. , the classification of some pesticides has been adjusted to take account of severe hazards to health other than acute toxicity [13].

Table 1.3 : GHS Classification of Pesticides by Hazard

GHS Category	Classification criteria			
	Oral		Dermal	
	LD_{50} (mg/kg bw)	Hazard Statement	LD_{50} (mg/kg bw)	Hazard Statement
Category 1	< 5	Fatal if swallowed	< 50	Fatal in contact with skin
Category 2	5 - 50	Fatal if swallowed	50 - 200	Fatal in contact with skin
Category 3	50 - 300	Toxic if swallowed	200 - 1000	Toxic in contact with skin
Category 4	300 - 2000	Harmful if swallowed	1000 - 2000	Harmful in contact with skin
Category 5	2000 - 5000	May be harmful if swallowed	2000 - 5000	May be harmful in contact with skin

1.4 Consequences

1.4.1 On Human-beings

Pesticides can be found, often in small amounts, almost anywhere worldwide. In addition to use in agriculture and forestry, pesticides are used in many public places, including office buildings, restaurants, schools, parks, golf courses, and along roads, railroads, and power lines. A great number of pesticide compounds have been found to contaminate water resources, ambient air, fog, rain, and soils in numerous studies. Most non occupational exposure comes from food or home pesticide use, such as pet treatments, extermination of household pests, removal of lice, and garden and lawn treatments [29].

There are three main routes of exposure: oral, dermal, and inhalation. Ingestion of food or water containing pesticides is oral exposure. Inhalation exposure can occur by breathing air containing pesticides as vapor, aerosol, or small particles. Dermal exposure occurs when the skin comes in contact with pesticides [29].

1.4.1.1 Acute toxicity

Most pesticide poisoning cases involve either organophosphate or carbamate insecticides. Both chemical groups affect humans by inhibiting acetyl cholinesterase, an enzyme essential to proper functioning of the nervous system.

Fungicides as a class are probably responsible for a disproportionate number of irritant injuries to skin and mucous membranes, as well as dermal sensitization, rather than frequent or severe systemic poisonings because many of them have low toxicity in mammals and are formulated as suspensions of wettable powders or granules and are inefficiently absorbed [29].

A large number of organochlorine insecticides (not more authorized in developed countries, but some of them still used in developing countries) are the active ingredients of various home and garden products and some agricultural and environmental pest control products. Other most hazardous compounds, such as DDT, aldrin, dieldrin, or heptachlor, are still environmental contaminants due to their high environmental persistence. The main target of acute toxic action of organochlorine pesticides is the nervous system, where these compounds induce a hyperexcitable state. Severe intoxication by these compounds causes myoclonic jerking movements, and then generalized tonic-clonic convulsions followed by coma and respiratory depression [29].

1.4.1.2 Long-Term Health Effects

1.4.1.2.1 Neurological effects

Findings from many epidemiological studies have provided support to the hypothesis that pesticide exposure may increase risk of Parkinson's disease, a late onset progressive neurological disorder associated with selective degeneration of nigrostriatal dopaminergic neurons. Organochlorines, organophosphorus compounds, chlorophenoxy acids/esters, and botanicals have been identified as specific classes of pesticides posing a risk for the occurrence of Parkinson's disease [29].

1.4.1.2.2 Carcinogenicity

Pesticides are currently classified by international agencies and committees for their potential carcinogenic properties on the basis of the available evidence from human (epidemiological) and experimental studies. Several agrochemical ingredients have been classified as potentially carcinogenic to humans. Some pesticides with sufficient or limited evidence of carcinogenicity such as chlordecone, DDT, pentachlorophenol, captafol, and aldicarb have been banned or their use has been restricted in some countries. These compounds are widespread environmental pollutants due to their bioaccumulation and persistence in nature. Residues of these pesticides have been detected in the food chain and in different biological media in humans. A large number of organophosphate pesticides are not classifiable as to their carcinogenicity to humans. Few pesticides belonging to the chemical families of carbamates or dithiocarbamates have been demonstrated as animal carcinogens. The principal hazard of these compounds is the presence of impurities and the metabolic and degradation products of thiocarbamates: the production of carcinogenic N-nitroso compounds by the reaction of many thio- and alkyl-carbamates, such as molinate, triallate, and butylate, with nitrite. As an example, N-nitrosocarbaryl, a derivative of carbaryl, is a potent carcinogen in rats. Ethylene thiourea, a degradation product of ethylene bisdithiocarbamate fungicides, such as mancozeb, maneb, metiram, zineb, and others, is a potent thyroid carcinogen in rats, although it is not considered a human carcinogen. Captafol, a chloroalkyl compound widely used as fungicide, demonstrates a carcinogenic effect with sufficient evidence on multiple target organs in two rodent species [14].

1.4.1.2.3 Allergies

Allergic effects are harmful effects that some people develop in reaction to substances that do not cause the same reaction in the most other people. Allergic reactions are not through to occur during a person's first exposure to a substance. Later exposures result in the allergic response. This process is called sensitization, and substances that cause people to become allergic to them are known as sensitizers. Some people become allergic to pesticides. Allergic effects to pesticides include: systemic effects, such as asthma or even life threatening shock, skin irritation, such as rush, blisters, or open sores, and eye and nose irritation, such as itchy, watery eyes and sneezing [15].

1.4.1.2.4 Endocrine System

Many chemicals that have been identified as endocrine disruptors are pesticides.

EDCs act mainly by interfering with natural hormones because of their strong potential to bind to estrogen or androgen receptors. In particular, EDCs can bind to and activate various hormone receptors and then mimic the natural hormone's action (agonist action). EDCs may also bind to these receptors without activating them. This antagonist action blocks the receptors and inhibits their action. Finally, EDCs may also interfere with the synthesis, transport, metabolism and elimination of hormones, thereby decreasing the concentration of natural hormones. For example, thyroid hormone production can be inhibited by some ten endocrine disruptor pesticides (amitrole, cyhalothrin, fipronil, ioxynil, maneb, mancozeb, pentachloronitrobenzene, prodiamine, pyrimethanil, thiazopyr, ziram, zineb) [16].

1.4.1.2.5 Reproductive System

The presence of pesticides in the body for a longer time also affects reproductive capabilities by altering the levels of male and female reproductive hormones. Consequently, it results in stillbirth, birth defects, spontaneous abortion and infertility [32].

1.6.1.2.6 Other Organs

Lon-term exposure to pesticide also damages liver, lungs, kidneys and may cause blood diseases [17].

1.4.2 On Environment

Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants.

1.4.2.1 Water Contamination

Pesticides can reach surface water through runoff from treated plants and soil. Contamination of water by pesticides is widespread. The results of a comprehensive set of studies done by the U.S. Geological Survey (USGS) on major river basins across the country were that 90 percent of water and fish samples from all streams contained one, or more often, several pesticides. Pesticides were found in all samples from major rivers with mixed agricultural and urban land use influences and 99 percent of samples of urban streams. The USGS also found that concentrations of insecticides in urban streams commonly exceeded guidelines for protection of aquatic life. According to USGS, more pesticides were detected in urban streams than in agricultural streams [4].

Groundwater pollution due to pesticides is a worldwide problem. According to the USGS, at least 143 different pesticides and 21 transformation products have been found in ground water, including pesticides from every major chemical class. During one survey in India, 58% of drinking water samples drawn from various hand pumps and wells around Bhopal were contaminated with organochlorine pesticides above the EPA standards. Once ground water is polluted with toxic chemicals, it may take many years for the contamination to dissipate or be cleaned up. Cleanup may also be very costly and complex, if not impossible [4].

1.4.2.2 Soil Environment

A major fraction of the pesticides that are used for agriculture and other purposes accumulates in the soil. The indiscriminate and repeated use of pesticides further aggravates this soil accumulation problem. Several factors such as soil properties and soil micro-flora determine the fate of applied pesticides, owing to which it undergoes a variety of degradation, transport, and adsorption/desorption processes. The degraded pesticides interact with the soil and with its indigenous microorganisms, thus altering its microbial diversity, biochemical reactions and enzymatic activity. The above can lead to the disturbance in soil ecosystem and loss of soil fertility [18].

1.6.2.3 Atmosphere

Aerosol particles are omnipresent in the lower boundary layer of the atmosphere. They have typical atmospheric lifetimes of about three to ten days. During applications, a significant fraction of applied pesticides, about 15 to 40%, is dispersed in the atmosphere by volatilization or spray drift processes. Pesticides travel in the atmosphere with long range atmospheric transport and deposition from their emission area. The fate of pesticides is influenced by their partition between the gas phase and particulate phase. Considering the low volatility of majority of the commonly used pesticides, they are often adsorbed on the surface of atmospheric particles. They may undergo different transport and transformation processes resulting in the generation of secondary products that could be even more hazardous than the primary emitted pesticides. These aspects are central to atmospheric composition changes, air quality and associated climate change [19].

1.4.2.4 Nontarget Organisms

All taxonomic levels of organisms can be affected by pesticides. Green plants include the angiosperms, gymnosperms, ferns, mosses, and algae, the non green are fungi and bacteria. Animals include mammals, birds, reptiles, amphibians, fish, insects, crustacea, worms, coelenterates, and protozoa. All have been shown to be affected by one or more pesticides. There is great variability in species sensitivity to a particular pesticide, as well as great variation in the toxicity of different pesticides to a species. Additionally, for any species, sensitivity to a given pesticide varies with sex, age, nutritional background, stress, health, and the microenvironment. This complexity is important in the evaluation of the precise effect of a pesticide on a species or group of species, but it should not obscure the basic principle that most organisms are affected by some concentration of exposure to one or more pesticides [35].

Pesticides, by necessity, are poisons and would be expected to have adverse effects on any nontarget organism having physiological functions common with those of the target that are attacked or inhibited by the pesticide. These are predictable and dose-related responses. For instance, when a cholinesterase-inhibiting insecticide is applied to a field crop to control one or more pest species, it obviously will also kill other insect species not considered pests. Additionally, avian, mammalian, amphibian, and reptilian species coming in direct contact with the insecticide application may also be affected. Indirect effects can be observed when the insecticide is moved from the application site to another site. There it may be accumulated at several trophic levels to become toxic at the top of a food chain, or reach the secondary site in concentrations that are toxic to nontargets [35].

The overall effects of pesticides on nontargets can be categorized as follows: reduction of species numbers, alteration of habitat with species reduction, changes in behavior, growth changes, altered reproduction, changes in food quality and quantity, resistance, disease susceptibility, biological magnification [20].

1.5 Legislation

Although a vast increase has been seen in the enactment of pesticide legislation worldwide, it remains absent in approximately a quarter of countries in Africa and the Southeast Asian region, where present, regulations often lack comprehensiveness and the capacity to enforce these effectively. Developed nations, conversely, are increasingly implementing more stringent legislation [21].

Pesticide products are subject to extensive biological, chemical, and toxicological tests before authorization. The test requirements have been specified by the national agencies, and they are generally carried out according to internationally tested guidelines harmonized by the OECD (Organization for Economic Co-operation and Development) and developed by experts of OECD countries and the Joint FAO/WHO Meeting on Pesticide Residues and regularly updated based on evolving scientific knowledge.

In the EU, the application procedure, evaluation of the active substances, and their approval are covered by Regulation (EC) 1107/2009 of the European Parliament and the Council defining the legal framework concerning the placing of plant protection products on the market. Following the Regulation (EC) 1107/2009, more recent Commission Regulations specify the data required for active substances and plant protection products to be approved and placed on the market. For a new active substance (pesticide), the minimum submitted data should include the following information: identity of the active substance, physical and chemical properties of the active substance, analytical methods, toxicological and metabolism studies, residues in or on treated products, food and feed, and fate and behavior in the environment and ecotoxicological studies.

In the United Stated, the EPA registers the use of pesticides and establishes tolerances (the maximum amounts of residues that are permitted in or on a food. EPA, before the registration of a new pesticide or the authorization of a new use for a registered pesticide, must ensure that the pesticide, applied according to label directions, can be used with a reasonable certainty of no harm to human health and without posing unreasonable risks to the environment. To make such determinations, EPA requires more than a hundred different scientific studies and tests from applicants [22].

Most countries use Maximum Residues Limits (MRL) to regulate pesticides. As Codex Alimentarius reported, a MRL is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly in accordance with Good Agricultural Practice [23]. In principle MRLs are set on the basis of the following:

1. Supervised agricultural residue trials
2. Using appropriate consumer intake models
3. Data from toxicological tests on the pesticide that allow for the fixing of an “acceptable daily intake” (ADI)

If the estimated daily consumer intake for all commodities calculated under (2) is lower than the ADI calculated under (3), then the residue level under (1) is set as the MRL [24].

In EU, to ensure that MRLs are as low as possible, applicants seeking approval of a pesticide must submit scientific information about the minimum amounts of pesticide necessary to protect a crop and the residue level remaining on the crop after such treatment. The European Food Safety Authority (EFSA) then verifies that this residue is safe for all European consumer groups, including vulnerable groups such as babies, children and vegetarians. When there is a risk established for any consumer group, the MRL application will be rejected and the pesticide may not be used on that crop. Food safety thus has priority over plant protection. In many cases the amount of pesticide needed is much lower than the highest level that is still considered safe. In such cases the MRL is set at the lower level, thus ensuring that only the necessary (minimum) amount of pesticide is used. The quantities and when the pesticide may be used is defined by the relevant national authority and can be found on the label of the pesticide. Authorisations are granted on a national basis because the local and environmental conditions and the occurrence of pests (and therefore uses of pesticides) may differ. For example, in the southern Member States where it is warmer, there are more insects and thus more insecticides are needed. In other parts of the EU, it is more humid conditions that suit fungal infestation, and thus more fungicides are needed. When MRLs are set these differences should be taken into account. For crops grown outside the European Union, MRLs are set on request of the exporting country [18]. The main legislation on MRLs in EU is the Regulation (EC) 396/2005, which is referring to MRLs of pesticides in or on food and feed of plant and animal origin and Commission implementing rules, and its amendments. Up to now, in the EU

- Legislation covers pesticides currently or formerly used in agriculture in or outside the EU (around 1100)
- A general default MRL of 0.01 mg/kg has been applied where a pesticide is not specifically mentioned
- A database of approximately 145000 MRLs [25][26].

Except of EU, many countries and organisms have developed their own MRLs' database. Some examples of these are USA, Canada, Australia, Brazil, Argentina, Hong Kong, Chile, Korea and others [27].

In the EU, the Member States, according to the Regulation (EC) 396/2005, sample and analyze different combinations of product/pesticide residue annually within an EU multiannual coordinated control program (EU-MACCP) to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues. Additionally, each Member State shall design and perform its national monitoring control program annually.

EFSA has the responsibility of the monitoring program. The Member States, after implementation of the monitoring programs, of pesticide residue levels in food commodities, EU-MACCP, and National, submit their results to EFSA and the European Commission. According to Article 32 of Regulation (EC) 396/2005, EFSA is responsible for drawing up an annual report on pesticide residues on the basis of the results provided by the reporting countries. On the basis of the results of EU monitoring programs, EFSA may derive some recommendations aimed at improving the enforcement of the European pesticide residue legislation.

Moreover, the Rapid Alert System for Food and Feed (RASFF) has been established to provide the control authorities with an effective tool for exchange of information on measures taken to ensure food safety by establishing a network for the notification of a direct or indirect risk to human health deriving from food or feed.

The organization of the official controls within the EU is based on the general rules established by Regulation (EC) 882/2004 (EC, 2004), where the general requirements for methods of sampling and analysis and laboratories are laid down in Articles 11 and 12. According to Article 12 of this regulation, the competent authority of the Member States shall designate laboratories that may carry out the analysis of samples taken during official controls. However, they may only designate laboratories that operate and are assessed and accredited in accordance with the following European standards: ISO/IEC 17025:2005 (ISO/IEC, 2005), ISO/IEC 17011:2004 (ISO/IEC, 2004), and taking into account criteria for different testing methods laid down in Community feed and food law.

To contribute to a high quality and uniformity of analytical results of EU official control laboratories, an analytical network of European Reference Laboratories (EURLs), National Reference Laboratories (NRLs), and Official

National Laboratories (OFLs) is designated (EURL-NRL-OFL network). Currently, there are four EURLs established in the EU for pesticide residues: fruits and vegetables; cereals and feed; food of animal origin; and singleresidue methods [22].

In Greece, which is a State Member of EU, the Greek Ministry of Rural Development and Food is responsible for issues about pesticide use in agricultural activities. In fact, with law 4036/2012 ‘Pesticide marketing, sustainable use and other provisions’ Greece has approved the establishment of necessary supplementary measures for the implementation of the provisions of:

- Regulation (EC) No 1107/2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
- Regulation (EC) No 396/2005 of the European Parliament, on MRLs of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC and its implementing regulations,
- The incorporation in the Greek laws of the provisions of Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides, of the supplementary measures of the directive and the adoption of national measures in compliance with Directive requirements
- The establishment of other provisions in relation to pesticides [28]

As a result, Greece is updated with the last provisions of EU according to use of pesticides, has its own national action plan for sustainable use of plant protection products and uses the same MRLs with EU [29][30].

1.6 Method Validation

Except the legislation about pesticides in general that is described in Chapter 1.4, there is specific legislation that describes the guidelines concerning method validation for the determination of pesticide residues in foodstuffs.

In the EU, responsible for this part of legislation is the Document No SANTE/11813/2017. This guidance document was implemented by

1/1/2018 and describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with MRLs, enforcement actions, or assessment of consumer exposure to pesticides. Document No SANTE/11813/2017 replaced Document No SANTE/11813/2015. The new rules do not impose many differences in the method validation criteria. However, there is a differentiation in the criteria for acceptance of a method that is suitable for the determination of pesticides, and in particular for the assessment of the trueness parameter as well as the redefining of the role of ion ratios in the HRMS. According to them, the definition of method validation is ‘the process of characterising the performance to be expected of a method in terms of its scope, specificity, accuracy sensitivity, repeatability and within laboratory reproducibility. Some information on all characteristics, except within laboratory reproducibility, should be established prior to the analysis of samples, whereas data on reproducibility and extensions of scope may be produced from Analytical QC, during the analysis of samples. Wherever possible, the assessment of accuracy should involve analysis of certified reference materials, participation in proficiency tests, or other inter-laboratory comparisons’ [31][32].

The following units are describing the main guidelines on method validation as SANTE/11813/2017 defines.

1.6.1 Chromatographic Separation and Determination

Sample extracts are normally analysed using capillary gas chromatography (GC) and/or high performance or ultra performance liquid chromatography (HPLC or UPLC) coupled to mass spectrometry (MS) for the identification and quantification of pesticides in food and feed samples. Various MS detection systems can be used, such as a single or triple quadrupole, ion trap, time of flight or orbitrap. Typical ionisation techniques are: electron impact ionisation (EI), chemical ionisation (CI), atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). Different acquisition modes may be used such as full-scan, selected ion monitoring (SIM), selected reaction monitoring (SRM) and multiple reaction monitoring (MRM).

The minimum acceptable retention time for the analytes under examination should be at least twice the retention time corresponding to the void volume of the column. The retention time of the analyte in the extract should correspond to that of the calibration standard (may need to be matrix-matched) with a tolerance of ±0.1min, for both gas chromatography and liquid chromatography. Larger retention time deviations are acceptable where both retention time and peak shape of the analyte match with those of a suitable IL-IS (Isotopically Labeled - Internal Standard), or evidence from validation studies is available. IL-IS can be particularly useful where the chromatographic procedure exhibits matrix induced retention time shifts or peak shape distortions. Overspiking with the analyte suspected to be present in the sample will also help to increase confidence in the identification.

1.6.2 Identification using Mass Spectrometry

Mass Spectrometry detection can provide mass spectra, isotope patterns, and/or signals for selected ions. Although mass spectra can be highly specific for an analyte, match values differ depending on the particular software used which makes it impossible to set generic guidance on match values for identification. This means that laboratories that use spectral matching for identification need to set their own criteria and demonstrate that these are fit-forpurpose. Guidance for identification based on MS spectra is limited to some recommendations whereas for identification based on selected ions more detailed criteria are provided.

Reference spectra for the analyte should be generated using the same instruments and conditions used for analysis of the samples. If major differences are evident between a published spectrum and the spectrum generated within the laboratory, the latter must be shown to be valid. To avoid distortion of ion ratios the concentration of the analyte ions must not overload the detector. The reference spectrum in the instrument software can originate from a previous injection (without matrix present), but is preferably obtained from the same analytical batch.

In case of full scan measurement, careful subtraction of background spectra, either manual or automatic, by deconvolution or other algorithms, may be required to ensure that the resultant spectrum from the chromatographic peak is representative. Whenever background correction is used, this must be applied uniformly throughout the batch and should be clearly recorded.

Identification relies on the correct selection of ions. They must be sufficiently selective for the analyte in the matrix being analysed and in the relevant concentration range. Molecular ions, (de)protonated molecules or adduct ions are highly characteristic for the analyte and should be included in the measurement and identification procedure whenever possible. In general, and especially in single-stage MS, high m/z ions are more selective than low m/z ions (e.g. m/z < 100). However, high mass m/z ions arising from loss of water or loss of common moieties may be of little use. Although characteristic isotopic ions, especially Cl or Br clusters, may be particularly useful, the selected ions should not exclusively originate from the same part of the analyte molecule. The choice of ions for identification may change depending on background interferences. In HRMS, the selectivity of an ion of the analyte is determined by the narrowness of the mass extraction window (MEW) that is used to obtain the extracted ion chromatogram (EIC). The narrower the MEW, the higher the selectivity. However, the minimum MEW that can be used relates to mass resolution.

EICs of sample extracts should have peaks of similar retention time, peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch. Chromatographic peaks from different selective ions for the analyte must fully overlap

Different types and modes of mass spectrometric detectors provide different degrees of selectivity , which relates to the confidence in identification. The requirements for identification are given in the table 3.1.

The relative intensities or ratios of selective ions, expressed as a ratio relative to the most intense ion, that are used for identification, should match with the reference ion ratio. The reference ion ratio is the average obtained from solvent standards measured in the same sequence and under the same conditions as the samples. Standards in matrix may be used instead of solvent standards as long as they have been demonstrated to be free of interferences for the ions used at the retention time of the analyte. For determination of the reference ion ratio, responses outside the linear range should be excluded.

As long as sufficient sensitivity and selectivity are obtained for both ions, and responses are within the linear range, ion ratios in unit mass resolution MS/MS have shown to be consistent and should not deviate more than 30% (relative) from the reference value.

For accurate mass measurement / high resolution mass spectrometry, the variability of ion ratios is not only affected by S/N of the peaks in the extracted ion chromatograms, but may also be affected by the way fragment ions are generated, and by matrix. For example, the range of precursor ions selected in a fragmentation scan event ('all ions', precursor ion range of 100 Da, 10 Da, or 1 Da) results in different populations of matrix ions in the collision cell which can affect fragmentation compared to solvent standards. Furthermore, the ratio of two ions generated in the same fragmentation scan event tends to yield more consistent ion ratios than the ratio of a precursor from a full scan event and a fragment ion from a fragmentation scan event. For this reason, no generic guidance value for ion ratio can be given. Due to the added value of accurate mass measurement, matching ion ratios are less critical, however, they should be used as indicative.

Table 1.4 summarizes mass spectrometry identification requirements.

Table 1.4 : Identification requirements for different MS techniques, SANTE11813/2017

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	**Typical systems (examples)**		**minimum number of ions**	**other**
Unit mass resolution (LRMS)	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3^d Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within ±30% (relative) of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement (HRMS)	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm$^{a, b, c}$	S/N ≥ 3^d Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap.

a: preferably including the molecular ion, (de)protonated molecule or adduct ion

b: including at least one fragment ion

c: < 1 mDa for m/z < 200

d: in case noise is absent, a signal should be present in at least 5 subsequent scans

1.6.3 Analytical method validation and performance criteria

1.6.3.1 Linearity

Linearity is the method's ability to obtain test results, which are directly proportional to the concentration of analyte in the sample [33].

According to the lastest legislation, multi-level calibration, at least five levels, is preferred. An appropriate calibration function must be used (e.g. linear, quadratic, with or without weighing). The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region should not be more than ±20% [28].

$$Deviation\ of\ back\ calculated\ concentration\ (\%) = 100 * \frac{Cmeasured - Ctrue}{Ctrue}$$

In general, the use of weighted linear regression (1/x) is recommended, rather than linear regression.

The lowest calibration level (LCL) must be equal to, or lower than, the calibration level corresponding to the reporting limit (RL). The RL must not be lower than the LOQ [28].

1.6.3.2 Estimation of Matrix Effect

Matrix Effect defines as an influence of one or more co extracted compounds from the sample on the measurement of the analyte concentration or mass. It may be observed as increased or decreased detector response, compared with that produced by solvent solutions of the analyte. The presence, or absence, of such effects may be demonstrated by comparing the response produced from the analyte in a solvent solution with that obtained from the same quantity of analyte in the sample extract [28].

Matrix effects are known to occur frequently in both GC and LC methods and should be assessed at the initial method validation stage. Matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same type as the sample, should be used for calibration. An alternative practical approach to compensate for matrix effects in GC-analyses is the use of analyte protectants that are added to both the sample extracts and the calibration standard solutions in order to equalise the response of pesticides in solvent calibrants and sample extracts. The most effective way to compensate for matrix effects is the use of standard addition or isotopically labelled internal standards. In case of more than 20% signal suppression or enhancement, matrix-effects need to be addressed in calibration [28].

1.6.3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of determination as referred to in Reg. 396/2005 means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods; In this respect it can be regarded as the LOQ because the definition of LOQ, according to SANTE11813/2017, is the lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method [28].

1.6.3.4 Trueness

The measure of trueness is normally expressed as ‘bias’. The closeness of agreement between the average value obtained from a series of test results (i.e. the mean recovery) an accepted reference or true value.

In validation, trueness refers to average recovery for each spike level tested. A minimum of 5 replicates is required at the targeted LOQ or RL of the method, and at least one other higher level, for example, 2-10 times higher than the targeted LOQ or the MRL. Where the residue definition includes two or more analytes, then wherever possible, the method should be validated for all analytes included in the residue definition. Trueness must be between 70-

120%. . Recovery rates outside the range can be accepted if they are consistent (RSD ≤ 20%) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step) ,but the mean recovery should not be lower than 30 % or above 140 %. However, in these cases a correction for recovery is required or a more accurate method should be used, if practicable.

1.6.3.5 Precision

It is the closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation. It contains two subsections, Repeatabillity and Reproducibility.

1.6.3.5.1 Repeatability-Precision (RSD_r)

The definion refers that it is the precision (standard deviation) of measurement of an analyte (usually obtained from recovery or analysis of reference materials), obtained using the same method on the same sample(s) in a single laboratory over a short period of time, during which differences in the materials and equipment used and/or the analysts involved will not occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result Repeatability RSD_r is estimated for each spike level tested [28].

1.6.3.5.2 Within-laboratory reproducibility-Precision (RSD_{wR})

It is mentioned as the precision (standard deviation) of measurement of an analyte (usually by means of recovery or analysis of reference materials), obtained using the same method in a number of laboratories, by different analysts, or over a period in which differences in the materials and equipment will occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result. Within-lab-reproducibility (RSD_{wR}) is that produced in a single laboratory under these conditions. In a method validation, it must be below 20% [28].

1.6.3.6 Specificity

It is defined as the ability of the detector (supported by the selectivity of the extraction, clean-up, derivatisation or separation, if necessary) to provide signals that effectively identify the analyte. HRMS and MS^n can be both highly selective and highly specific. It estimated as the signal of reagent blank or blank control samples and must be below 30% of RL [34][35].

The summary of performance criteria in method validation are shown in Table 1.5.

Table 1.5: Validation parameters and criteria, SANTE 11813/2017 [28]

Parameter	What/how	Criterion	Comments
Sensitivity/linearity	Linearity check from at least five levels	Deviation of backcalculated concentration from true concentration ≤±20%	
Matrix effect	Comparison of response from solvent standards and matrix-matched standards		>20% signal suppression or enhancement, matrix-effects need to be addressed in calibration
LOD and LOQ	Lowest spike level meeting the method performance criteria for trueness and precision	≤ MRL	
Specificity	Response in reagent blank and blank control samples	≤30% of RL	
Trueness (bias)	Average recovery	70-120%	mean recoveries

	for each spike level tested		30-70% can be accepted if RSD ≤ 20% and the basis for this is well established
Precision (RSDr)	Repeatability RSDr for each spike leveltested	≤ 20%	
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20%	

CHAPTER 2
Determination of Pesticides in Oranges

2.1 In general, the Orange

The orange is the fruit of the citrus species *Citrus sinensis* and also called sweet orange. The sweet orange types are the most widely grown citrus fruits throughout the world and provide the greatest fruit marketing production. The many known cultivars can be subdivided into three main groups:

Common oranges: They are oblong to spherical Fruits are flattened at the base in most varieties (Figure 2.1) . The Valencia is the main cultivar grown, with harvesting from spring to autumn, depending on local climates. The fruit is dual purpose and suitable for both eating as fresh fruit or processing, while seedless types are also grown. Other common oranges are often seedy and mainly suitable for processing. For instance, Pera, Hamlin, and Pineapple. Navel oranges have navel-like structure at the stylar end, or apex and are also widely grown as winter seedless eating. The Washington navel with numerous clones or selections is the most important cultivar, while there are many others navels (over 50 distinct varieties or clones) available that spread the maturity period from early (like Fukumoto, Navelina, Leng, and Newhall) to late (Lane and Navelate), and to what are now being called ‘summer navels,’ following the location of many new very late maturing cultivars in Australia, like Powell, Chislett, and Barnfield. A new pink/red fleshed navel, called Cara Cara, is also creating marketing interest.
Blood or pigmented oranges have been popular in Mediterranean countries for their distinctive flavor and both rind, flesh, and juice pigments. Such varieties are Tarocco, Sanguinello, and Moro [36][37].

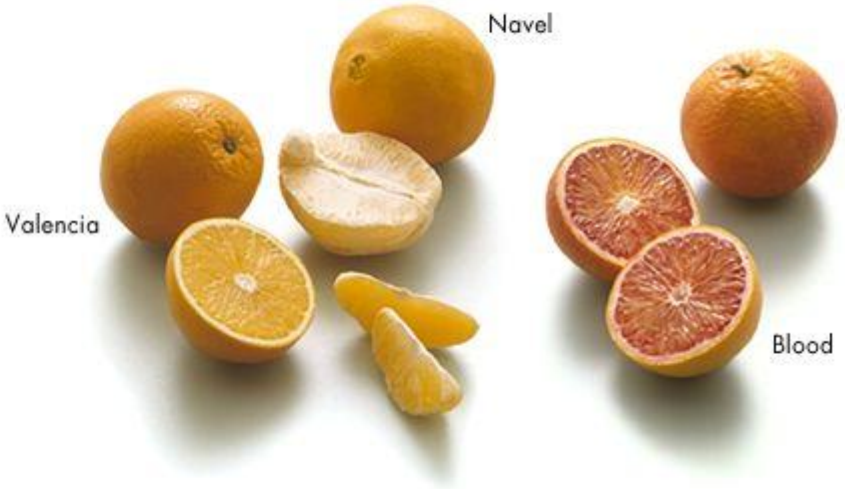


Figure 2.1: Main Types of Oranges.

Oranges may be consumed as fresh or dessert fruits that can be eaten out of hand. Juice, slices, segments, rind, and leaves are also used to garnish food. Also, as processed products like juice (fresh, chilled, frozen, canned, blended, or concentrated), syrups and cordials, segments and rind oil, or essence. After the juice is extracted, there remain residues that can be a source material from which valuable byproducts can be produced. The manufacture of fruit syrups and juices, jams, jellies, marmalade, chutney and sauces are traditional methods of preservation. Modern processes include canning, freezing, quick-freezing and dehydration [38][39].

Oranges contain thiamin, riboflavin, niacin, vitamin B-6, folate, pantothenic acid,phosphorus, magnesium, manganese, selenium and copper. Because of their high vitamin C content oranges are associated with boosting the immune system. They also contain choline, zeaxanthin, and carotenoids. Choline helps with sleep, muscle movement, learning and memory and maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat and reduces chronic inflammation. Zeaxanthin and carotenoids have antioxidant effects [40].

Oranges are cultivated in tropical and subtropical regions around the world. The trees can grow in a wide range of soil conditions, from extremely sandy to rather heavy clay loams, though they grow best in intermediate soil types. Orange trees are susceptible to diseases. These may affect the leaves or fruit and even kill the trees. Control methods include the eradication of infected trees, chemical suppression of disease-transmitting insects (use of pesticides), and using resistant rootstock for grafting.

Florida, Brazil and China are the biggest orange production countries [41]. In EU, citrus production is concentrated in the Mediterranean region. Spain and Italy represent the leading EU citrus producers, followed by Greece, Portugal, and Cyprus [42].

2.2 Sample Preparation Techniques

Sample preparation is a very important part of the analytical method because the sample must initially be cleaned up before the final analysis. Due to the low levels of pesticides that may be found in samples, the analyte concentration must be enriched before its instrumental determination [43].

Pesticides overall are compounds from different organic groups, having different chemical properties. As a result, there are two type of methods:

Multiresidue methods: cover a large number of pesticides
Single methods and common moiety methods: for a pesticide residue or a group of pesticides than cannot be determined using a multi-residue method
Single methods using derivatisation: For the analysis of some compounds derivatisation may be required. These derivatives may be prepared prior to chromatographic analysis or as part of the chromatographic procedure, either pre- or post-column [44].
Nowadays, the high number of possible pesticide residues in foodstuff indicates the need to develop multiresidue methods to cover compounds of different polarities and to allow routine analysis . Below, there is a description of some of the most used sample preparation techniques for pesticide residues determination.

2.2.1 Solvent Extraction

Solvent extraction is the most widely used technique, mainly because of its ease of use and wide ranging applicability. The extraction process varies slightly, depending on whether the sample is liquid or solid. Analysis of liquid samples has an advantage over analysis of solid samples that one fewer pretreatment step is usually required, because of their liquid state. The latter are usually repeatedly extracted with an immiscible organic solvent. Solid samples are usually homogenized before extraction, by mechanical grinding, mixing, rolling, agitating, stirring, chopping, crushing, macerating, mincing, pressing, pulverizing, or any other reasonable means of comminuting the sample. A portion is then blended or stirred with an organic solvent which is then homogenized with sodium sulfate to bind water present in the sample. The dried powder is then centrifuged and the supernatant is either concentrated or injected directly in the chromatographic system. Sample clean-up is usually performed before final chromatographic analysis.

Solvent extraction procedures have such as, they are laborious, time-consuming, expensive, and subject to problems arising from evaporation of large volumes of solvent and the disposal of toxic or inflammable solvents [45].

2.2.2 Solid Phase Extraction (SPE)

SPE (Solid Phase Extraction) is one of the most frequently used procedures for cleanup, extraction, and preconcentration of pesticide residues from different samples. Disposable cartridges for SPE were introduced more than 30 years ago. In addition to cartridges, SPE can be done in disc, pipette tips, and 96-well plates and is recognized as beneficial alternative to LLE, because it overcomes many drawbacks. The advantages of this technique are related to low solvent consumption, low costs, and reduction of processing time. The SPE can be performed in off-line or online mode (automated process). During SPE sample preparation, the steps are (Figure 2.2):

Conditioning of solid phase for activation of sorbents with the appropriate solvent
Loading the sample in order of retention the target compounds existing in sample
Washing with solvent for removal of interferences
Eluting with appropriate solvent in order to elute the target compounds

More frequently, the interferences are retained in the cartridge and the analytes pass through and are collected for analysis. In this occasion the third step is not done [22].

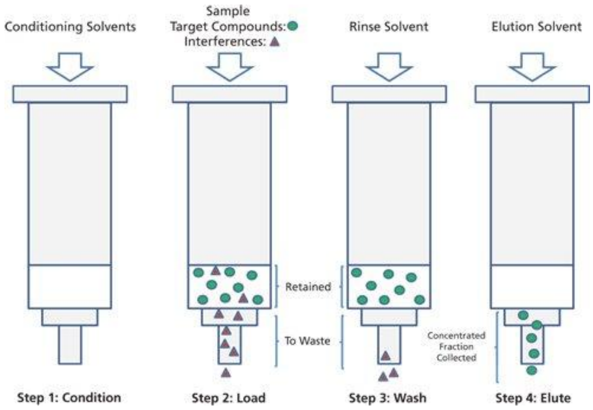


Figure 2.2 : Steps of SPE

2.2.3 Quechers

Quechers acronym stands for quick, easy, cheap, effective, rugged, and safe. QuEChERS furnishes high-quality results in a minimum number of steps and with low consumption of solvent and glassware. The original procedure consists in extraction of the homogenized sample by hand-shaking or vortex mixing with the same amount of acetonitrile to furnish a final extract sufficiently concentrated to remove the need for solvent evaporation. Gram quantities of salts, like sodium chloride and magnesium sulfate, are then added to the sample, with mixing, to drive partitioning of the analytes between the aqueous residue and the solvent. After simple vortex mixing and centrifugation, which results in perfect physical separation of the phases, clean-up and removal of residual water is performed simultaneously by use of a rapid procedure, called dispersive solid-phase extraction (dSPE), in which a primary–secondary amine (PSA) adsorbent and more anhydrous magnesium sulfate are mixed with the sample extract. Dispersive SPE is based on SPE methodology, but the adsorbent is added directly to the extract without conditioning and the clean-up is easily performed by shaking and centrifugation. The latest procedure requires less time than traditional SPE and simultaneously removes residual water and many polar matrix components, for example organic acids, some polar pigments, and sugars [45].

The original unbuffered version of Quechers evolved into two official methods using citrate buffering at a relatively low buffering capacity, CEN Standard Method EN 15662, or acetate buffering at higher concentration to give a greater buffering strength, AOAC Official Method 2007). Both versions lead to a pH around 5, which corresponds to a compromise to extract satisfactorily those pesticides that are sensitive under acidic or basic regardless of the matrix. These versions have been widely evaluated and adopted as routine methods in many laboratories around the world [45].

The QuEChERS method has the advantages of high recovery, accurate results, high sample throughput, low solvent and glassware usage (no chlorinated solvents), less labor and bench space, lower reagent costs, and ruggedness. Organic acids and other potential contaminants are removed during clean-up. The main disadvantage of QuEChERS is that for one grammar of sample per milliliter of final extract the concentration of the extract is lower than for the concentrated extracts obtained by use of most traditional procedures [45][46].

2.3 Chromatographic Techniques

Chromatographic methods are most widely used for analytical separation, identification and quantitation of pesticide residues in different matrices. Application of selective and highly sensitive detection systems is essential in food analysis because of the very low level of admissible concentrations of pesticide residues food samples [47]. The most common used chromatographic techniques for pesticides analysis are High Performance Liquid Chromagraphy (HPLC) and Gas Chromatography (GC).

2.3.1 Principles of HPLC and Instrumentation

Liquid Chromatography (Figure 2.3) is an analytical technique that is used to separate a mixture in solution into its individual components. The separation relies on the use of two different phases or immiscible layers, one of which is held stationary while the other moves over it. The mobile phase is liquid. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture. HPLC is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped through a column that contain the stationery phase. An HPLC instrument consists of: an injector, a pump, a column, a detector [48]

A recording of detectors' response with time forms a chromatogram.

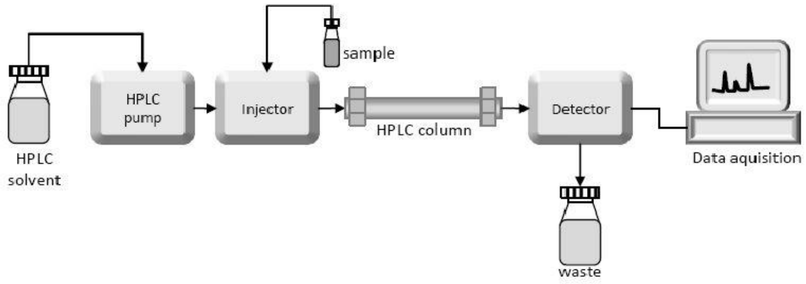


Figure 2.3: HPLC Instrumentation

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

Depending on the mechanism of separation there are the following types of Chromatography:

adsorption chromatography: separation is due to a series of adsorption/desorption steps. The stationary phase is solid.

ion-exchange chromatography: separation of ionic components of the mixture because they are electrostatically retained in different strength with converse ionic static phase groups

molecular exclusion chromatography: separation based on molecule size (molecular weight)

affinity chromatography: separation based on the highly selective interaction of a molecule of the mixture with a molecule chemically bounded (immobilized) to the solid static phase

partition chromatography: separation is based on solute partitioning between two liquid phases

Partition chromatography can divide into:

- Normal Phase: when liquid stationary phase more polar than liquid mobile phase, and
- Reversed Phase: when mobile phase is more polar than liquid stationary phase [17].

2.3.1.1 RP-UPLC

In this work we used a chromatographic system of Reversed Phase Ultra Performance Liquid Chromatography (RP-UPLC). UPLC enhance mainly in three areas: “speed, resolution and sensitivity. UPLC applicable for particle less than 2μm in diameter to acquire better resolution, speed, and sensitivity compared with HPLC. The separation and quantification in UPLC is done under very high pressure (up to 100M Pa). As compare to HPLC, under high pressure it is observed that not any negative influence on analytical column and also other components like time and solvent consumption is less in UPLC [49].

In RPLC the stationary phase (Figure 2.4) is less polar than the mobile phase and the interaction between analyte and the stationary phase has a predominantly hydrophobic (apolar) character. The most commonly used stationary phase in RPLC is silica gel in which octadecyl silica chains are covalently bound to the free hydroxyl groups, indicated as a C18 phase. Other commonly used stationary phases are silica gels modified using octyl (indicated, e.g., as a C8 phase), hexyl, butyl, or ethyl groups. Occasionally organic polymer-based phases are also used. Modified silica gels may be used up to several hundred bars pressure and across a pH range of 2–8. Care must be taken to select the right pH, as the chemically bound groups begin to hydrolyze at pH below 2 and the silica gel begins to dissolve at pH higher than 8–9. Retention of compounds occurs by apolar interaction between the analyte and the immobilized octadecyl silica chain. Most compounds exhibit hydrophobic character to some extent and thus they can be analyzed by RPLC. Even strongly polar or ionic substances can be analyzed by RPLC if the pH is adjusted so that the analyte will be in neutral form. The ability of RPLC to separate apolar and very polar analytes in one run makes it possible to determine pesticides simultaneously with their usually distinctly more polar metabolites [48]. The surface of C18 phases always contains unreacted silanol groups, which may form secondary polar interactions with the analyte. This is generally disadvantageous in RPLC as it often causes peak broadening. An important improvement is the introduction of the so-called end-capping procedure: The residual silanol groups in the C18 phase are reacted with monofunctional chlorosilane, which decreases surface polarity. This very popular stationary phase is called C18ec, where the notation “ec” stands for end-capped [50].

Mobile phases in RPLC are mostly polar solvents such as water, acetonitrile, methanol, and isopropanol. In RPLC apolar solvents have high solvent strength. Accordingly, the order of solvent strength is water< acetonitrile<methanol<ethanol<acetone (from weak to strong). The most commonly used solvent mixture is a water–acetonitrile gradient, in which the amount of acetonitrile is increased during a chromatographic run to elute first the polar components and then the more strongly bound apolar compounds. Mixtures containing a wide range of compounds may be studied by a fast gradient starting from high water content and ending at high acetonitrile content. RPLC is widely applicable, although pH control must often be

applied. Most important application areas include peptide and protein analysis (proteomics), drugs and their metabolites, fatty acids, and also volatile compounds such as aldehydes and ketones, although these require derivatization [50].

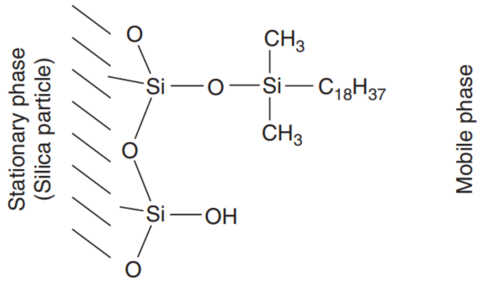


Figure 2.4: Typical RP stationary phase

2.3.2 Principles of GC and Instrumentation

In gas chromatography (Figure 2.5), firstly the sample is vaporized, then is injected in the column. The mobile phase consists of a chemically inert gas and does not reacting with analytes. There are two types of gas chromatography:

- gas-solid chromatography (GSC): the stationary phase is solid. The retention of analytes is results of adsorption/desorption steps with the solid phase.
- gas-liquid chromatography (GLC): the stationary phase is liquid that is held on a finely-divided inert solid support. The retention of analytes is based on solute partitioning between mobile (gas) and liquid phase.

GLC is most common used in sciences and simply referred as gas chromatography (GC) [21].

A GC instrument, has simple components. Usually helium, hydrogen or nitrogen gas compressed in cylinders is used as the carrier gas (mobile phase).Flow of the carrier gas into a temperature controlled sample injection is controlled by pressure regulators and gas metering valves. A GC column is attached to the injection port and samples are introduced into the carrier gas steam at a temperature sufficient to insure vaporization of all components. Typically, the sample is introduced with a microliter syringe which is forced through a rubber septum at the injection port. A detector attached directly to the column exit monitors individual sample components as they are eluted from the column. The detector must be insensitive to carrier gas, while detecting sample components that are eluted. A recording of its response with time forms a chromatogram [51].

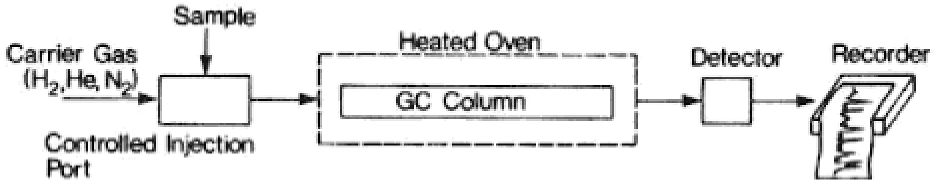


Figure 2.5: GC Instrumentation

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

2.3.2.1 GLC

In the experiments of this master thesis, it is used gas liquid chromatography system. The popularity of GC is based on a favourable combination of very high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity. GC plays an important role in analytical chemistry because including the analysis of emerging organic pollutants, such as polychlorinated alkanes (PCAs) and polybrominated diphenylethers (PBDEs) but also pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) [52]. GC is a separation technique capable of separating highly complex mixtures based primarily upon differences of boiling point/ vapor pressure and of polarity.

The mobile phase in a gas chromatographic system is referred to as the carrier gas. The carrier gas is inert and does not interact with the analytes. The choice of a carrier gas is limited based on the type of detector used, or when several gases may be suitable, on its efficiency and availability. The most commonly used carrier gases for capillary gas chromatography are helium (He), hydrogen (H_2), and nitrogen (N_2). The efficiency of a chromatographic system with a given carrier gas varies with the flow rate (in ml/min), also expressed in terms of average linear velocity (in cm/sec). That is to say, one carrier gas may not always be more efficient than another, but it may be more efficient for a given range of average linear velocities. Therefore, the selection of an appropriate carrier gas depends on how it performs under specific operating conditions [53].

The column is where the chromatographic separation takes place. The liquid stationary phase is coated on the inside wall of the capillary column and the inert mobile phase flows through the hollow tube. There are two general types of column, *packed* and *capillary*. Packed columns contain a finely divided, inert, solid support material coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm. . Typical dimensions for capillary columns range from 15 to 60 meters in length and from 0.20 to 0.53 mm in internal diameter. Capillary columns can be one of two types (Figure 2.6):

- wall-coated open tubular (WCOT) or
- support-coated open tubular (SCOT).

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

The chemical nature of the stationary phase has a great impact on the quality of the separation. The stationary phase usually has a thickness ranging from 0.1 to 1 µm [53][54].

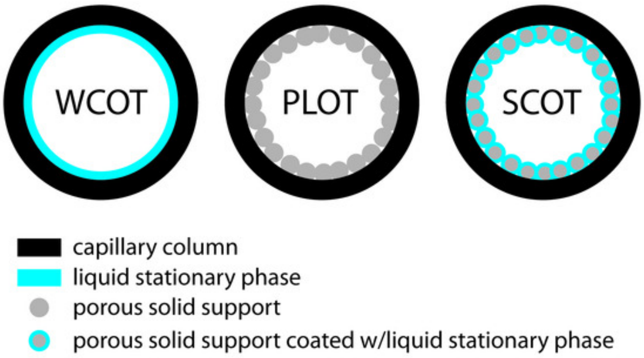


Figure 2.6 : Types of capillary collumns

2.4 Mass Spectrometry

Mass Spectrometry is combined with chromatographic techniques and is a useful tool for pesticide residue analysis and in analytical chemistry in general. Mass spectrometry is based upon the in vacuum separation of ions, in the gas phase, according to their mass-to-charge (m/z) ratio. Mass spectrometry made use of a technique of ionization to ionize the substances to be analyzed. There are several types of ionization [55]. Mass Spectrometry can be divided into two groups, depending on the mass measurement:

Low Resolution Mass Spectrometry (LRMS)
High Resolution Mass Spectrometry (HRMS)

2.4.1 Ionization Techniques

2.4.1.1 Electron Impact Ionization (EI)

Electron impact ionization is by far the most commonly used ionization method. The effluent from the GC enters enclosed ion source. Electrons "boiled" from a hot wire or ribbon (filament) are accelerated typically by 70 V (and thus have 70 eV of energy) before entering the ion source through a small aperature. When these electrons pass near neutral molecules, they may impart sufficient energy to remove outer shell electrons, producing additional free electrons and positive (molecular) ions. The energy imparted by this type of ionization is high and, with only rare exceptions, causes part of or all of the molecular ions to break apart into neutral atoms and fragment ions. This ionization technique produces almost exclusively positively charged ions (Figure 2.7) [56].

$$M + e^- \longrightarrow M^{+*} + 2\,e^-$$

$$M^{+*} \longrightarrow F^+ + N^*$$

Figure 2.7: Mechanism of EI.

2.4.1.2 Chemical Ionization (CI)

In chemical ionization new ionized species are formed when gaseous molecules interact with ions. Chemical ionization may involve the transfer of an electron, proton, or other charged species between the reactants. These reactants are the neutral analyte M and ions from a reagent gas.

Assuming reasonable collision cross sections and an ion source residence time of 1 μs, a molecule will undergo 30–70 collisions at an ion source pressure of about 2.5×10^2 Pa. The 10^3 –10^4 -fold excess of reagent gas also shields the analyte molecules effectively from ionizing primary electrons which is important to suppress competing direct EI of the analyte. There are four general pathways to form ions from a neutral analyte M in CI (Figure 2.8):

Proton transfer	$M + [BH]^+ \longrightarrow [M+H]^+ + B$
Electrophilic Addition	$M + X^+ \longrightarrow [M+X]^+$
Anion Abstraction	$M + X^+ \longrightarrow [M-A]^+ + AX$
Charge Exchange	$M + X^{+*} \longrightarrow M^{+*} + X$

Figure 2.8: Main mechanism pathways of CI.

Although proton transfer is generally considered to yield protonated analyte molecules, $[M+H]^+$, acidic analytes may also form abundant $[M–H]^–$ ions by protonating some other neutral. Electrophilic addition chiefly occurs by attachment of complete reagent ions to the analyte molecule, e.g., $[M+NH4]^+$ in case of ammonia reagent gas. Hydride abstractions are abundant representatives of anion abstraction, e.g., aliphatic alcohols rather yield $[M–H]^+$ ions than $[M+H]^+$ ions. Whereas reactions 1–3 result in even electron ions, charge exchange (reaction 4) yields radical ions of low internal energy which behave similar to molecular ions in low-energy electron ionization [57].

2.4.1.3 Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric pressure chemical ionisationis an analogous ionisation method to chemical ionisation. The significant difference is that APCI occurs at atmospheric pressure and has its primary applications in the areas of ionisation of low mass pharmaceutical compounds (APCI is not suitable for the analysis of thermally labile compounds). In APCI, the analyte solution is introduced into a pneumatic nebulizer and desolvated in a heated quartz tube before interacting with the corona discharge creating ions. It can be in two modes ionization modes positive or negative. Ionization in positive-ion mode occurs by reaction of the analyte with protonated solvent molecules, generally giving rise to an abundant protonated analyte molecule $[M+H]^+$ or adduct ions like $[M+NH4]^+$, if ammonium salts are added to the solvent. In the negative-ion mode, ions are generated by proton abstraction by oxygen ions O2* or by the formation of adducts with anions such as acetate or chloride present in the sample or solvent.

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

Assuming nitrogen is the sheath and nebulizer gas with atmospheric water vapour present in the source, then the type of primary and secondary reactions that occur in the corona discharge (plasma) region during APCI are as shown in the scheme. The most abundant secondary cluster ion is $(H_2O)_2H^+$ along with significant amounts $(H_2O)_3H^+$ and H_3O^+. The reactions listed above (Figure 2.9) are ways to account for the formation of these ions during the plasma stage. The protonated analyte ions are then formed by gas-phase ion-molecule reactions of these charged cluster ions with the analyte molecules. This results in the abundant formation of $[M+H]^+$ ions [58][59].

$$N_2 + e^- \longrightarrow N_2^+ + 2e^-$$

$$N_2^+ + 2N_2 \longrightarrow N_4^+ + N_2$$

$$N_4^+ + H_2O \longrightarrow H_2O^+ + 2\,N_2$$

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH^*$$

$$H_3O^+ + H_2O + N_2 \longrightarrow [H_2O]_2H^+ + N_2$$

$$[H_2O]_nH^+ + H_2O + N_2 \longrightarrow [H_2O_2]_{(n+1)}H^+ + N_2$$

Figure 2.9: Reactions in APCI

2.4.1.4 Electro Spray Ionization (ESI)

ESI is a process by which a solution is sprayed into a high electric field at atmospheric pressure. Charged droplets result from the nebulization of the solution in an electric field, with both solvent and analyte ions being detected. This is a widely applied technique. ESI is a liquid-phase ionization technique, which does not require the evaporation of a neutral analyte, but rather the formation of preformed ions in solution. Therefore, ESI is the method of choice for the ionization of analytes that would easily thermally decompose. In order to achieve preformed analyte ions in solution, the composition of the sample solution (or LC mobile phase) has to be adjusted in order to convert a neutral analyte into an ion in solution. Basic analytes, for example, are ionized by the addition of an acid to the solution. The electrospray process in greatly limited in terms of flow-rate that can be nebulized. The use of a nebulizing gas allows higher flow-rates to be used. However, higher flow-rates also require the use of heat, for example, by application of a concurrent, countercurrent, or cross flow of hot gas, for the desolvation of charged droplets in order to promote the release of analyte ions into the gas phase. The high efficiency of the technique and its compatibility with LC and other liquid separation methods gave rise to much interest in the pharmaceutical applications of the technique. As the mass analyzer separates ions according to their mass-to-charge ratio (m/z) rather than their mass, both Sample solution Charged aerosol sample ions Mass analyzer Atmospheric pressure Vacuum Principle of electrospray ionization inside an atmospheric pressure ion source (Figure 2.10). As a result, in addition to increasing sensitivity, ESI effectively extends the mass range of analytes amenable to MS by more than an order of magnitude to beyond 150 kDa [30].

An important issue in the application of ESI in the analysis of analytes in complex biological samples is the occurrence of ionization suppression or enhancement effects. These so-called matrix effects are due to influence of co-eluting matrix constituents on the liquid-phase analyte ionization and on the transfer of preformed analyte ions from the liquid to the gas phase. [30].

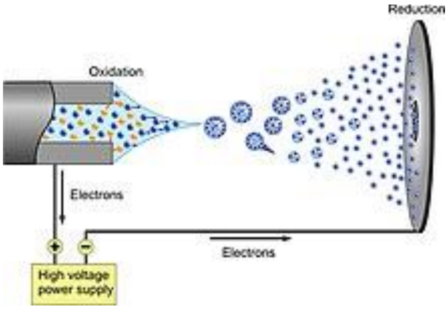


Figure 2.10: ESI Mechanism

2.4.2 LRMS

LRMS measurements provide information about nominal mass of the analyte i.e., the m/z for each ion is measured to single-digit mass units. The most common LRMS instruments that have been used in pesticide residues analysis are Quadrupole, Triple Quadrupole, and Ion Trap.

2.4.2.1 Quadrupole (Q)

A quadrupole analyzer (Figure 2.11) uses a combination of radio frequency alternating current (AC) and direct current (DC) voltages as a mass filter, for separating ions. The quadrupole consists of four parallel rods. The positive DC voltage is applied on two opposite rods, and the same value of the negative DC voltage is applied on the remaining two rods. The AC is connected to all four rods. Combined DC and RF potentials on the quadrupole rods can be set to pass only a selected m/z ratio. All other ions do not have a stable trajectory through the quadrupole mass analyzer and will collide with the quadrupole rods, never reaching the detector. The single quadrupole is certainly the simplest, cheapest, most robust, and ubiquitous mass analyzer in research and development laboratories, but it suffers from a limited sensitivity, resolving power and mass accuracy [60].

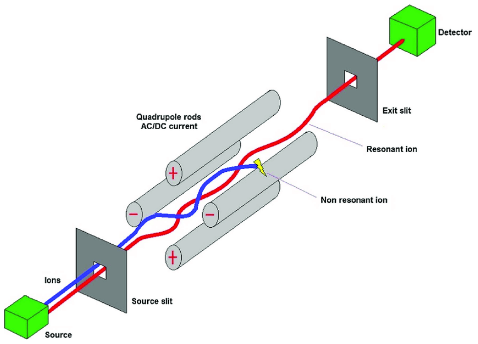


Figure 2.11: Quadrupole mechanism

2.4.2.2 Triple Quadrupole (TQ or QqQ)

A QqQ mass spectrometer (Figure 2.12) offers MS/MS in which the first and third quadrupoles act as mass filters, while the second quadrupole is used for fragmentation of the precursor ion through interaction with a collision gas (usually nitrogen or argon). The main MS/MS scan modes are product ion, precursor ion, neutral loss, single reaction monitoring (SRM), multiple reaction monitoring (MRM), and MSn scans.

The main benefits of analysis in MS/MS mode are increased selectivity, improved S/N, lower limits of quantitation (LOQ), wider linear range, and improved accuracy.

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

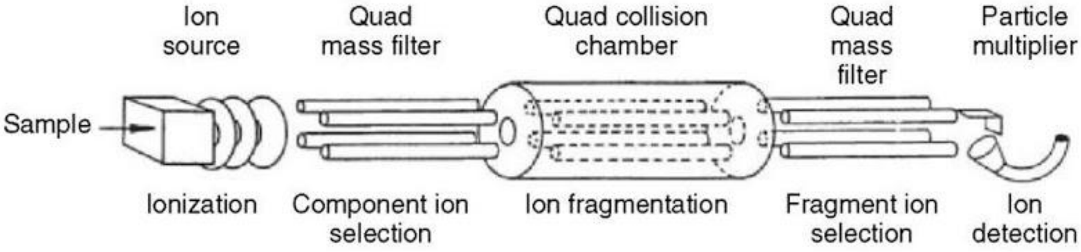


Figure 2.12: Linear QqQ instrument parts.

2.4.2.3 Ion Trap (IT)

An ion trap (Figure 2.13) may be described as a quadrupole that has undergone a solid of rotation. A typical ion trap comprises two endcap electrodes and a ring electrode, all of hyperbolic or hemispherical cross-section. The end-cap electrodes contain small-diameter holes for allowing ions to enter and leave the trap. Ions are confined inside the trap by a radio-frequency field of constant frequency but variable power. The ions may be detected, according to their m/z ratio, by applying voltages sufficient to eject them from the trapping field [62].

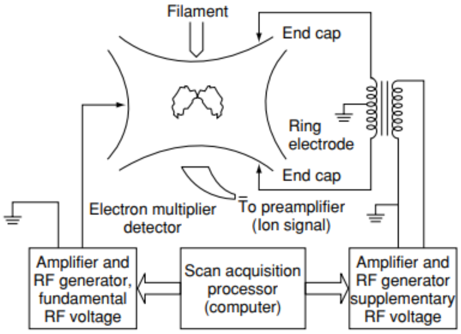


Figure 2.13: Schematic diagram of an IT

2.4.3 HRMS

HRMS provides information about the exact mass of the analyte, i.e., the m/z of each ion is measured from four to six decimal points. As a result, co-elutting molecules with the same nominal mass can be identified. The HRMS instruments that have been used mostly in pesticides analysis are Time of Flight (TOF) and Orbitrap mass analyzers.

2.4.3.1 TOF

TOF (Figure 2.14) is based on the fact that ions with the same energy but different mass travel with different velocities. Basically, ions formed by a short ionization event are accelerated by an electrostatic field to a common energy and travel over a drift path to the detector. The lighter ones arrive before the heavier ones, and a mass spectrum is recorded. Measuring the flight time for each ion allows the determination of its mass. This cycle is repeated with a repetition rate that depends on the flight time of the highest mass to be recorded. The enhancement in the mass resolution is obtained by using reflectron (ion mirror). The reflectron is a series of ring electrodes with increasing voltage that creates retarding fields. The higher-energy ions reaching the reflectron area penetrate more deeply inside, and this results in extension of the time until they are reflected. Due to this phenomenon, the ions of the same m/z value with different initial energies hit the detector at almost the same time. The flight times of the ions separated in a field-free region are proportional to the square root of the respective m/z value.

The inherent characteristics of TOF/MS are its high sensitivity in scan mode (all ions are detected), theoretically unlimited mass range as well as high acquisition speed (the duty cycle of modern instruments can attain 100 Hz). In addition, high-end TOF instruments afford resolving power of 40,000-60,000 and mass accuracies below 2 ppm. The only drawback to TOF analyzers is its limited dynamic range and quantitative performance [31,32,34].

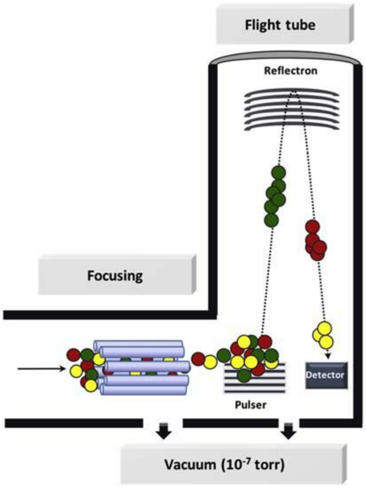


Figure 2.14: Scheme of TOF instrument

2.4.3.2 Orbitrap

The Orbitrap mass analyzer (Figure 2.15) consists essentially of three electrodes. Outer electrodes have the shape of cups facing each other and electrically isolated by a hair-thin gap secured by a central ring made of a dielectric. A spindle-like central electrode holds the trap together and aligns it via dielectric end-spacers. When voltage is applied between the outer and the central electrodes, the resulting electric field is strictly linear along the axis and thus oscillations along this direction will be purely harmonic. At the same time, the radial component of the field strongly attracts ions to the central electrode.

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

The orbitrap analyzer offers very high resolving power in the range 100,000-240,000, and excellent mass accuracy below 1 ppm. Drawback of orbitrap analyzers is its low acquisition rate [60][63].

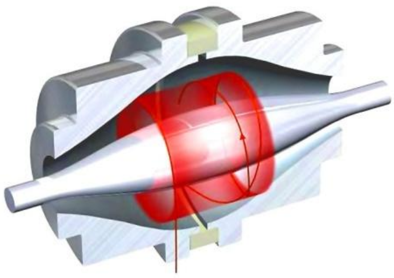


Figure 2.15: Scheme of Orbitrap

2.4.3.3 Hybrid Instruments

The coupling of two different analyzers is known as hybrid instrument. An example is QqTOF (Figure 2.16) where the first quadrupole is mass selective device, the second serves as a collision cell and the third is a TOF analyzer. An advantage is the high resolving power of TOF, typically in the range 20,000-40,000. As a result, interfering peaks from ions having the same nominal mass can be resolved, thus improving the signal to noise ratio.

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

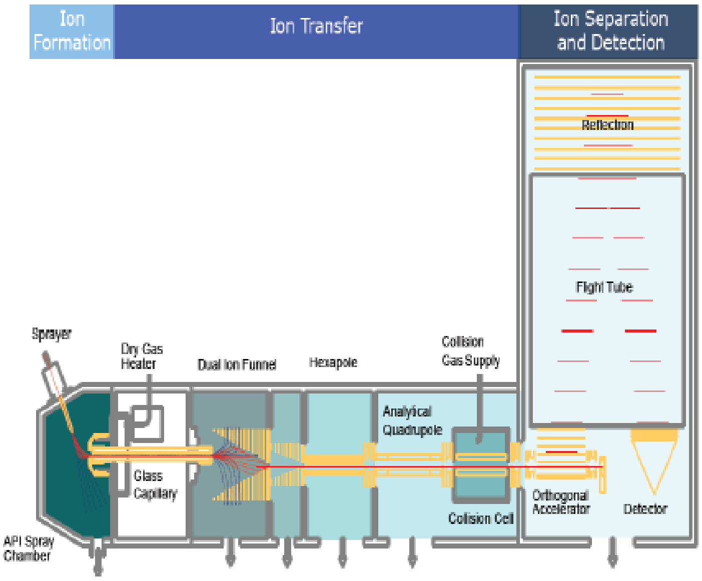


Figure 2.16: Scheme of QqTOF instrument (Maxis Impact, Bruker)

2.5 Acquisition Modes in HRMS

2.5.1Data Dependent Acquisition (DDA)

DDA is mode of data collection in MS/MS in which a fixed number of precursor ions whose m/z values were recorded in a survey scan (FS single-mass) are selected in real time using predetermined rules, such as intensity threshold or suspect inclusion list and are subjected to a second stage of mass selection in an MS/MS analysis. After acquiring the product ion mass spectra, the system returns back to the survey scan [82],[83].

We use this type of acquisition for the development of a GC-APCI-QTOF Database.

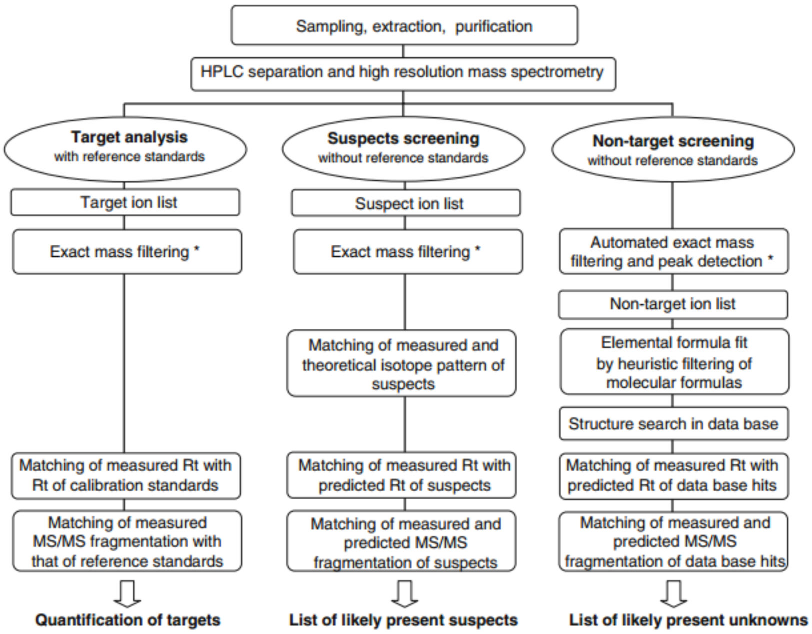
2.5.2 Data Independent Acquisition (DIA)

This acquisition mode could be considered as a simple and generic mode, which is based on nonspecific CID. Therefore, the MS/MS spectra are obtained in a nonselective manner. Full-scan spectra at different collision energies are obtained in one injection. This acquisition provides accurate mass data of parent compounds and fragment ions in a single run using two alternating scans, one at low and one at high collision energy. By applying low energy (LE) in the collision cell, fragmentation is performed. A full-scan spectrum is obtained that provides information for the parent ion (the (de)-protonated molecule) and, in some cases, the adduct ions and the in-source fragments. By applying high energy (HE) in the collision cell, fragmentation is performed and a spectrum similar to MS/MS experiments is obtained. This approach is called all-ions MS/MS, MSE or bbCID, according to the QTOF manufacturer [83].

2.6 Data Analysis Workflows in HRMS

2.6.1 Target screening

In this approach (Figure 2.17), an in-house developed database is used for the screening of a large number of compounds. The information included in the database is based on the analysis of the available reference standards. The reference standard is necessary for comparison of the retention time, the MS spectrum profile (precursor ion, adducts, in-source fragments), as well as the MS/MS spectrum (fragment ions and ion ratios) . Quantitation can be performed in full-scan mode, but requires greater effort than in LC-LRMS methods where Single Reaction Monitoring (SRM) mode is used [82][84].



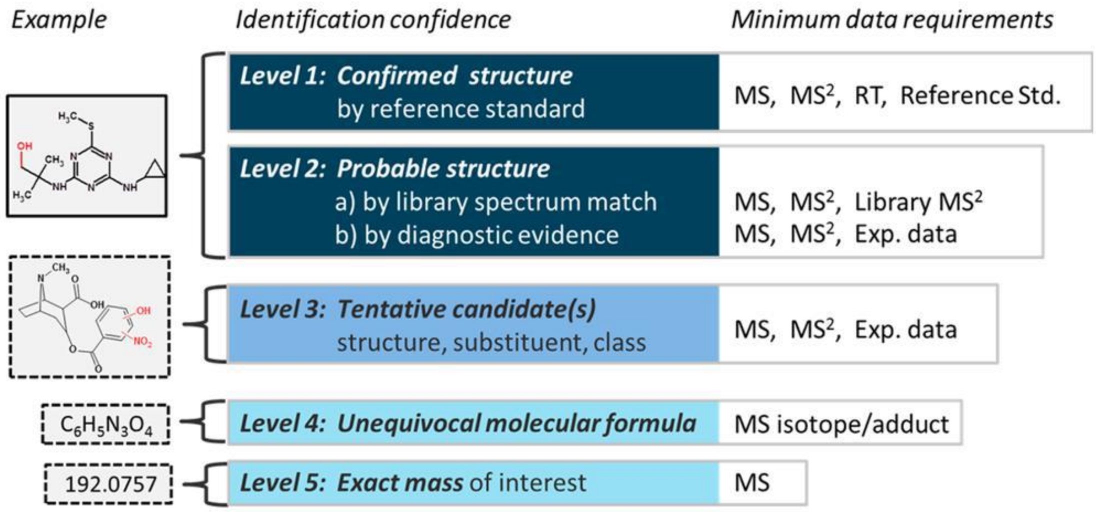
*Note that the *m/z* range of the extraction window for the exact mass filtering depends on the mass accuracy and the resolving power of the mass spectrometer used

Figure 2.17 : Systematic workflow for target, suspect and non-target screening by LC-HRMS

2.7 Confidence in the identification procedure

2.7.1 Confidence in target screening

The confirmation of positive findings in target screening can be performed by attributing identification points (IPs). According to the 2002/657/EC guideline, 4 IPs are required for unequivocal confirmation, and for HRMS instruments with resolution higher than 10,000, the precursor ion earns 2 IPs and the product ions earn 2.5 IPs [86]. This means that one single HRMS/MS transition can confirm the detection of a substance, which is risky when there are several co-eluting isomers [87]. Another fact is that resolving power may largely vary between HRMS instruments, which makes the definition of general criteria difficult [85]. More precise criteria for the use of mass accuracy and mass resolution have to be implemented to define clearly the requirements for a reliable confirmation in LC-HRMS [83]. Bletsou et al. [84] proposed an identification points system for HRMS analysis in order to take full advantage of the capabilities of HRMS instruments (Figure 2.18).



Note: MS^2 is intended to also represent any form of MS fragmentation (e.g., MS^e, MS^n).

Figure 2.18: Identification confidence levels in HRMS

2.8 Determination of Pesticides in Orange – Analytical Methods Performed

Methods for the analysis of pesticides have made significant progress in the last years mostly because of developments in chromatographic instrumentation. The need for rapid high-resolution methods of analysis is as pressing today as it ever was. A combination of MS with chromatographic equipment is essential for comprehensive analysis and fulfils the EU requirements for identification, quantification and verification of the important pesticides. Even with such powerful instrumental techniques, however, the risk of interference increases with the complexity of the matrix studied, so sample preparation before instrumental analysis is still mandatory in many applications, for example food analysis.

The extraction process is the first and major limiting step in the pesticide residue analysis. In most of these the extraction procedure usually involves sample homogenization with an organic solvent, alone or mixed with water or pH-adjusted water, using a homogenizer, blender, or sonicator. In addition to these classical extraction techniques, other more recent approaches, for example QuEChERS, supercritical fluid extraction (SFE), pressurized-liquid extraction (PLE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), solid-phase extraction (SPE), solid-phase microextraction (SPME) and stir-bar-sorptive extraction (SBSE) have resulted in new possibilities in sample treatment and advantages such as a substantial reduction of the extraction time and incorporation into on-line flow-analysis systems.

In chromatographic techniques the old detectors have been replaced by mass spectrometry analyzers. The most commonly used GC detectors are element selective detectors such as the ECD, NPD, FPD confront issues with matrix related interferences while ELSD, FID, TSD, AED find some limited use. The most commonly used LC detectors are DAD, UV-Vis and FD. The LC–MS arnd GC-MS techniques can be LRMS or HRMS. The most studies so far in the determination of pesticides in fruits have used LRMS such as Q, TQ, and IT analyzers. HRMS analyzers such as TOF, Orbitrap or hybrid systems like QTOF have not been studied a lot on target screening but have been used more for suspect and non target screening [45][64]

Table 2.1: Representative applications of chromatographic determination of pesticide residues in fruits and vegetables with different sample preparation techniques.

Matrix	Analytes	Extraction Method	Extractions Solvent	Clean-up	Instrumental Technique	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Recovery (%)	RSD (%)	Ref
Lettuce, Maize grain, Wheat grain, whole orange	Chlorantraniliprole, Cyantraniliprole	Citrate buffered QuEChERS	ACN	d-SPE PSA,MgSO$_4$, and C18	HPLC-MS/MS		10	87-107	≤8	[65]
Tomato, pepper, and orange	90 pesticides	Citrate buffered QuEChERS	ACN	dSpe:	LC -QqQ-MS		5-50	52-120	2-15	[66]
Vegetables and fruits samples	60 pesticides	Solvent extraction And use of sodium chloride	ACN:MeOH mixture (90:10 v/v)	SPE using GCB/PSA	UPLC-ESI-TOF/MS	0.3-3.8	0.8-11.8	74-111	<13.2	[67]
Vegetables and fruits samples	199 pesticides	Citrate buffered QuEChERS	ACN	dSpe: MgSO$_4$, PSA	UPLC-QTOF/MS	10-50	-	-	-	[68]
Oranges	Carbusulfan and its metabolites	Solvent extraction	DCM	no	LC-TQ-MS	0.4-3	1-10	-	-	[69]
Oranges	6 pesticides	Solvent extraction	EtOAc	no	LC-QIT-MS	5-200	10-300	72-92	12-19	[70]
Oranges	6 pesticides	Solvent extraction	EtOAc	no	LC-TQ-MS	5-200	-	70-94	8-19	[70]
Oranges	Carbusulfan and its metabolites	PLE	DCM	no	LC-IT-MS		10-70	55-90	8-19	[71]
Fruit and	43 Multiclass	Solvent	MeOH/H2O	SPE (Oasis	LC-TQ-MS		10	70-110	<15	[72]

Vegetables	pesticides and nine metabolites	Extraction	with (0.1% HCOOH)	HLB)						
Lettuce and orange	229 pesticides	QuEChERS	ACN	dSpe: MgSO$_4$, PSA	LC-TQ-MS GC-IT-MS		<10	70-120	<10	[73]
Fruit and Vegetables	30 multi-class Pesticides	Solvent Extraction	DCM	no	GC-MS-MS		0.01-6.4	70-110	<17	[74]
Fruit and Vegetables	90 pesticides	Solvent extraction	Acetone	SPE	GC-MS		10-50	>80	<10	[75]
Fruit and Vegetables	4 Neonicotinoid pesticide	Solvent extraction	Acetone	SPE (Extrelut-NT20)	LC-MS	20-100	100-500	74.5-105	1.5-15	[76]
Fruits and Vegetables	504 pesticides	Citrate buffered QuEChERS	ACN	dSPE: MgSO$_4$, PSA	UPLC–QTOF–MS					[68]
Fruits and Vegetables	130 multiclass pesticides	ASE	EtOAc	no	GC-MS/MS		10-50	70-120	<20	[77]
Fruits and Vegetables	125 pesticides	buffer citrate QuEChERS	ACN	dSPE	LC-QTOF-MS		10-50		<20	[78]
Fruits and Vegetables	24 pesticides	Modified QuEChERS	EtOAc	dSPE: MgSO$_4$, PSA	GC-ECD GC-NPD		5-10	93	10	[79]
Fruits and Vegetables	130 pesticides	Acetate buffered QuEChERS	ACN (1% Acetic acid)	no	LC-Orbitrap		10-20			[45]
Fruits and Vegetables	74 pesticides	Solvent extraction	EtOAc	No	LC–MS/MS		10	80-118	3-20	[80]
Fruits and Vegetables	142 pesticides	Acetate buffered QuEChERS	ACN (1% Acetic acid)	dSPE: MgSO$_4$, PSA,C18	GC-MS-MS		10-100	70-110	<10	[81]

CHAPTER 3
Scope and Objectives

Pesticides are mainly used to prevent crops from pests. However, farmers do not always apply the guidelines of Good Agricultural Practise and pesticide residues remain on the plants. As a result, these residues through the food chain end up to human organism or accumulate in the environment. If pesticide residues concentrations are above the MRLs are dangerous for human health.

The orange is a citrus fruit which has a large nutritional value. As with other citrus fruits, orange pulp is an excellent source of vitamin C. Oranges contain diverse phytochemicals, including , flavonoids and numerous volatile organic compounds producing orange aroma. For this reason, orange has a major role in daily diet, all over the world. Orange can be consumed raw as a fruit. Furthermore, it can be used in cooking and confectionery recipes. Therefore, it has to be legally not contain pesticide residues or their concentrations must be below MRLs.

In Greece, there is a large agricultural production of oranges. Part of them is exported abroad in other world markets. The other part is distributed in the interior trade.

Analytical chemistry plays a decisive role in the fight against the fruit contamination of pesticides. Various analytical methods for the determination of pesticide residues have been developed. Most of them using chromatographic techniques coupled with LRMS (LC-QqQ, GC-QqQ). Therefore, chromatographic techniques combined with HRMS (LC-QToF,GC-QToF) were selected to study if they can be used as an alternative in pesticide routine analysis.

So, the aim of this master thesis is the development of a multivariate method for the determination of pesticide residues using HRMS techniques. Particularly, the objectives of this study are:

- Development of a multiresidue method for the determination of pesticide residues in orange based on Quechers AOAC using UPLC-ESI-QToF, GC-APCI-QToF techniques
- Validation of the above method for a certain number of pesticides of different polarities
- Development of a Database for target screening of pesticides for GC-APCI-QToF technique
- The target screening in representative number of orange samples in order to investigate if the pesticide residues are below legal limits

EXPERIMENTAL PART

CHAPTER 4
Materials and Methods

4.1 Chemicals and Reagents

All solvents were special purity, Pesticide Grade for pesticide residue analysis. For UPLC-ESI-QTOF system all solvents were UPLC-MS grade. Methanol (MeOH) hypergrade for LC-MS was purchased from Sigma-Aldrich (Steinheim, Germany), Acetonitrile (ACN) was purchased from Honeywell (New Jersey,USA), whereas 2-propanol and Ethyl acetate (EtoAC) of LC-MS grade was from Fisher Scientific (Geel, Belgium). Distilled water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Regenerated cellulose syringe filters (RC, pore size 0.2 μm, diameter 15mm) were purchased from Phenomenex (Torrance, CA, USA). Ammonium acetate and Sodium formate 99% purity were purchased by Sigma-Aldrich (Steinheim, Germany). For GC-APCI-QTOF system Hexane for Pesticide residue analysis was purchased from Honeywell (New Jersey,USA) and Acetone Pestipure was ordered from Carlo Erba (Barchelona, Spain).

For experimental procedure:

Standards stock solutions of individual pesticides at a concentration of 1000 mg L^{-1} were purchased from Bruker Daltonik GmbH (Bremen, Germany).

Standards of Hexachlorobutadiene, Dichlorvos, Alpha-HCH, Hexachlorobenzene, Beta-HCH, Lindane, delta-HCH, Heptachlor, Aldrin, Dicofol, Isodrin, Alpha-Endosulfan, Dieldrin, Endrin, 4,4'-DDT, 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, Endosulfan-sulfate and the internal standard Triphenyl phosphate (TPP), (>99%purity) were ordered from Fluka-Sigma-Aldrich (Steinheim, Germany). Standard stock solutions of them were prepared by dissolving 0.01g of the crystalline standards with Hexane in 10 mL volumetric flask, so the concentration was 1000 mg L^{-1} and stored at −20 °C.

Working solutions of 1000 ng L^{-1} were prepared. The working solutions contained all the pesticides in total. 10 μL of each pesticide from standard stock solution were transferred into a 1 mL vial and diluted with appropriate solvent (MeOH, EtoAc, Hexane) depending on pesticides compounds' solubility. The solutions stored at -20 °C.

Acetic acid (HAc) ≥99% was used and ordered from Honeywell (New Jersey,USA). Furthermore, Magnesium sulphate ($MgSO_4$) anhydrous was ordered from Mallinckrodt (New York, USA), Sodium acetate (NaAc), PSA Silica and DSC-18 (C18) were from Supelco (Bellefonte, USA).

4.2 Sampling and Storage

29 orange samples were collected from local super markets, flea markets in Athens and home grown trees. 11 of the samples were from local super markets divided into 3 samples of organic oranges and 8 samples of commercial oranges. 16 samples were bought from flea markets and 2 samples were from home grown orange trees. In total, there were two different species of orange, navel and valincia. All samples were from Greece. Each sample was about 1 kg and after homogenization by a multi, a part of the sample was transferred in a sterile container and stored in a refrigerator at 4°C until sample preparation. Table 4.1 summarizes the details of total orange samples with their analysis code.

Table 4.1: Orange samples details with their analysis code.

No	Matrix	Analysis code	Type	Species	Sampling place	Origin
1	Oranges	OSM1	Navel	Organic	Super market	Greece
2	Oranges	OSM2	Navel	Organic	Super market	Greece
3	Oranges	OSM3	Navel	Organic	Super market	Greece
4	Oranges	SM4	Navel	Commercial	Super market	Greece
5	Oranges	SM5	Navel	Commercial	Super market	Greece
6	Oranges	SM6	Navel	Commercial	Super market	Greece
7	Oranges	SM7	Navel	Commercial	Super market	Greece
8	Oranges	SM8	Navel	Commercial	Super market	Greece
9	Oranges	SM9	Navel	Commercial	Super market	Greece

10	Oranges	SM10	Valencia	Commercial	Super market	Greece
11	Oranges	SM11	Valencia	Commercial	Super market	Greece
12	Oranges	FM1	Navel	Commercial	Flea market	Greece
13	Oranges	FM2	Navel	Commercial	Flea market	Greece
14	Oranges	FM3	Navel	Commercial	Flea market	Greece
15	Oranges	FM4	Navel	Commercial	Flea market	Greece
16	Oranges	FM5	Navel	Commercial	Flea market	Greece
17	Oranges	FM6	Navel	Commercial	Flea market	Greece
18	Oranges	FM7	Navel	Commercial	Flea market	Greece
19	Oranges	FM8	Navel	Commercial	Flea market	Greece
20	Oranges	FM9	Navel	Commercial	Flea market	Greece
21	Oranges	FM10	Navel	Commercial	Flea market	Greece
22	Oranges	FM11	Navel	Commercial	Flea market	Greece
23	Oranges	FM12	Navel	Commercial	Flea market	Greece
24	Oranges	FM13	Navel	Commercial	Flea market	Greece
25	Oranges	FM14	Navel	Commercial	Flea market	Greece
26	Oranges	FM15	Navel	Commercial	Flea market	Greece
27	Oranges	FM16	Navel	Commercial	Flea market	Greece
28	Oranges	HG1	Valencia	Organic	Home grown	Greece
29	Oranges	HG2	Valencia	Commercial	Home grown	Greece

4.3 Sample Preparation

The QuEChERS procedure applied was based on that proposed in the AOAC official method 2007.01 [1]. The final method was:

5 g of sample were weighted in a 50 mL polypropylene centrifuge tube. 50 μL of TPP 1000 μg/L were spiked into the sample, as internal standard. Afterwards 5 mL of ACN (modified with 1%HAc) were transferred into the tube and shaken by hand for 30 s. Then, 2 g anhydrous $MgSO_4$ and 0.5 g anhydrous NaAc were added and immediately shaken vigorously by hand to prevent formation of $MgSO_4$ agglomerates. Then, the tube was centrifuged at 3000 rpm for 2 min. For the cleanup step, 4 mL of the ACN supernatant were transferred into a a 15 mL polypropylene centrifuge tube containing 600 mg

MgSO4, 200 mg PSA and 200 mg C18. The tubes were shaken on a Vortex for 30s and centrifuged at 3000 rpm for 2 min.

3 mL of the extract (acetonitrile) were divided into two test tubes (each test tube contained 1.5 mL of the extract), for UPLC-ESI-QTOF and GC-APCI-QTOF analysis respectively. Extracts from both test tubes were evaporated under a gentle nitrogen steam near to dryness.

For UPLC-ESI-QTOF analysis, the first tube was reconstituted to 0.15 mL with a final propotion of MeOH:H_2O (50:50).

For GC-APCI-QTOF analysis In the second tube before the evaporation was added 15 μL of iso-octane used as a keeper for the volatile compounds. After the evaporation the tube was reconstituted to 0.15 mL with a final propotion of Hexane:Acetone (50:50).

Both the extracts were filtered through a 0.2 μm RC syringe filter and were ready for injection in chromatographic systems.

The final method was optimized for GC-APCI-QTOF system with the following tests.

Insted of a 15 g sample/15 mL ACN (1% HAc) we used 5g sample/5 mL ACN (1% HAc) and, accordingly, the amounts of salts MgSO4,NaAc from 6 and 1.5g that AOAC suggests were 2 and 0.5 g respectively.

Instead of non preconcentration in the final extract a preconcentration by a factor of 10 was tested.

The reconstitution solution tested with only Hexane, only Acetone and Hexane:Acetone (50:50).

4.4 HRMS Analysis

The analysis of oranges and the validation of the method were carried out in a UHPLC-ESI-QTOF system and a GC-APCI-QTOF system.

4.4.1 UHPLC-ESI-QTOF

For LC part of the analysis, an ultrahigh-performance liquid chromatography (UHPLC) system (Figure 4.1) was used, consisting of:

- a HPG-3400 pump (Dionex Ultimate 3000 RSLC, Thermo Fischer Scientific,)
- an Autosampler
- a hybrid quadrupole time of flight mass spectrometer (QTOF-MS) (Maxis Impact, Bruker Daltonics)

Method validation and orange samples' extracts were analyzed in RPLC. Mass spectra acquisition and data analysis was processed with Data Analysis 4.4 and TASQ 1.4 (Bruker Daltonics). The QTOF-MS system is equipped with an Electrospray (ESI) source, operating in both positive and negative ionization mode.

In RPLC, chromatographic system concluded a column Acclaim RSLC C18 (2.1 × 100 mm, 2.2 μm) from Thermo Fischer Scientific preceded by a guard column of the same packaging material, thermostated at 30^oC. For positive ionization mode (PI), the mobile phases are water:methanol 90:10 (solvent A) and methanol (solvent B) both amended with 5mM ammonium formate and 0.01% formic acid. For negative ionization mode (NI), the mobile phases consisted of water:methanol 90:10 (solvent A) and methanol (solvent B) both acidified with 5mM ammonium acetate. The gradient elution program was the same for both ionization modes, starting with 1% B with a flow rate of 0.2 mL min^{-1} for 1 min and it increases to 39 % in 2 min (flow rate 0.2 mL min^{-1}), and then to 99.9 % (flow rate 0.4 mL min^{-1}) in the following 11 min. Then it keeps constant for 2 min (flow rate 0.48 mL min^{-1}), then initial conditions were restored within 0.1 min, kept for 3 min and then the flow rate decreased to 0.2 mL min^{-1}. The injection volume was set up to 5 μL.

When RP chromatographic system was used, the operation parameters of ESI were the following: capillary voltage, 2500 V for positive and 3000 V for negative mode; end plate offset, 500 V; nebulizer pressure, 2 bar (N_2); drying gas, 8 L min^{-1} (N_2); and drying temperature, 200 °C.

In LC analysis, QTOF used a data independent acquisition mode called broadband collision-induced dissociation (bbCID) and recorded spectra between the range m/z 50-1000 while the scan rate was 2Hz. The Bruker bbCID mode provides MS and MS/MS spectra simultaneously, using two different collision energies. At low collision energy (4 eV), MS spectra (fullscan) were acquired while at high collision energy (25 eV), fragmentation is taking place at the collision cell resulting in MS/MS spectra.

A QTOF-MS external calibration was daily performed with a sodium formate solution, and a segment (0.1−0.25 min) in every chromatogram was used for internal calibration, using a calibrant injection at the beginning of each run. The sodium formate calibration mixture consists of 10 mM sodium formate in a mixture of water:isopropanol (1:1). The theoretical exact masses of calibration ions with formulas $HCOO(NaCOOH)_{1-14}$ in the range of 50−1000 Da were used for calibration. The instrument provided a typical resolving power of 36,000−40,000 during calibration (39274 at m/z 226.1593, 36923 at m/z 430.9137, and 36274 at m/z 702.8636).

4.4.2 GC-APCI-QTOF

For GC part of the analysis, we used a gas chromatography system combined with:

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

GC was operated in splitless injection mode (Restek Split liner w/Glass Frit (4mm x 6.3 x 78.5)) and the splitless purge valve was activated 1 min after injection. The injection volume was 1 μL. A Restek Rxi-5Sil MS column of 30 m (0.25 mm i.d. x 0.25 μm film thickness) was used with Helium as carrier gas in a constant flow of 1.5 mL min^{-1}.

The GC oven was programmed as follow: 55°C initial hold for 3 min, increase at a rate of 15°C min^{-1} to 180°C, then increase with a step of 6.5°C min^{-1} to 280°C and hold for 5 min followed by an increase of 10°C min^{-1} to 300°C and hold for 5.28 min. The temperature of splitless injector port, GC-MS transfer line and MS source was maintained at 280, 290 and 250°C, respectively.

The QTOF mass spectrometer was automatically calibrated with Perfluorotributylamine (FC43) prior to each injection. MS/MS spectra was generated by an AutoMS/MS acquisition (data dependent acquisition) method in positive ionization mode. With this method a full scan and the MS/MS spectra of the 5 most abundant ions were acquired. A mass range between 30 and 1000 m/z was scanned with a spectra rate of 8 Hz. The same spectra rate used for bbCID mode while GC is coupled with QTOF.

It is used both AutoMS/MS acquisition for the developing of GC-APCI-QTTOF database for target screening and bbCID mode for samples screening and method validation.

Figure 4.1. The UHPLC-QTOF-MS system

4.5 Method Validation

Method Validation was performed for the entire method in both ionization modes for LC-ESI-QTOF while in GC-APCI-QTOF only in positive mode, with requirements which SANTE/11813/2017 defines. The method was validated for 197 pesticides as they are described in Table 4.2 which contains and their

EU MRLs in oranges. The validation levels were 10 μg kg^{-1} and 20 μg kg^{-1}. Validated parameters were:

Linearity was studied for each analyte by analyzing standard solution in six levels 20, 50, 100, 150, 200, 300 μg L^{-1}. Calibration curves were estimated using linear regression.

Limits of Detection and Quantitation for instrument and method were determined theoretically by using the data from regression analysis calculating the standard deviation of intercept from calibration curves and spiked curves respectively. Also, for the analytes in method the LOQ is the level of spike that complete the following parameters: RSD_{rw}<20%, RSD_r<20%, R%=70-120%

Repeatability of instrument and method was determined by comparing RSD_r of analyzing the same standard solution (100 μg L^{-1}) and the same spiked level samples (10 and 20 μg kg^{-1}) respectively.

Reproductivity of the method was determined by RSD_{rw} comparing the same level spiked samples (10 μg kg^{-1}) analyzing in different days.

Truness of the method was determined comparing the recoveries of two spiked levels (10 and 20 μg kg^{-1}).

Matrix effect was determined by comparing the response of analyte between matrix matched samples and standard solution.

Table 4.2: Validated Pesticides with their type, chemical group and EU MRL in oranges.

Pesticides	type	group	MRL (mg kg^{-1}) in orange	Pesticides	type	group	MRL (mg kg^{-1}) in orange
2.4-DDT	Insecticide	Organochlorine	0.05*	Inabenfide	Herbicide, Plant growth regulator	Unclassified	-
4.4-DDD	Insecticide, Metabolite	Organochlorine		Iprodione	Fungicide	Dicarboximide	0.01*
4.4-DDE	Metabolite	Organochlorine		Isodrin	Insecticide	Cyclodiene	-
4.4-DDT	Insecticide	Organochlorine		Isoprothiolane	Fungicide, Plant growth regulator	Phosphorothiolate	0.01*
Acephate	Insecticide	Organophosphate	0.01*	Isoproturon	Herbicide	Urea	0.01*
Acrinathrin	Insecticide, Acaricide	Pyrethroid	0.02*	Isopyrazam	Fungicide	Pyrazole	0.01*
a-HCH	Insecticide, Acaricide, Veterinary substance	Organochlorine	0.01*	Lufenuron	Insecticide, Acaricide, Veterinary substance	Benzoylurea	0.01*
Aldrin	Insecticide	Organochlorine	0.01*	Malaoxon	Metabolite	Organophosphate	-
Ametoctradin	Fungicide	Triazolopyrimidine	0.01*	Malathion	Insecticide, Acaricide, Veterinary substance	Organophosphate	2
Amitraz	Insecticide, Acaricide, Veterinary substance	Amidine	0.05*	Mandipropamid	Fungicide	Mandelamide	0.01*
Atrazine	Herbicide	Triazine	0.05*	MCPA	Herbicide, Metabolite	Aryloxyalkanoic acid	0.05*
Atrazine-desethyl	Metabolite	Triazine	-	Mecoprop	Herbicide	Aryloxyalkanoic acid	0.05*
Azinphos-ethyl	Insecticide, Acaricide	Organophosphate	0.02*	Mephosfolan	Insecticide, Acaricide	Organophosphate	-
Azinphos-methyl	Insecticide, Acaricide, Mollusicide	Organophosphate	0.05*	Mepiquat	Plant growth regulator, Herbicide	uarternary ammonium compou	0.02*
Benfuracarb	Insecticide	Carbamate	0.01*	Mecarbam	Insecticide, Acaricide	Organophosphate	0.01*
Benodanil	Fungicide	Anilide	-	Mesosulfuron methyl	Herbicide	Sulfonylurea	0.01*
b-HCH	Insecticide, Acaricide, Veterinary substance	Organochlorine	0.01*	Mesotrione	Herbicide	Triketone	0.01*
Bifenthrin	Insecticide, Acaricide	Pyrethroid	0.05	Metabenzthiazuron	Herbicide	Urea	-
Bitertanol	Fungicide	Triazole	0.01*	Metaflumizone	Insecticide, Veterinary substance	Semicarbazone	0.05*
Boscalid	Fungicide	Carboxamide	2	Metalaxyl	Fungicide	Phenylamide	0.7
Bromophos-ethyl	Insecticide	Organophosphate	0.01*	Methacrifos	Insecticide, Acaricide	Organophosphate	0.01*
Bromophos-methyl	Insecticide	Organophosphate	-	Methamidophos	Insecticide, Acaricide, Metabolite	Organophosphate	0.01*
Bromopropylate	Acaricide	Benzilate	0.01*	Methidathion	Insecticide, Acaricide	Organophosphate	0.02*
Bromxynil	Herbicide, Metabolite	Hydroxybenzonitrile	0.01*	Methomyl	Insecticide, Acaricide, Metabolite	Carbamate	0.01*

Pesticides	type	group	MRL (mg kg^{-1}) in orange	Pesticides	type	group	MRL (mg kg^{-1}) in orange
Butocarboxim	Insecticide	Carbamate	-	Methoprotryn	Herbicide	Triazine	-
Capropamide	Fungicide	Cyclopropanecarboxamide	-	Methoxychlor	Insecticide, Veterinary substance	Organochlorine	0.01*
Captafol	Fungicide	Phthalimide	0.02*	Methoxyfenozide	Insecticide	Diacylhydrazine	2
Carbaryl	Insecticide, Plant growth regulator	Carbamate	0.01*	Metolachlor	Herbicide	Chloroacetamide	0.05*
Carbendazim	Fungicide, Metabolite	Benzimidazole	0.2	Metribuzin	Herbicide	Triazinone	0.1*
c-HCH-Lindane	Insecticide, Acaricide, Veterinary substance	Organochlorine	0.01*	Monocrotophos	Insecticide, Acaricide	Organophosphate	0.01*
Chlormquat			0.01*	Myclobutanil	Fungicide	Triazole	3
Chlorothalonil	Fungicide	Chloronitrile	0.01*	Nitralin	Herbicide	Dinitroaniline	-
Chlorpyrifos-ethyl (Chlorpyrifos)	Insecticide	Organophosphate	1.5	N-phenylurea	Metabolite	Unclassified	-
Chlorpyrifos-methyl	Insecticide, Acaricide	Organophosphate	2	Omethoate	Insecticide, Acaricide, Metabolite	Organophosphate	0.01*
Chlorfenvinphos	Insecticide, Acaricide, Veterinary substance	Organophosphate	0.01*	o-o DDE	Metabolite	Organochlorine	
cis-Chlordane	Insecticide	Organochlorine	0.01*	o-p DDE	Metabolite	Organochlorine	
Climbazol	Fungicide, Other substance	Conazole	-	Oxamyl	Insecticide, Acaricide, Nematicide	Carbamate	0.01*
Cyanazine	Herbicide	Triazine	-	Paraoxon-ethyl			
Cycloate	Herbicide	Thiocarbamate	-	Paraoxon-methyl			see parathion methyl
Cyflufenamid	Fungicide	Amidoxine	0.02*	Parathion-ethyl	Insecticide, Acaricide	Organophosphate	0.05*
Cyfluthrin	Insecticide	Pyrethroid	0.02*	Parathion-methyl	Insecticide	Organophosphate	0.01*
Cyhalofop-P-butyl	Herbicide	Aryloxyphenoxypropionate	0.02*	Penconazole	Fungicide	Triazole	0.05*
Cyhalothrin	Insecticide, Veterinary substance	Pyrethroid	0.2	Pentabromobenzyl acrylate			-
Cypermethrin	Insecticide, Veterinary substance	Pyrethroid	2	Pentabromoethylbenzene			-
Cyproconezole	Fungicide	Triazole	0.05*	Pentachlorobenzene	Metabolite	Unclassified	-
Cyprodinil	Fungicide	Anilinopyrimidine	0.02*	Perpethrin/PERMETHRIN	Insecticide, Veterinary substance	Pyrethroid	0.05*
Cyromazine	Insecticide, Veterinary substance	Triazine	0.05*	Phosalone	Insecticide, Acaricide	Organophosphate	0.01*
Daminozide	Plant growth regulator	Unclassified	0.06*	Phosmet	Insecticide, Acaricide, Veterinary substance	Organophosphate	0.5
Deet	Insecticide, Repellent	Unclassified		Phosphamidon	Insecticide, Acaricide	Organophosphate	0.01*

Pesticides	type	group	MRL (mg kg^{-1}) in orange	Pesticides	type	group	MRL (mg kg^{-1}) in orange
Deltamethrin	Insecticide, Metabolite, Veterinary substance	Pyrethroid	0.04	Phoxin/Phoxim	Insecticide, Other substance , Veterinary substance	Organophosphate	0.01*
Demeton-S-Me-Sulfon	Insecticide, Acaricide, Metabolite	Organophosphate	0.01*	Picoxystrobin	Fungicide	trobilurin type- methoxyacrylat	0.01*
d-HCH	Insecticide, Acaricide, Veterinary substance	Organochlorine	-	Pinoxaden	Herbicide	Unclassified	0.02*
Diazinon	Insecticide, Acaricide, Repellent, Veterinary substance	Organophosphate	0.01*	Pirimicarb	Insecticide	Carbamate	3
Dichlofluanid	Fungicide	Sulphamide		Pirimiphos-Eth	Insecticide, Acaricide	Organophosphate	-
Dichlorvos	Insecticide, Acaricide, Metabol	Organophosphateite	0.01*	p-p DDA			-
Dicofol	Acaricide	Organochlorine	0.02*	Procymidone	Fungicide	Dicarboximide	0.01*
Dieldrin	Insecticide, Metabolite	Chlorinated hydrocarbon	see aldrin	Prometryn	Herbicide	Triazine	-
Difenacarm (Difenacoum)	Rodenticide	Hydroxycoumarin		Propaquizafop	Herbicide	Aryloxyphenoxypropionate	0.05*
Dimethoate	Insecticide, Acaricide, Metabolite	Organophosphate	0.01*	Propazine	Herbicide	Triazine	-
Diniconazole (Et2)	Fungicide	Triazole	0.01*	Propenofos/PROFENOFOS	Insecticide, Acaricide	Organophosphate	-
Dinitramin	Herbicide	Dinitroaniline	-	Propham	Herbicide, Plant growth regulator	Carbamate	0.01*
Dinoseb	Herbicide	Dinitrophenol	0.02*	Propoxycarbazone sodium	Herbicide	Triazolone	0.02*
Dinoterb	Herbicide	Dinitrophenol	0.01*	Prothiofos	Insecticide	Organophosphate	-
Disulfoton	Insecticide, Acaricide	Organophosphate	0.01*	Pyridaben	Insecticide, Acaricide	Pyridazinone	0.5
Endosulfan alpha	Insecticide, Acaricide	Organochlorine	0.05*	Pyridalyl	Insecticide	Pyridalyl	0.01*
Endosulfan sulphate	Metabolite	Unclassified		Pyrifenox	Fungicide	Pyridine	-
Endrin	Insecticide, Avicide, Rodenticide	Organochlorine	0.01*	Pirimiphos-methyl	Insecticide, Acaricide	Organophosphate	0.01*
Ethafluralin	Herbicide	Dinitroaniline	0.01*	Pyroxsulam	Herbicide, Graminicide	Triazolopyrimidine	0.01*
Ethiofencarb	Insecticide	Carbamate	-	Quinalphos	Insecticide, Acaricide	Organophosphate	0.01*
Ethion	Insecticide, Acaricide, Metabolite	Organophosphate	0.01*	Quinoxyfen	Fungicide	Quinoline	0.02*
Ethoprophos	Insecticide, Nematicide	Organophosphate	0.02*	Quintozene	Fungicide	Chlorophenyl	0.02*
Etrimfos	Insecticide	Organophosphate	-	Quizalofop-P	Metabolite	Aryloxyphenoxypropionate	0.05*
Fenamiphos	Nematicide	Organophosphate	0.02*	Quizalofop-P-ethyl	Herbicide	Aryloxyphenoxypropionate	-
Fenarival (Fenarimol)	Fungicide	Pyrimidine	0.02*	S18-Spirotetramate Met cis enol			see spirotetramate
Fenitrothion	Insecticide	Organophosphate	0.01*	19-Sprirotetramate Met cis-Ketohydroxy			see spirotetramate
Fenoxycarb	Insecticide	Carbamate	2	S20-Spirotetramate Met monohydroxy			see spirotetramate
Fenoxaprop-P-ethyl	Herbicide	Aryloxyphenoxypropionate	-	Simazin (CH2Cl2)	Herbicide	Triazine	0.01*

Pesticides	type	group	MRL (mg kg^{-1}) in orange	Pesticides	type	group	MRL (mg kg^{-1}) in orange
Fenthion	Insecticide, Veterinary substance, Avicide	Organophosphate	0.01*	Simeconazole	Fungicide	Conazole	-
Fenthion sulfoxide	Insecticide, Metabolite	Organophosphate	SEE FENTHION	Spinosad	Insecticide	Micro-organism derived	0.3
Fenvalerate	Insecticide, Acaricide, Termiticide, Veterinary substance	Pyrethroid	0.02*	Spirotetramat	Insecticide	Tetramic acid	1
Flamprop (vial name)/Flamprop-methyl (stock solution name)	Herbicide, metabolite	Aryaminopropionic acid	-	Spiroxamine	Fungicide	Morpholine	0.01*
Fluazuron	Acarcide, Insecticide, Ioxodicide	Benzoylphenyl urea	-	Sulfentrazone	Herbicide	Aryl triazolinone	-
Flubendiamide	Insecticide	Benzene-dicarboxamide	0.01*	TCMTB (Busan)	Fungicide, Microbiocide, Other substance	Mercaptobenzothiazole	-
Fludioxonil	Fungicide	Phenylpyrrole	10	Tebuconazole	Fungicide, Plant growth regulator	Triazole	0.9
Flufenoxuron	Insecticide, Acaricide	Benzoylurea	0.3	Tepraloxydim	Herbicide	Cyclohexadione	0.1*
Flumethrin	Acaricide, Insecticide, Sheep dip, Ectoparasiticide	Pyrethroid	-	Terbufos	Insecticide, Nematicide	Organophosphate	0.01*
Fluthiacet-methyl	Herbicide	Imine	-	Terbuthylazine	Herbicide, Microbiocide, Algicide	Triazine	0.1
Folpet	Fungicide	Phthalimide	0.03*	Tetradifon	Acaricide, Insecticide	Bridged diphenyl	0.01*
Foramsulfuron	Herbicide	Pyrimidinylsulfonylurea	0.01*	Tetrachlorvinphos	Insecticide, Acaricide, Veterinary substance	Organophosphate	-
Furmecyclox	Fungicide, Other substance	Furamide	-	Tetrasul	Acaricide, Insecticide, Nematicide	Bridged diphenyl	-
Glyphosate	Herbicide	Phosphonoglycine	0.5	Thiacloprid	Insecticide, Molluscicide	Neonicotinoid	0.01*
Halosulfuron methyl	Herbicide	Pyrazolium	0.01*	trans-Chlordane	Insecticide	Organochlorine	see cis chlordane
Haloxyfop	Herbicide, Metabolite	Aryloxyphenoxypropionate	0.01*	Trichlorfon (Dylox)	Insecticide, Veterinary substance	Organophosphate	0.01*
Haloxyfop-ethoxyethyl	Herbicide	Aryloxyphenoxypropionate		Trifluralin	Herbicide	Dinitroaniline	0.01*
Haloxyfop-methyl	Herbicide	Aryloxyphenoxypropionate		Triadimefon	Fungicide, Metabolite	Triazole	0.01*
Heptachlor	Insecticide	Organochlorine	0.01*	Tritosulfuron	Herbicide	Sulfonylurea	0.01*
Heptachlor Epoxide	Metabolite	Unclassified		Lambda-cyhalothrin	Insecticide	Pyrethroid	0.2
Hexachlorobenzene	Fungicide, Biocide, Metabolite, Wood preservative	Chlorinated hydrocarbon	0.01*	Pyriproxyfen	Insecticide, Veterinary substance	Unclassified	0.6
Hexachlorobutadiene			-				
Imidacloprid	Insecticide, Veterinary substance	Neonicotinoid	1				

* Indicates lower limit of analytical determination

4.6 Target screening of Orange Samples

After the analysis of both validation set and real samples, in LC-ESI-QTOF and GC-APCI-QTOF systems, the raw data were processed by Bruker TASQ Client 2.1. In TASQ method, the Extracted Ion Chromatogram for each analyte that belongs to database was created with a mass error window of 0.005Da.

LC database for pesticide screening for positive ionization mode of ESI consists of 942 compounds. For negative ionization mode of ESI the database has 321 analytes.

The data from real samples in LC were processed with methods that concudes whole databases in positive and negative ionization, while for validated samples was created a method that consists of the pesticides that have been used in the validation procedure.

4.7 Development of a GC-APCI-QTOF database

Although for LC data there were databases for target screening of pesticides in GC data there was not any database. For this reason we created a virtual database for GC-APCI-QTOF system for positive ionization mode of APCI. This database contains analytes of different groups of compounds that can be analysed by GC-APCI-QTOF, for instance pesticides, PCBs, PCNs, PBDEs, dioxins etc. For this reason mix of standards were prepared and analysed with GC-APCI-QTOF in data dependent acquisition mode AutoMS/MS which gives the information of the MS spectra and MS/MS spectra of five most abundant ions each time. The processing of raw data was carried out by software Data Analysis 5.0.

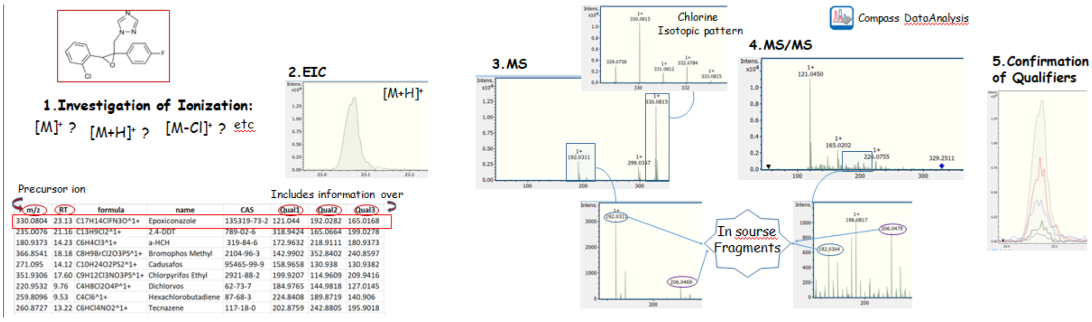
The main workflow (Figure 4.2) composed of the following steps:

Calibration
Investigation of Ionization

Extracted Ion Chromatogram - determination of Rt

MS and MS/MS spectra processing – determination of precursor and qualifier ions while information about in source fragmentation.

The final database has information for CAS number, the molecular formula precursor ion and qualifier ions and their formulas of each compound.



m/z	RT	formula	name	CAS	Qual1	Qual2	Qual3
330.0804	23.13	C17H14ClFN3O^1+	Epoxiconazole	135319-73-2	121.044	192.0282	165.0168
235.0076	21.16	C13H9Cl2^1+	2,4-DDT	789-02-6	318.9424	165.0664	199.0278
180.9373	14.23	C6H4Cl3^1+	a-HCH	319-84-6	172.9632	218.9111	180.9373
366.8541	18.18	C8H9BrCl2O3PS^1+	Bromophos Methyl	2104-96-3	142.9902	352.8402	240.8597
271.095	14.12	C10H24O2PS2^1+	Cadusafos	95465-99-9	158.9658	130.938	130.9382
351.9306	17.60	C9H12Cl3NO3PS^1+	Chlorpyrifos Ethyl	2921-88-2	199.9207	114.9609	209.9416
220.9532	9.76	C4H8Cl2O4P^1+	Dichlorvos	62-73-7	184.9765	144.9818	127.0145
259.8096	9.53	C4Cl6^1+	Hexachlorobutadiene	87-68-3	224.8408	189.8719	140.906
260.8727	13.22	C6HCl4NO2^1+	Tecnazene	117-18-0	202.8759	242.8805	195.9018

Figure 4.2: General workflow of developing the database.

4.8 Qualification

The qualification of pesticides in samples was the criteria as SANTE/11813/2017 determines for GC-APCI-QTOF and LC-ESI-QTOF analysis:

Retention time of analyte must be below 0.1min in sample in comparison with retention time of the same analyte in standard solution.

At least 2 ions of which one is the molecular ion, or protonated or deprotonated molecular ion and the second is a qualifier fragment ion with mass accuracy below 5ppm. These extraxted ions chromatograms must be fully overlap.

As a result, TASQ method parameters were:

Retention Time difference: 0-0.2 min (because in samples matrix may change retention time)

Mass accuracy: < 5 mDa

mSigma: a term for isotopping fitting of a compound due to its molecular formula : <200.

An identification example of Chlorpyrifos in orange extract is shown in figure 4.3.

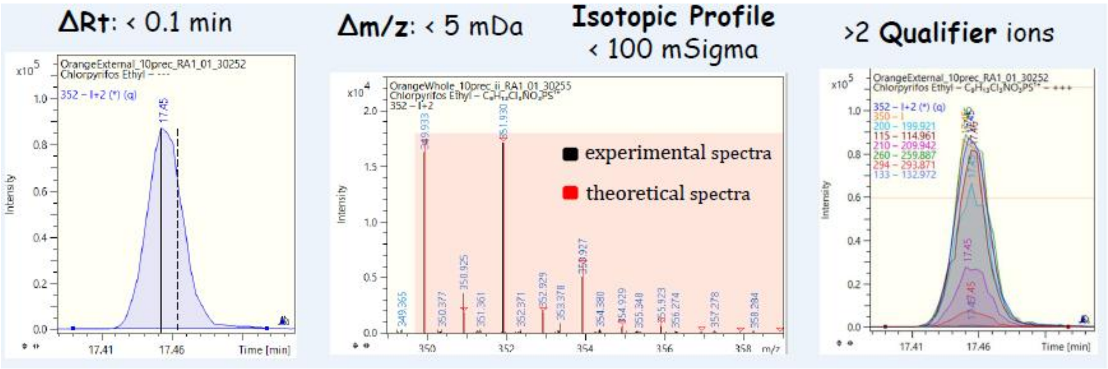


Figure 4.3: Chlorpyrifos peak with its isotopic profile and qualifier ions.

4.9 Quantification

Quantification of the analytes based on procedural standard calibration method. This approach can compensate for matrix effects and low extraction recoveries associated with certain pesticide. It is only applicable when a series of samples of the same type, in our method oranges, are to be processed within the same batch. Procedural standards prepared by spiking a series of blank test portions with different amounts of analytes, prior to extraction. The procedural standards, then, analysed in exactly the same way as the samples.

Procedural standards calibration curves, that resulted, are used with the peak areas of the pesticide in positive samples for the estimation of the amount of each compound.

CHAPTER 5
Results and Discussion

5.1 General Observations

After the analysis of tests samples in GC-APCI QTOF, it is decided that the sample preparation will be applied with a sample mass of 5 g of orange with use of 5 mL ACN (1% CH_3COOH). The peak areas between the sample masses tests of 15g and 5g of oranges had no statistical differences. As a result, it is chosen a more environmentally friendly procedure due to the fact that the reagents used are one third of the initially supposed to be used.

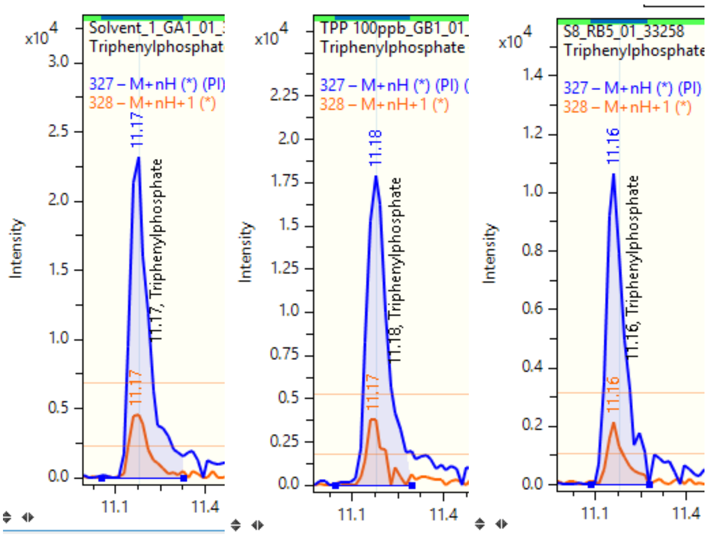
Furthermore, it is decided in sample preparation to have a final preconcentration by a factor of ten than not to have. In the preconcentrated sample case all the spiked analytes (10 μg kg^{-1}) were detected on the final test sample. On the other hand, not having a final preconcentration many compounds that were spiked not detected and the peak areas of detected compounds were less than the peak areas of the same analytes in the test with the preconstitution.

Finally, the final reconstitution for GC-APCI-QTOF analylis selected to be the solution hexane:acetone (50:50). In this case polar and not polar analytes detected. For reconstitution with hexane many polar compounds not detected while, in the reconstitution with acetone non polar compounds not detected or their peak areas were too low.

5.2 Internal Standard

The internal standard method is used to improve the accuracy of a quantification. Because isotopically labeled internal standards had not been purchased, it is decided to use Triphenyl Phosphate (TPP) as internal standard for both GC-APCI-QTOF and LC-ESI-QTOF analyses due to the fact that TPP can be identified in positive ionization mode for both analyses.

However, in LC-ESI-QTOF observed that TPP had contaminated the system figure 5.1 as in solvent samples (not TPP spiked) there was peak areas for TPP that agreed in all identification criteria.Furthermore the carry-over was much more than the amount that we have spiked in the sample.



i ii iii

Figure 5.1: Peak of TPP in i) a solvent injection (not spiked with TPP), ii)standard of TPP of 100 μg L^{-1} iii)sample spiked with TPP of 10 μg kg^{-1} initial concentration.

As a result TPP was used only for GC-APCI-QTOF analysis. To confirm that TPP can be used correctly as internal standard for the whole list of analytes we investigate the improvement of linearity, recovery and repeatability in both cases using TPP and not using it.

In linearity test, it is compared the coefficient of determination of each analyte calibration curve for levels 20-300 μg L^{-1}. In first occasion for not using internal standard, it is used the absolute areas of analytes and in the occasion of using TPP used the relative areas calculating by the below equation.

$$Relative\ Area = \frac{Absolute\ Area\ of\ Analyte}{Area\ of\ TPP}$$

The result was that all the analytes improved the coefficient of determination in their curves while using the relative areas. The comparison is given in figure

5.2. Without using TPP the percentage of analytes had coefficient of determination below 0.9 was 49%.

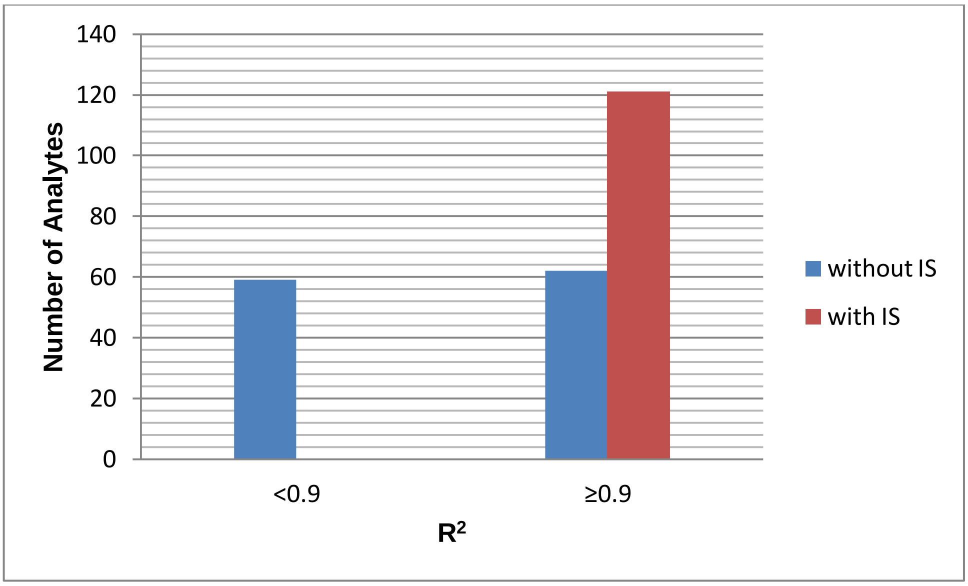


Figure 5.2: Comparison of coefficient of determinatioon while using IS or not GC-APCI-QTOF.

In repeatability test, it is compared the relative standard deviation (RSD_r) for level 10 µg Kg^{-1} spiked sample. The majority of the analytes's RSD_r improved as it can be seen at figure 5.3. While there is a percentage of 17% of the analytes that even with the use of TPP, still have RSD_r above 20% as SANTE 11813/2017 defines as limit.

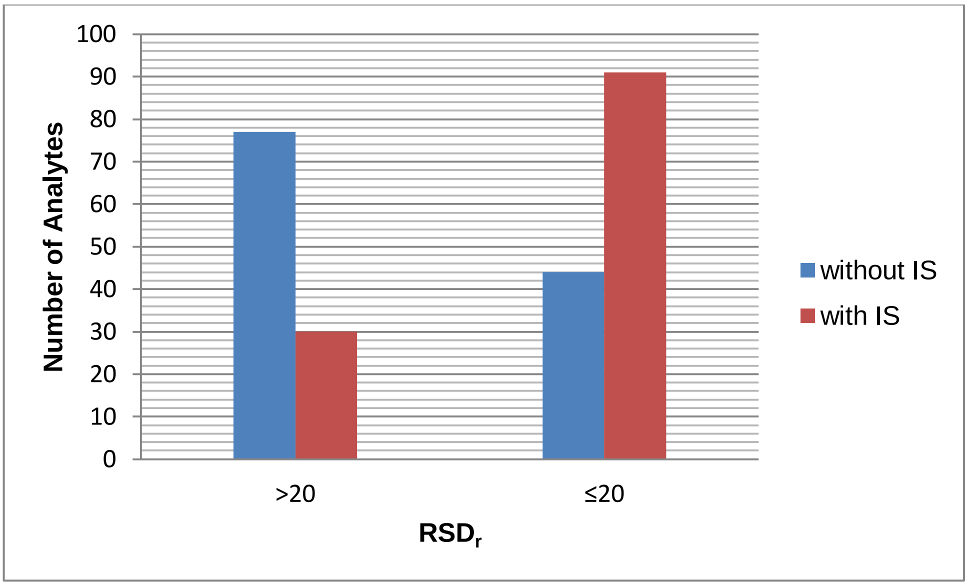


Figure 5.3: Comparison of relative standard deviation while using IS or not in GC-APCI-QTOF.

Finally, in recovery tests, it is compared how recoveries were improved in level 10 µg kg^{-1} spiked sample. The use of internal standard TPP increases the recoveries of all analytes. However, not using IS the percentage of recoveries above 140% is only 3% of the analytes while using TPP it is increased to 13%. Furthermore, the majority of analytes (87%) have recoveries between 30-140% which is acceptable according to SANTE 11813/2017. Without using TPP only 54% of analytes have acceptable recoveries.

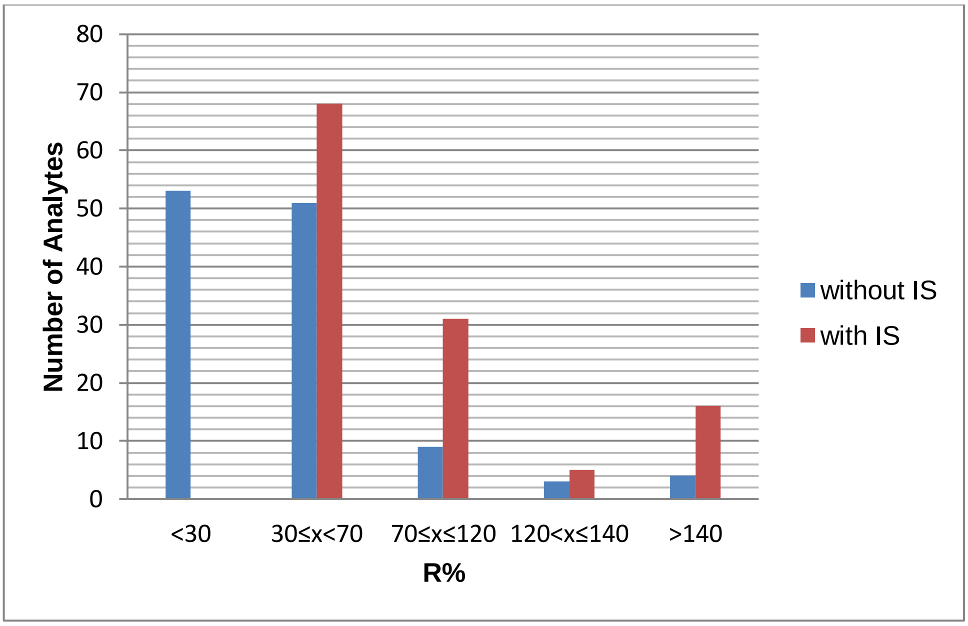


Figure 5.4: Comparison of percentages of recoveries while using IS or not in GC-APCI-QTOF.

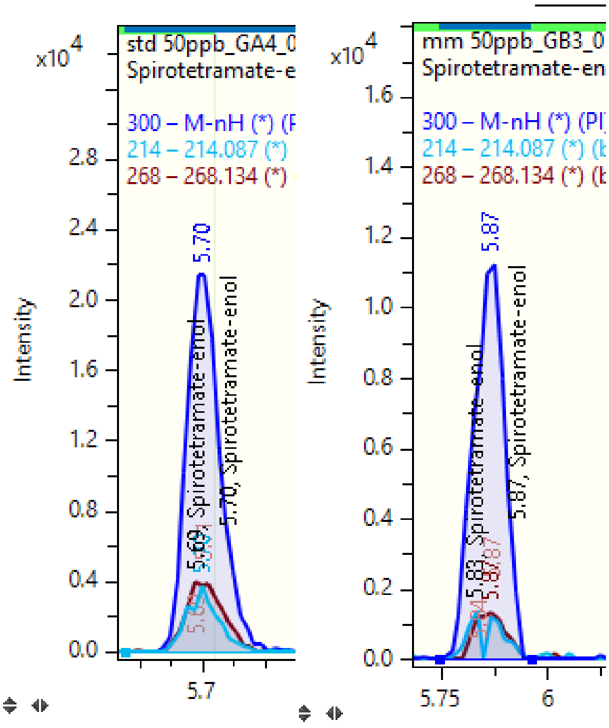
To conclude, TPP can be used as internal standard in GC-APCI-QTOF analysis because improves the criteria of recovery and repeatability for the majority of the analytes and linearity for all analytes.

5.3 Method Validation

In this study, the determination of 197 pesticides was investigated by a common sample preparation based on QuEChERS AOAC method and analysis in both positive and negative ionization mode for LC-ESI-QTOF and positive mode for GC-APCI-QTOF. From the statistical data obtained from the validation of the methods it is deduced the existence of a number of compounds, different for each method, for which it was impossible to determine. Validation was carried out according to SANTE11813/2017 as it expressed in chapter 4.5.

5.3.1 Retention Time (Rt)

Retention times of the analytes in the extracts produced by the application of each method were compared to those obtained from the calibration standards without exceeding the tolerance required by the legislation (0.1min) for most of the analytes for all methods. However, Spirotetramate-enol in negative ionization with LC-ESI-QTOF has a difference in retention time of 0.17min between calibration standard and matrix matched sample (figure 5.5).



i ii

Figure 5.5: Chromatograms of Spirotetramate-enol in i) Calibration standard of 50 µg L^{-1} ii) in matrix matched standard of 50 µg L^{-1}, in negative ionization of LC-ESI QTOF.

We can assure that both chromatograms belong to Spirotetramate-enol because all the other qualification crireria are applied.

5.3.2 Linearity of Calibration Curves

The methods were tested for linearity by preparing six standard calibration solutions of 20, 50, 100, 150, 200 and 300 µg L^{-1} for each analyte (Figure

5.6). These standards were made in solvent, the respective solution for each method. For LC-ESI-QTOF it is used MeOH:H_2O (50:50) while for GC-APCI-QTOF it is used Hexane:Acetone (50:50).

The regression lines were determined by the least squares method, according to the following equation:

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

y: Peak area of each analyte

a: the intercept

b: the slope

C: concentration of standard analyte

S_a: Standard deviation of intercept

S_a: Standard deviation of slope

The coefficients of determination (R^2) for all calibration curves were also calculated. The values of all the above parameters are listed in Tables AI.1, AI.2, AI.3 in Annex I. Figure 5.7 summarizes the number of analytes according to their coefficient of determination.

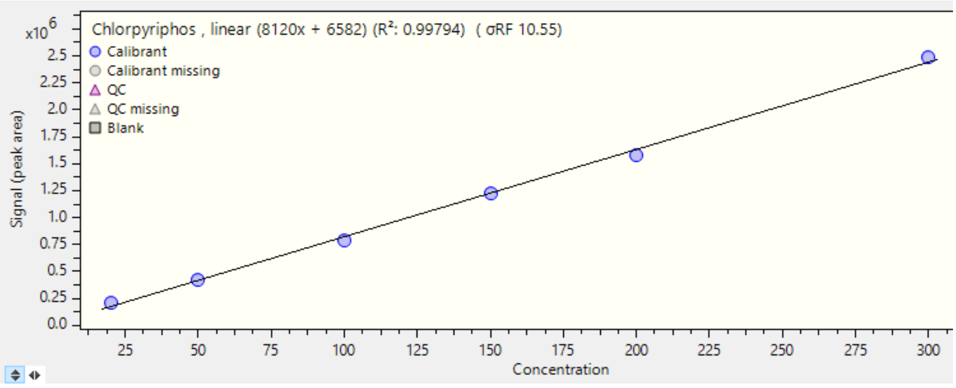


Figure 5.6: Calibration curve of Chlorpyrifos in positive ionization LC-ESI-QTOF

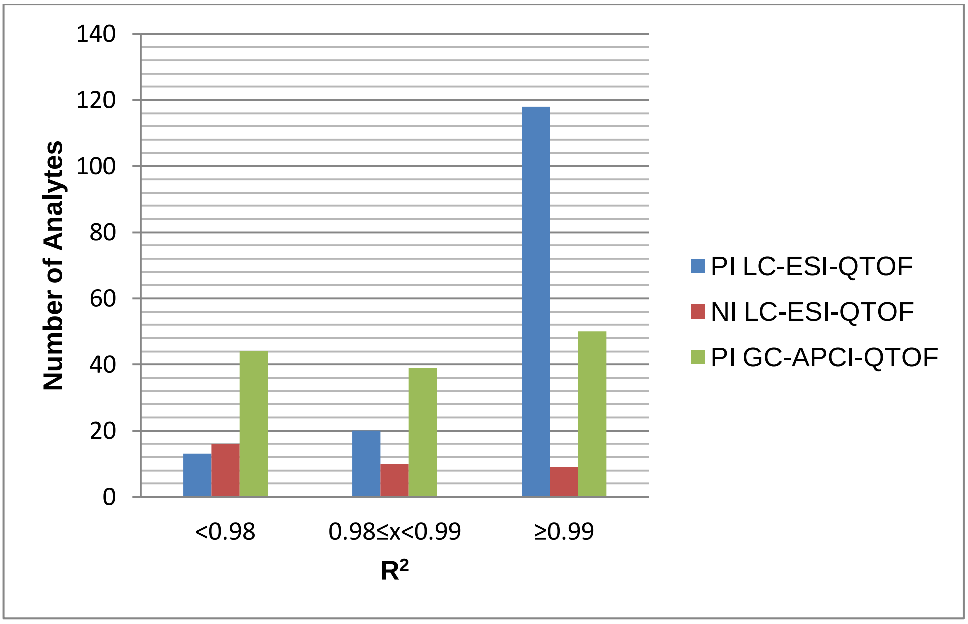


Figure 5.7: Coefficient of determination of analytes' calibration curves in all methods of analysis.

LC-ESI-QTOF analysis with positive ionization has better linearity in comparison with negative ionization and positive ionization with GC-APCI-QTOF analysis because 91% of pesticides have coefficient of determination above 0.98 while 55% and 67%, respectively.

Common analytes for positive and negative ionization with LC-ESI-QTOF were compared in order to understand which ionization mode is more preferable. For this reason we compared the slopes of calibration curves for these analytes (Figure 5.8). The slope determines the sensitivity of each method for the analyte. The results are that 60% of the analytes are more sensitive in positive ionization mode while the other 40% in negative ionization mode. A potential explanation why this is happening is the structure of each analyte. Some of the analytes are proton acceptors (more sensitive in positive ionization) while others are proton donors (more sensitive in negative ionization).

Moreover, common analytes in positive ionization mode for both LC-ESI-QTOF and GC-APCI-QTOF were compared. In this situation we cannot use the slopes of calibration curves as in GC analysis the slopes calculating using IS while in LC analysis without using IS. For this reason, as a try of comparison we use the mean of absolute areas of standard of 100 μg L^{-1} that had been analysed three times for both LC-ESI-QTOF and GC-APCI-QTOF (Figure 5.9). The majority of the analytes have higher absolute areas in LC analysis. This may be due to the solubility of the analytes in reconstitution solution because more polar pesticides will have better solubility in LC MeOH:H_2O 50:50 than in GC analysis Acetone:Hexane 50:50. Moreover, the amount of analye on column is different between LC and GC analysis. In LC analysis 5 μL are injected on column while in GC analysis 1 μL. As a result for the 100 μg L^{-1} standard in LC analysis it will be 500 pg on column per analyte while in GC analysis 100 pg on column.

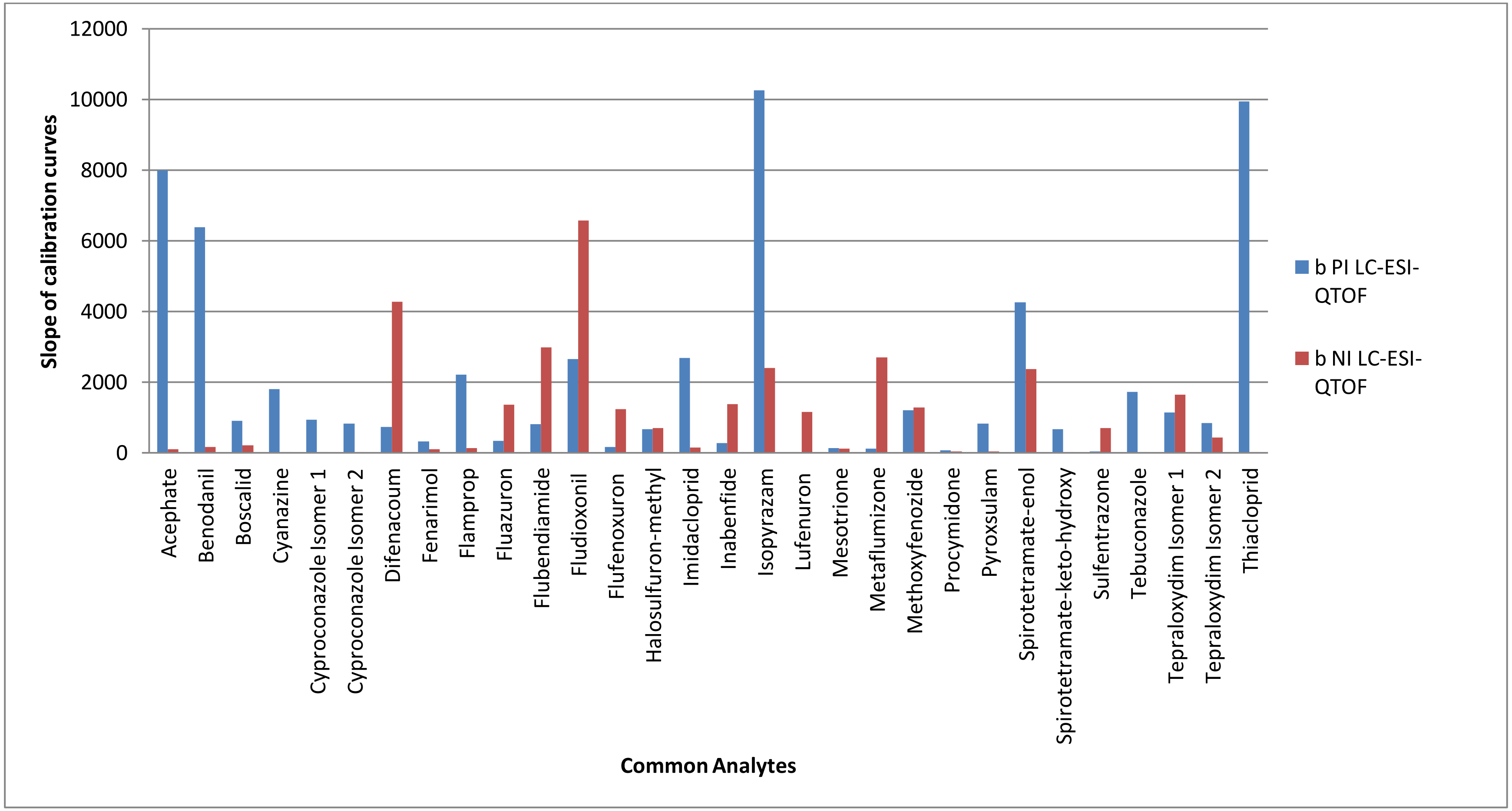


Figure 5.8: Comparison of the slopes of Calibration curves in common analytes for positive and negative ionization with LC-ESI-QTOF.

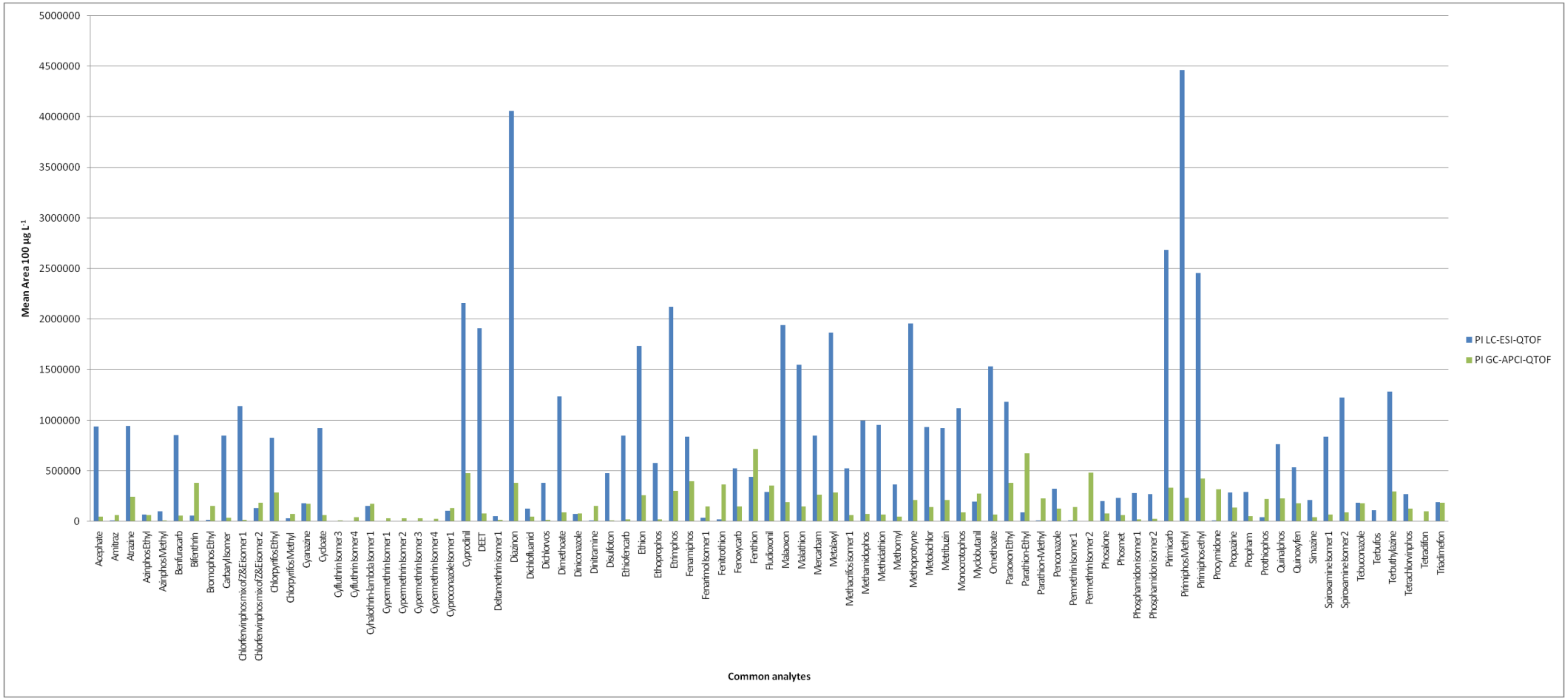


Figure 5.9: Comparison of the mean absolute areas of 100 µg L^{-1} in common analytes for positive ionization with LC-ESI-QTOF and GC-APCI-QTOF.

5.3.3 Matrix Effects

Orange is a complex matrix because during the extraction procedure not only pesticides are extracted but also other components of it. These components increase the noise in the instrumental analysis techniques. In following figure 5.10, are the the base peak chromatograms of a blank sample (orange) in the three method of analysis we used.

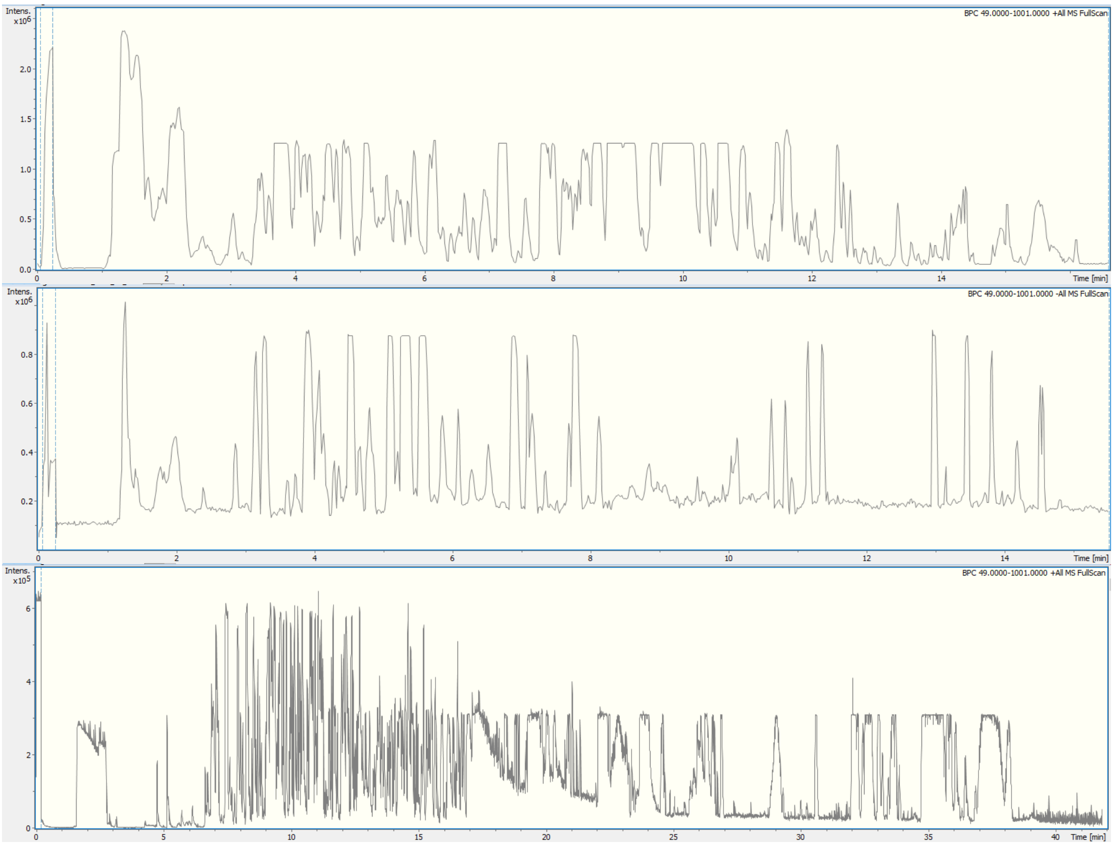


Figure 5.10: Base peak chromatograms of a blank sample from positive and negative ionization with LC-ESI-QTOF and positive ionization with GC-APCI-QTOF (from above).

For evaluation of the effect of matrix effect, matrix matched calibration curves were made in extracts of each method for six levels: 20, 50, 100, 150, 200, and 300 μg L^{-1}, as in calibration curves. Matrix matched standards prepared using the extracts of the blank sample. The appropriate amount of standard for each concentration was evaporated. Then, it is reconstituted with extracts of blank sample. The phenomenon of matrix effect was expressed with the percentage increase or decrease of the signal, ME%, based on the formula:

$$ME\% = 100 \times (\frac{b_m}{b_s} - 1)$$

In which,

b_m: slope of matrix matched curve for an analyte

b_s: slope of calibration curve for an analyte

The matrix effect of the analytes may be considered to be strong when ME% <-50% or ME%> +50%, moderate when -50% <ME% <-20% or + 20% <ME% <+ 50% and weak when -20% <ME% <+20%. The results of the study on the matrix effect of the analytes appear in figure 5.11.

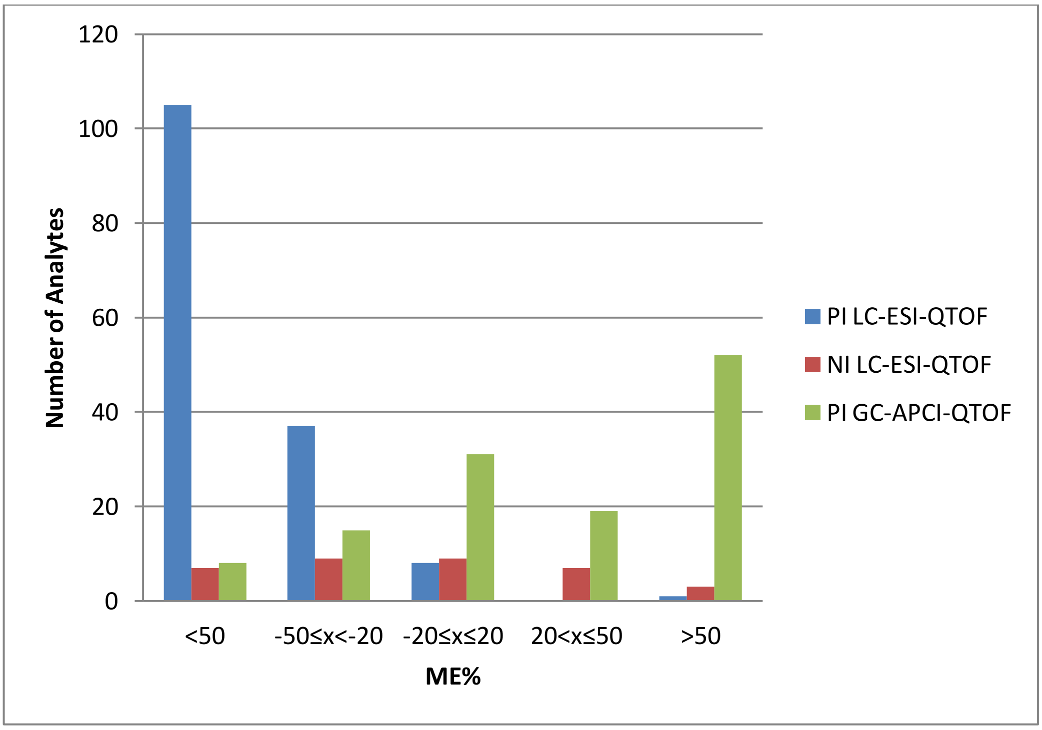


Figure 5.11: Matrix effects of analytes in all methods of analysis

It can be observed that with positive electrospray ionization most of the analytes have matrix effects below 50%. So, matrix induced signal suppression for the majority of the analytes. This may occur because in the source there are many other components of the sample except of pesticides that can be ionized. As a result many molecules of pesticides cannot be ionized in samples while in calibration standards without matrix components more molecules of pesticides are ionized. On the other hand in positive ionization with GC-APCI-QTOF the majority of analytes have matrix effects above 50%. In this case matrix induced signal enhancement for most of the analytes. This phenomenon occurs because components of matrix mask the active sites of GC leading more molecules of analytes in the detector.

5.3.4 Method Accuracy

The accuracy was checked by measuring the recovery for each plant protection product after sample preparation of the spiked samples at two concentration levels, 10 μg L^{-1} and 20 μg L^{-1}. Methods were applied a total of 18 times for each spiking level (six times for each spiking level for three working days).

In this work the recovery for all methods applied is given by the equation:

$$\%R = \frac{Peak\ area\ of\ spiked\ sample}{Peak\ area\ of\ matrix\ matched\ sample} \times 100$$

The results of recoveries are summarized in the figures 5.12–13 for both 10 and 20 μg kg^{-1} levels.

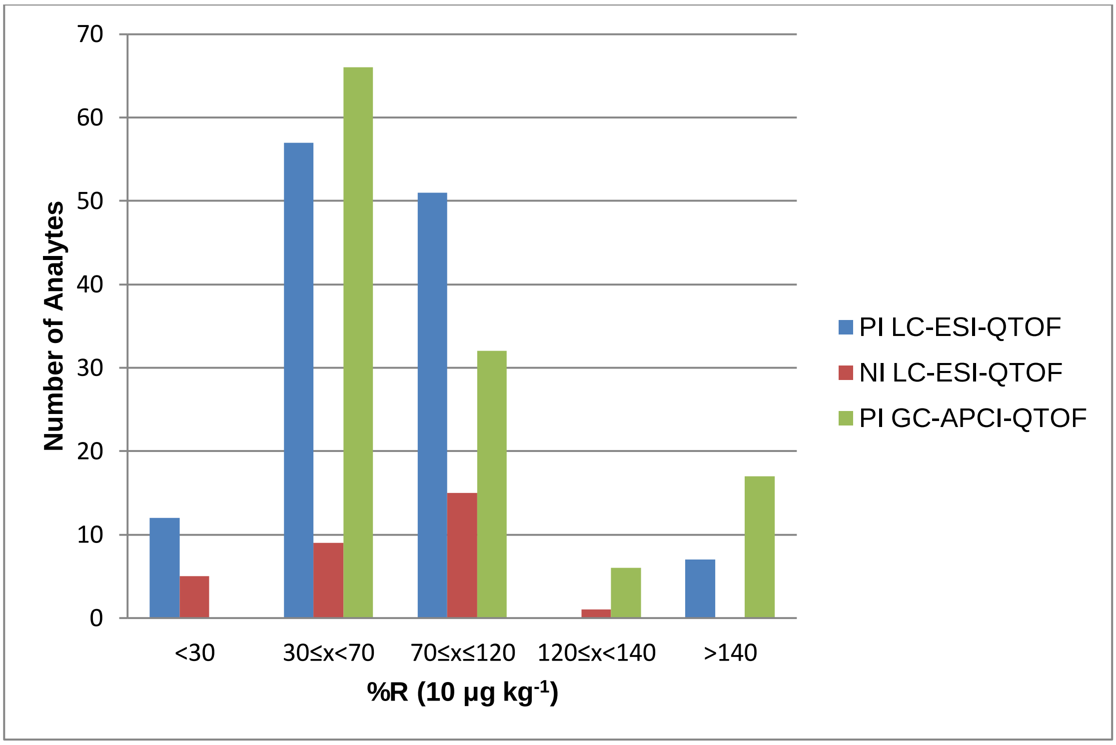


Figure 5.12: Recoveries of the analytes for all methods of analysis for the level of 10 µg kg^{-1}.

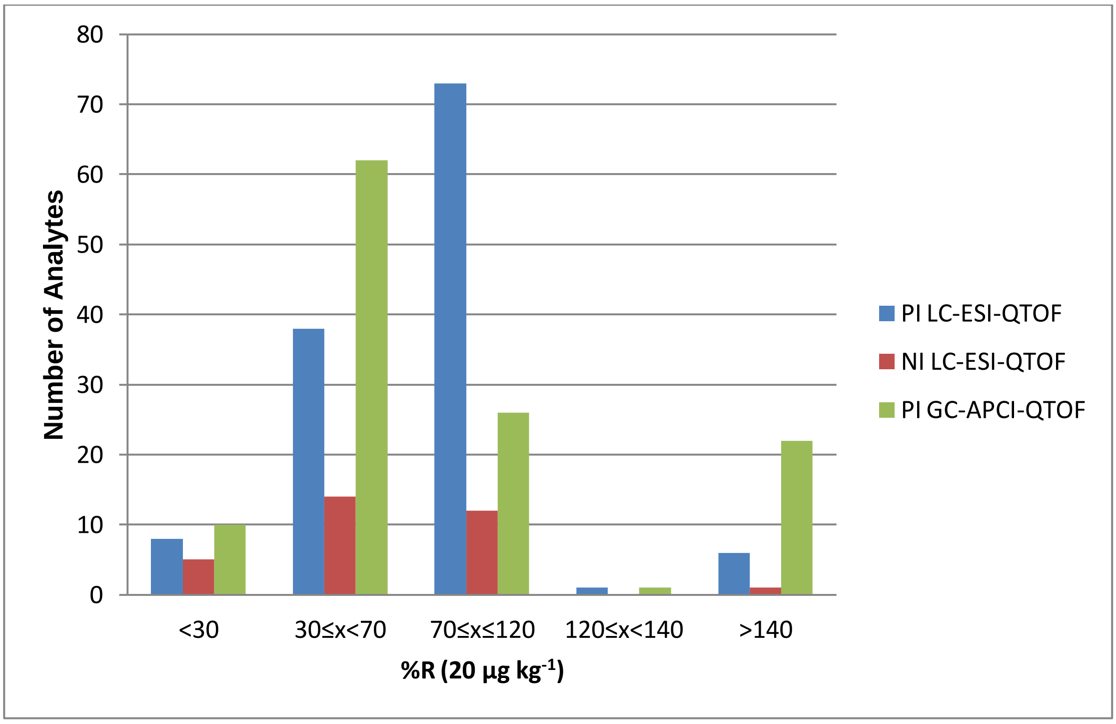


Figure 5.13: Recoveries of the analytes for all methods of analysis for the level of 20 µg kg^{-1}.

According to SANTE, 70% to 120% are acceptable recoveries. However, recoveries from 30-70% and 120%-140% can be accepted only if their RSD are below 20%. As it can be seen, the majority of the analytes have recoveries in the categories of 30-140 %. More specifically, for positive ionization with LC-ESI-QTOF, 85% and 89% of the analytes have recoveries from 30% to 140% in levels 10 and 20 μg kg^{-1}, respectively. In the same way the percentages od the analytes with the negative ionization with LC-ESI-QTOF were 83% and 83%. For positive ionization with GC-APCI-QTOF were 86% and 73%. The reason why the recoveries are not in 70-120% may be,except of the difficulty of analyte extraction from the matrix of oranges, the part of evaporation until dryness at the sample preparation. The more volatile analytes (available for GC analysis) are more vulnerable to evaporate part of them.

5.3.5 Limits of Detection and Quantification

Limit of Detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level. Limit of Quantification (LOQ) is the lowest concentration of the analyte that has been validated with acceptablele accuracy by applying the complete analytical method. In this thesis, we estimated the LOD and LOQ for the instrument (ILOD) and method (MLOD). For the estimation we used the below equations:

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

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

In which,

S_a: Standard deviation of intercept

b: the slope of curve

For ILODs S_a and b were from calibration curves while in MLODS were from procedural standard calibration curves. The results of ILODs and ILOQs are in Tables AI.1, AI.2, AI.3 and for MLODs, MLOQs Tables AI.4, AI.5, AI.6 in Annex I for all the methods of analysis.

In figures 5.14-5.17 are categorized the LODs and LOQs for instrument and method.

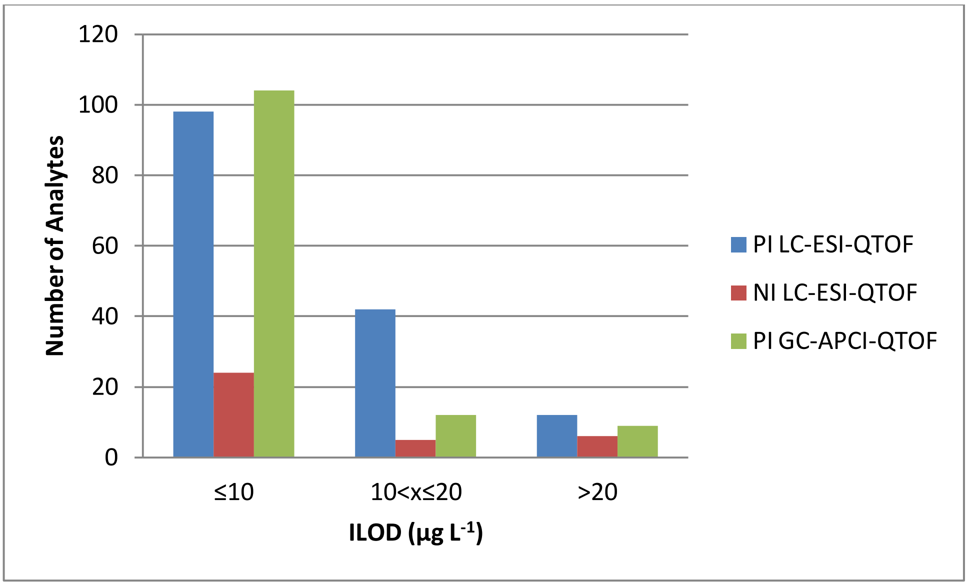


Figure 5.14 : ILODs for the analytes for all methods of analysis.

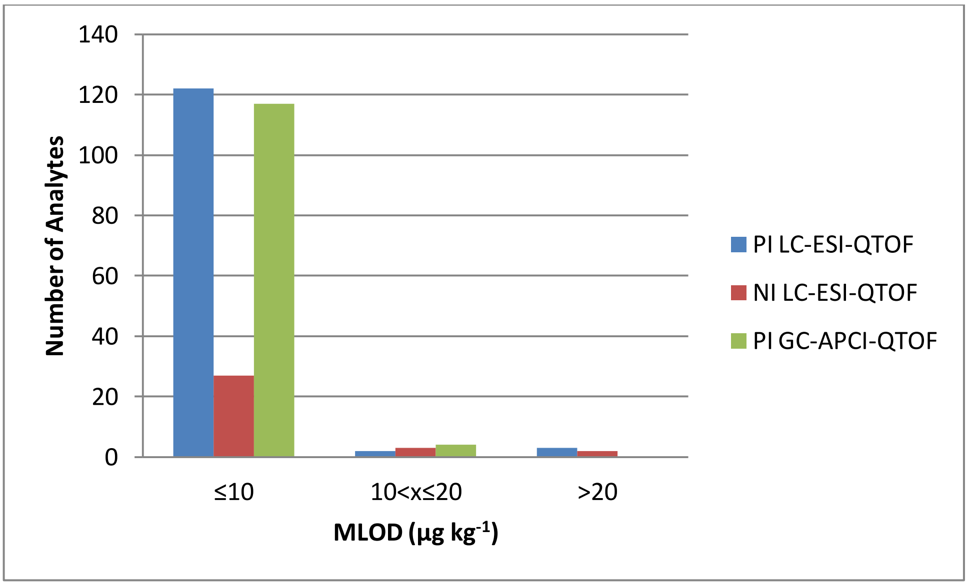


Figure 5.15 : MLODs for the analytes for all methods of analysis.

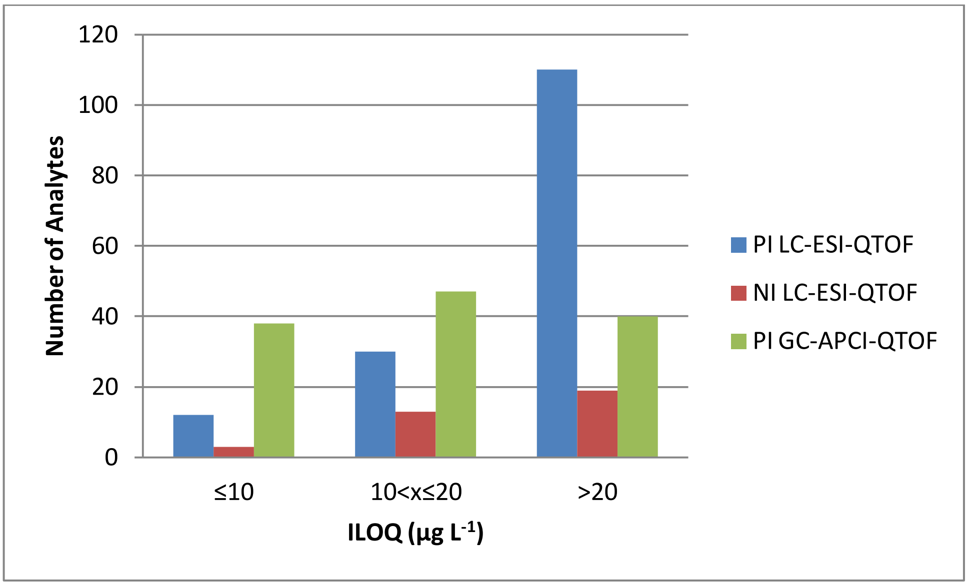


Figure 5.16 : ILOQs for the analytes for all methods of analysis.

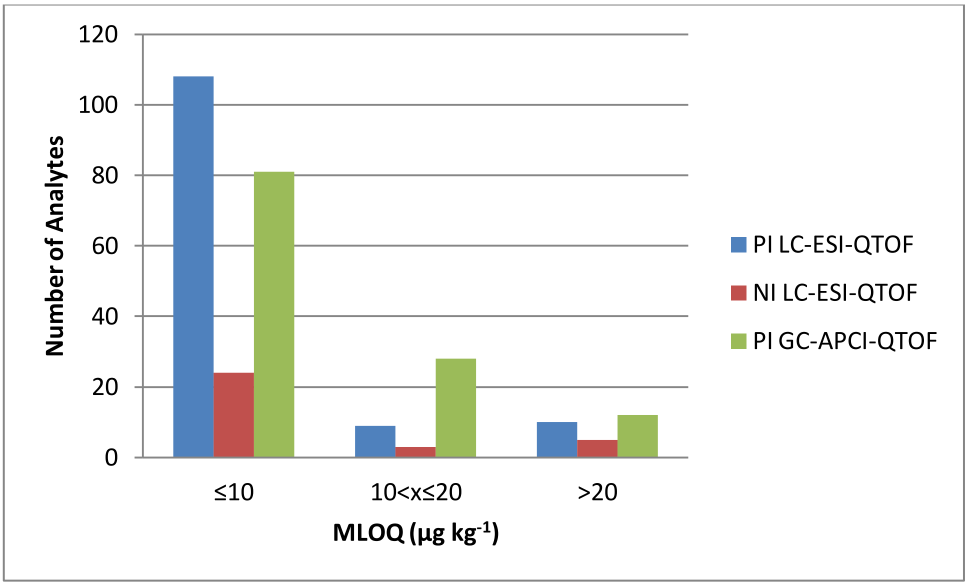


Figure 5.17 : MLOQs for the analytes for all methods of analysis.

As it can be concluded, for all methods of analysis the majority of analytes have ILODs below 10 μg kg^{-1}. However, ILOQs for many analytes are above 20 μg kg^{-1}. In pesticide residues analysis must be equipped with methods that can detect at least 10 μg kg^{-1} residues of pesticides in samples and this would be difficult because we have to confront with lower than 100% recoveries in most of the cases. The solution to this problem was the final preconcentration by the factor of ten in sample preparation. We managed to have a method that has MLODs below 10 μg kg^{-1}. More specifically, the MLOQs below 10 μg kg^{-1} of the analytes were 85%, 75%, 67% for the analytes in positive and negative ionization with LC-ESI-QTOF and positive ionization with GC-APCI-QTOF,respectively. In the same way, above 20 μg kg^{-1} the percentages of the analytes were 8%, 16% and 10% respectively. Thus, the majority of the analytes have MLODs and MLOQs below 10 μg kg^{-1} which is the general MRL for pesticides.

5.3.6 Precision

Precision is a combination of Repeatability and Intra-laboratory Reproducibility

5.3.6.1 Repeatability

According to the SANTE/11813/2017 Directive, method repeatability can be estimated by the relative standard deviation (% RSD_r) of recovering of six repetitive measurements at each spike level on the same day. In Figures 5.18-19 are the RSD_r of all analytes in two levels 10 and 20 μg kg^{-1}.

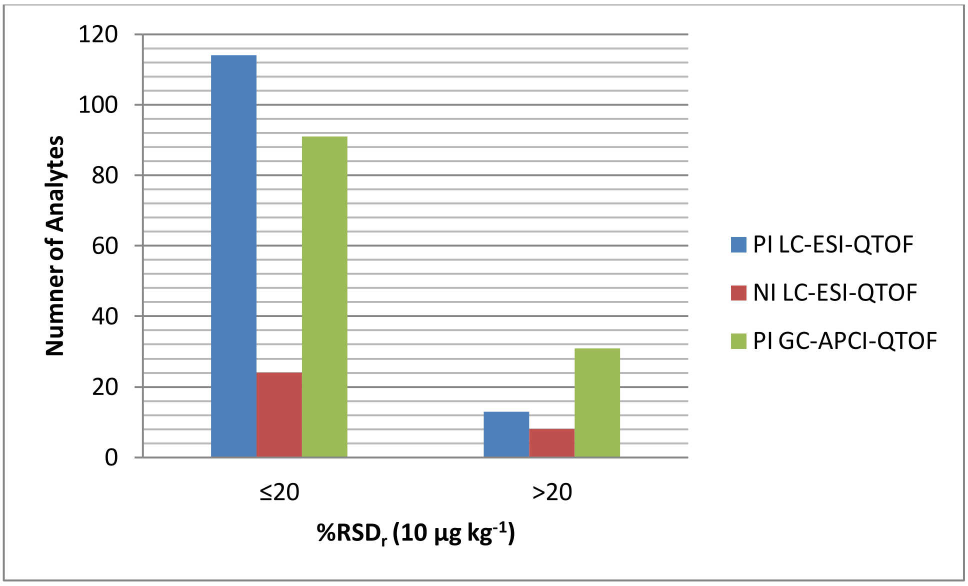


Figure 5.18: Distribution of RSD_r of all analytes in level of 10μg kg^{-1} for all methods of analysis.

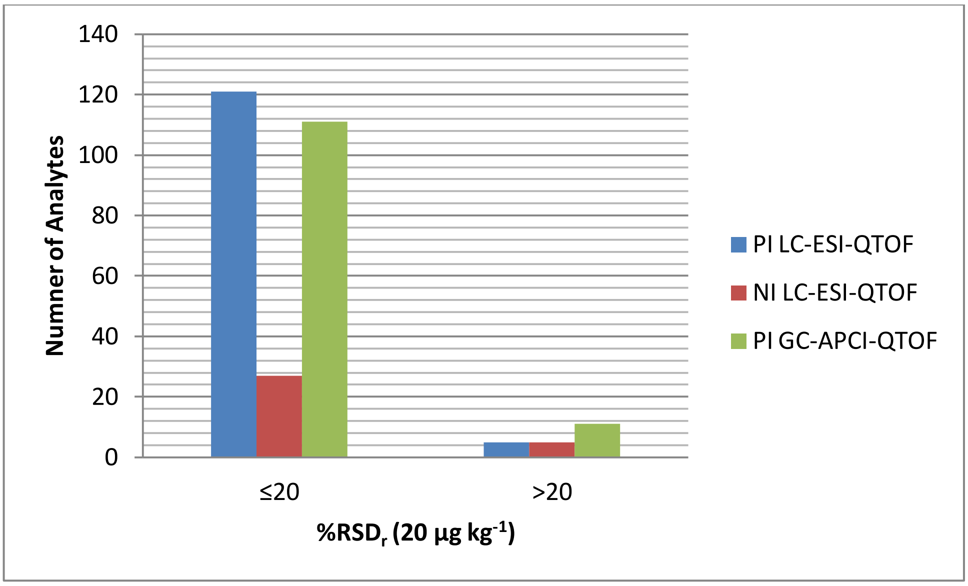


Figure 5.19: Distribution of RSD$_r$ of all analytes in level of 20µg kg^{-1} for all methods of analysis.

According the SANTE acceptable are the values of RSD$_r$ below 20%. For the level of 10µg kg^{-1}, 90% of the analytes' RSD$_r$ analyzed with positive ionization with LC-ESI-QTOF are below 20%. For the negative ionization with LC-ESI-QTOF and positive ionization with GC-APCI-QTOF the percentages were 75% and 74% of the analytes respectively. For the level of 20µg kg^{-1} the results are better for all the methods of analysis. More specifically, 96%, 84% and 91% are the percentages of the analytes which have RSD$_r$ below 20% for positive and negative ionization with LC-ESI-QTOF and positive ionization with GC-APCI-QTOF. This is happening because little differences sample preparation in low concentrations leads to bigger errors. The uncertainity is bigger in lower concentrations of analytes.

It is also estimated the instrumental RSD$_r$ by analyzing three times the same sample. Figure 5.20. summarizes the instrumental RSD$_r$ for all analytes for each instrument.

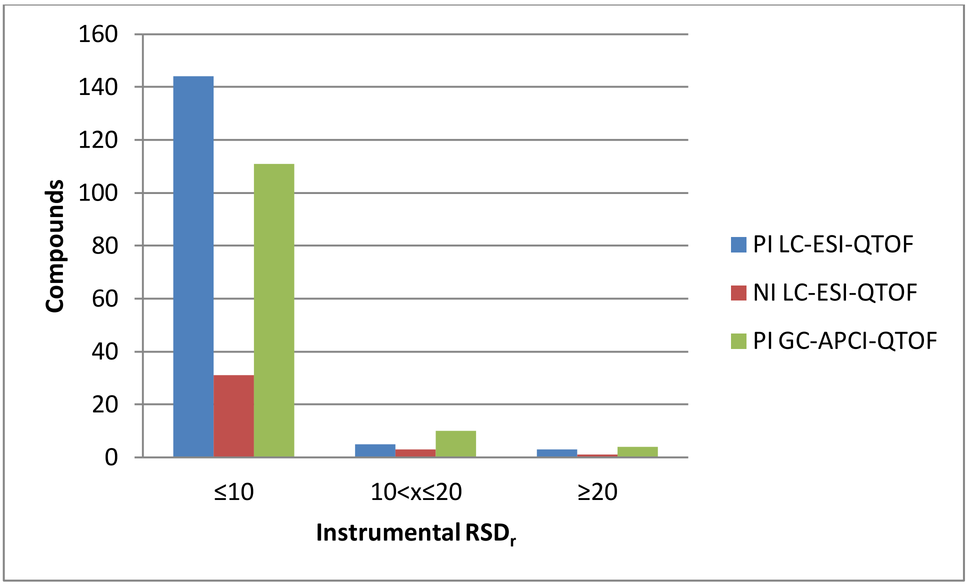


Figure 5.20: Distribution of Instrumental realative deviation RSD$_r$ of all analytes in each method.

The majority of the analytes has an instrumental RSD$_r$ below 10% for all the methods. In positive ionisation LC-ESI-QTOF only 2% of the pesticides has Instrumental RSD$_r$ above 20%, while in negative ionisation and GC-APCI-QTOF method 3% and 3%, respectively. As a result, all of the instruments are repeatable enough and suitable for pesticide analysis.

5.3.6.2 Intra-laboratory Reproducibility

It is estimated by the relative standard deviation (% RSD$_{wR}$) of the recovery from eighteen recurrent measurements at spike level of 10 µg kg^{-1} and 20 µg kg^{-1} over three different days. The values of RSD$_{wR}$ have been summarized in Figure 5.21-22.

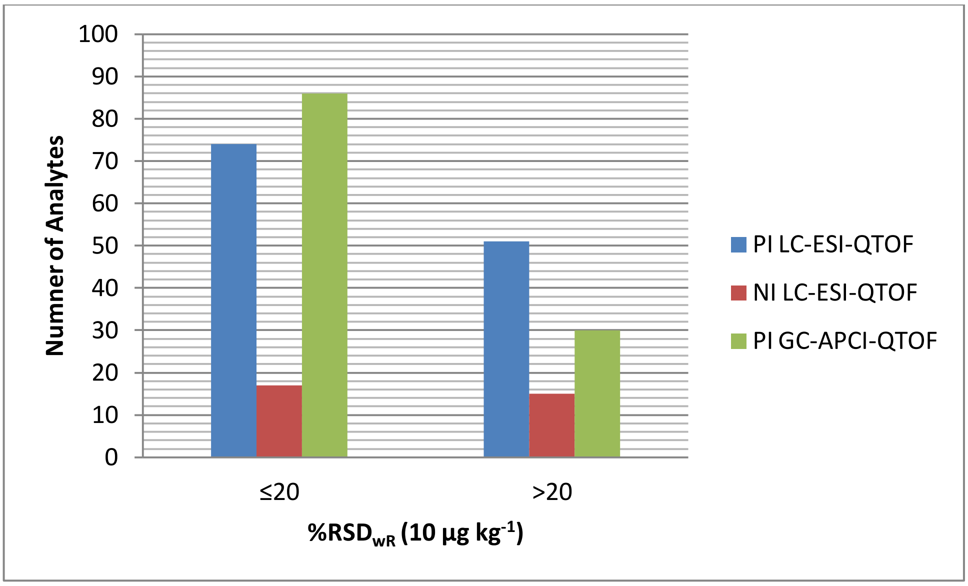


Figure 5.21: Distribution of RSD$_{wR}$ of all analytes in level of 10µg kg^{-1} for all methods of analysis.

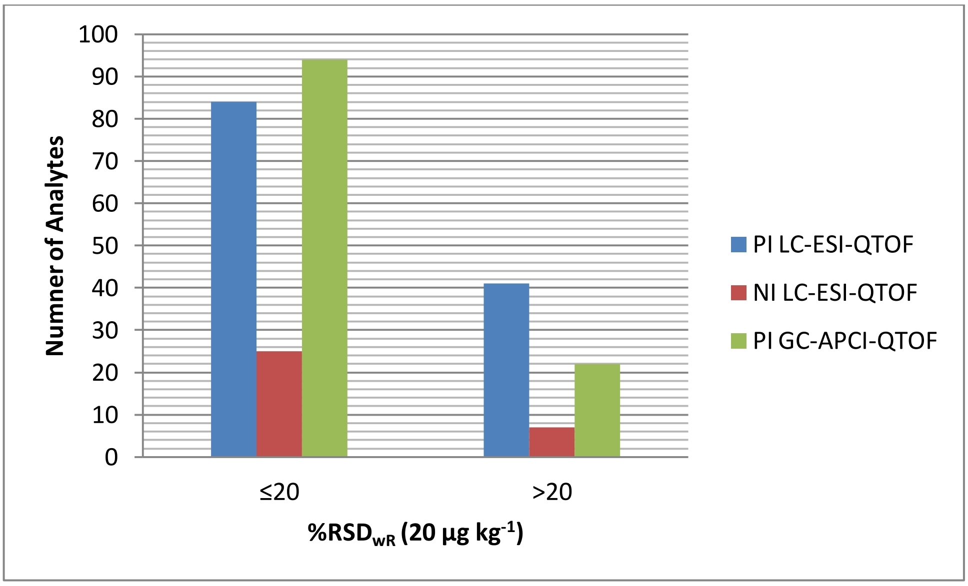


Figure 5.22: Distribution of RSD$_{wR}$ of all analytes in level of 20µg kg^{-1} for all methods of analysis.

The data from validation of recoveries, matrix effect and precision for the spiked levels 10 and 20 µg L^{-1} for the methods are summarized in Annex I on the Tables AI.4-AI.6

5.4 Application of target screening in Real Samples

Greek oranges from local supermarkets, flea markets and home grown trees were collected for pesticide analysis in order to investigate if the farmers follow good agricultural practice in their products. Organic and commercial oranges were analyzed. The positive samples are shown in Table 5.4.

The databases used for target screening contained 942 pesticides for positive ionization with LC-ESI-QTOF, 321 for negative ionization with LC-ESI-QTOF and 275 for positive ionization with GC-APCI-QTOF.

A total of 7 pesticides were identified in analyzed samples. All of them were under their corresponding MRLs but also pesticide residues detected on organic products (OSM1) that normally they must not have. This may happen because in many supermarkets the organic oranges are set next to commercial oranges. As a result, they may be contaminated by the commercial oranges. Especially when organic and commercial oranges are not packed, the possibility of contamination is higher due the fact that people may mix them as they have access to collect them by hand. In most of the positive samples the detected pesticides were 2 substances or more. Even though the residues are well under their MRLs they may have a synergistic action in human health. Pyriproxyfen and Chlorpyrifos are the pesticides that detected in most samples.

All the detected pesticides are commonly used in citrus crops. Moreover, in Greece there is specific directive for citrus crops about which of these pesticides must use for the prevention of specific microorganisms, how many times each year must they applied on crops and how many days must be applied before the harvest.

5.5 Compartment dependent concentration of pesticides

Because orange consists of many parts such as peel, juice, the edible part that are very different between them, we did an application to see if the concentration of the pesticides is the same in all of these parts. For this reason, we choose the sample FM 16 in which detected only Chlorpyrifos to

Table 5.4: Results of determination of pesticide residues in greek oranges.

Pesticides ($\mu g\ kg^{-1}$)	**SAMPLES**														**MRL** ($\mu g\ kg^{-1}$)	**MLOD** ($\mu g\ kg^{-1}$)	**MLOQ** ($\mu g\ kg^{-1}$)
	OSM1	**SM4**	**SM6**	**SM7**	**FM1**	**FM4**	**FM7**	**FM10**	**FM11**	**FM13**	**FM14**	**FM15**	**FM16**	**HG2**			
Chlorpyrifos		8.4	4.2	12.2				11.2		6.0			10.7		1500	0.99	3.0
Dimethoate					0.4	1.4									10	0.31	0.93
Fenoxycarb		37.1													2000	0.4	1.2
Imidacloprid														32.2	1000	4.1	13
Phosmet		105	24.1	13.7			1.3							0.7	1500	0.2	0.61
Pyriproxyfen	38.9	1.1	96.7				13.3	50.2	6.6		20.0				600		
Spirotetramate-enol						7.5		6.7				2.6			1000	0.79	2.4

see if the concentration of Chlorpyrifos is the same in whole orange and in individually parts of the matrix. Three oranges of FM16 that had not homogenized separated in four pieces each. From the first piece of each orange we cut the peel, collect them and homogenized. From the second pieces we collected only the edible part and homogenized it. The third pieces squeezed by hand to collect only the juice of them. The fourth pieces used as they were because we wanted the whole mass of the orange as we did in our method.

After the sample preparation, samples contained the extract of each individual matrix collected and analyzed in positive ionization with GC-APCI-QTOF.

After target screening data analysis, the resuts were that the concentration of Chlorpyrifos is not equally distributed in the individual parts of orange. In Figure 5.23, it is observed that in peel there is the most abount of Chlorpyrifos. The concentration in the peel is approximately twofold in comparison with the concentration in the whole mass. This is happening because the total concentration is diluted as in edible part and juice the concentration of Chlorpyrifos is very low.

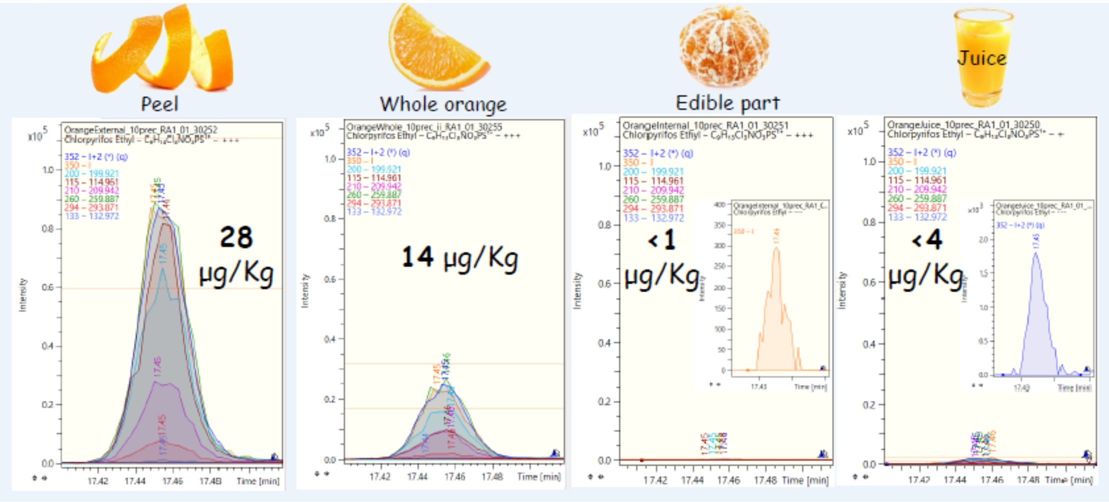


Figure 5.23: Concentration of Chlorpyrifos in different parts of orange.

CHAPTER 6
CONCLUSIONS

In this study, a common sample preparation method for the determination of pesticide residues in oranges was developed. The samples were analyzed in positive and negative mode with LC-ESI-QTOF and in positive ionization mode with GC-APCI-QTOF. The method was validated for its performance criteria for linearity, precision, matrix effects, LODs, LOQs. Finally, the aforementioned method was applied in orange samples from the market.

More specifically, the QuEChERS based method that developed is appropriate for the extraction of pesticides with different chemical properties. A database for target screening for positive ionization with GC-APCI-QTOF was developed containing 275 pesticides. The method validated for 197 analytes for linearity, precision, truness estimated LODs and LOQs and matrix effect. For linearity the best results were for positive ionization with LC-ESI-QTOF. For precision, positive ionization with LC-ESI-QTOF and GC-APCI-QTOF proves to be equivalent techniques. In positive ionization with LC-ESI-QTOF the majority of analytes has signal suppression. Opposite, positive ionization with GC-APCI-QTOF the most analytes have signal enhancement. MLODs, MLOQs are below the MRLs of European Union.

HRMS proves to be a better technique than LRMS, which is mostly used in routine analysis, for the determination of pesticide residues because it has the advantage of retrospective analysis and the ensurance of the identification of the analytes.

Target analysis in real samples results that the residues were below MRLs although in organic samples detected pesticide traces. With that seems that farmers must be informed for the good practice of agriculture. It is concluded that the higher concentration of Chlorpyrifos in orange matrix is set on the peel.

ABBREVIATIONS AND ACRONYMS

Table 6: Abbreviations and acronyms

FAO	Food and Agriculture Organization
MRL	Maximum Residues Limits
EPA	Environmental Protection Agency
MoA	Mode of Action
IRAC	Insecticide Resistance Action Committee
WHO	World Health Organization
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
LD_{50}	median Lethal Dose
OECD	Organization for Economic Co-operation and Development
EU	European Union
EC	European Comission
ADI	Acceptance Daily Intake
EFSA	European Food Safety Authority
HRMS	High Resolution Mass Spectrometry
LRMS	Low Resolution Mass Spectrometry
HPLC	High Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
EI	Electron Impact Ionization
CI	Chemical Ionization
ESI	Electrospray Ionization
APCI	Atmospheric Pressure Chemical Ionization
IL-IS	Isotopically Labelled Internal Standars
MS	Mass Spectrometry
MEW	Mass Extracted window
TOF	Time of Flight
LCL	Lowest Calibration Level
RL	Reporting Limit
GC	Gas Chromatography
LC	Liquid Chromatography

LOD	Limit of Detection
LOQ	Limit of Quantification
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
LLE	Liquid Liquid Extraction
dSPE	Dispersive solid-phase extraction
Quechers	Quick Easy Cheap Efficient Rugged Safe
CEN	European Committee for Standardization
AOAC	Association of Official Analytical Chemists
RP	Reversed phase
GSC	Gas-solid chromatography
GLC	Gas-liquid chromatography
TQ	Triple Quadrupole
IT	Ion Trap
CID	collision induced dissociation
DDA	Data Depentent Acquisition
DIA	Data Indepentent Acquisition
ECD	Electron Capture detector
NPD	Nitrogen-phosphorus detector
FPD	Flame photometric detector
ELSD	Evaporative light scattering detector
FID	Flame Ionization detector
TSD	Thermionic sensitive detector
AED	Atomic emission detector
QTOF	Quadrupole time of flight mass spectrometer
EtoAC	Ethyl Acetate
ACN	Acetonitrile
HAc	Acetic acid
FC43	Perfluorotributylamine

ANNEX I

Table AI.1: Calibration curves for 20-300 µg L^{-1}, ILODs, ILOQs and instrumental RSD_r for positive ionization with LC-ESI-QTOF.

Analyte	b ($\times10^2$)	S_b ($\times10^2$)	a ($\times10^4$)	S_a ($\times10^4$)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r	Analyte	b ($\times10^2$)	S_b ($\times10^2$)	a ($\times10^4$)	S_a ($\times10^4$)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r
Acephate	79.9	3.0	8.2	5.0	0.994	7.7	23	5.9	Malaoxon	165.1	4.3	23.2	7.1	0.997	10	29	2.7
Ametoctradin	130.2	2.9	12.9	4.8	0.998	15	46	1.0	Malathion	138.0	1.1	11.6	1.8	0.9997	4.4	13	2.4
Amitraz	11.2	1.5	6.6	2.6	0.93	9.2	28	9.2	Mandipropamid	44.58	0.65	1.6	1.1	0.9992	1.3	3.9	0.16
Atrazine	84.2	2.0	11.6	3.3	0.998	9.0	27	3.5	Mecarbam	76.0	1.5	5.5	2.4	0.999	9.5	29	3.0
Atrazine-desethyl	23.26	0.85	1.9	1.4	0.995	9.5	29	6.4	Mephosfolan	139.9	2.3	7.1	3.8	0.999	8.9	27	2.1
Azinphos-ethyl	7.26	0.29	-0.40	0.48	0.994	4.4	13	4.9	Mesosulfuron-methyl	10.52	0.17	0.32	0.28	0.9990	4.6	14	0.28
Azinphos-methyl (Guthion)	9.71	0.19	0.65	0.32	0.998	1.1	3	1.4	Mesotrione	1.261	0.065	0.16	0.12	0.992	31	94	8.5
Benfuracarb	84.7	2.1	7.4	3.4	0.998	6.5	20	3.0	Metaflumizone	1.089	0.022	0.012	0.037	0.998	6.6	20	8.9
Benodanil	63.9	1.6	8.0	2.7	0.997	10	32	2.1	Metalaxyl	163.5	4.6	19.7	7.6	0.997	5.9	18	6.6
Bifenthrin	5.70	0.29	-0.13	0.48	0.990	4.7	14	3.8	Methabenzthiazuron	73.2	1.9	13.0	3.2	0.997	9.3	28	2.5
Bitertanol	5.83	0.28	-0.27	0.47	0.991	16	49	2.2	Methacrifos Isomer 2								
Boscalid	9.02	0.48	-0.23	0.79	0.99	9.2	28	2.1	Methacrifos Isomer 1	42.9	1.2	6.0	2.0	0.997	10	29	0.74
Bromophos-ethyl	1.597	0.090	-0.10	0.15	0.99	18	54	3.6	Methamidophos	91.4	3.6	14.3	6.0	0.994	6.8	21	3.5
Butocarboxim	25.2	1.1	-1.2	1.8	0.993	6.9	21	3.8	Methidathion	84.6	2.0	9.1	3.4	0.998	4.1	13	5.4
Butoxycarboxim									Methomyl	49.6	1.9	-7.2	3.1	0.994	4.7	14	4.1
Carbaryl	81.5	2.1	0.0	3.4	0.997	6.8	21	5.4	Methoprotryne	193.5	5.7	12.5	9.4	0.997	5.7	17	4.2
Carbendazim	2.099	0.063	0.04	0.10	0.996	10	29	4.7	Methoxyfenozide	12.1	1.1	-2.9	1.8	0.97	6.6	20	0.64
Chlorfenvinphos (E/Z) Isomer 1	92.7	3.7	21.2	6.1	0.994	12	36	1.0	Metolachlor	88.8	2.0	6.2	3.4	0.998	11	34	4.0
Chlorfenvinphos (E/Z) Isomer 2	12.64	0.35	0.43	0.58	0.997	15	44	2.2	Metribuzin	78.5	2.9	12.5	4.8	0.995	11	33	1.6
Chlormequat	87.7	3.9	-0.9	6.5	0.992	3.8	12	6.6	Monocrotophos	99.39	0.97	11.7	1.6	0.9996	5.4	16	5.6
Chlorpyriphos	81.2	1.8	0.7	3.1	0.998	11	34	3.3	Myclobutanil	16.07	0.84	2.8	1.4	0.99	9.3	28	1.8
Chlorpyriphos-methyl	2.82	0.17	0.35	0.28	0.99	14	42	7.2	Nitralin	0.697	0.086	0.19	0.16	0.96	66	199	5.5
Cyanazine	17.99	0.29	-0.08	0.49	0.999	8.0	24	3.4	Omethoate	138.3	4.7	14.3	7.8	0.995	15	46	4.6
Cycloate	101.0	4.8	-5.3	7.9	0.991	5.8	18	4.4	Oxamyl	32.7	1.1	-1.4	1.9	0.995	7.1	21	0.39
Cyflufenamid	40.0	1.2	5.1	1.9	0.997	8.8	27	1.4	Paraoxon	104.1	3.8	14.1	6.3	0.995	10	30	3.6

Analyte	**b ($\times 10^2$)**	**S_b ($\times 10^2$)**	**a ($\times 10^4$)**	**S_a ($\times 10^4$)**	**R^2**	**ILOD (µg L^{-1})**	**ILOQ (µg L^{-1})**	**%RSD_r**	**Analyte**	**b ($\times 10^2$)**	**S_b ($\times 10^2$)**	**a ($\times 10^4$)**	**S_a ($\times 10^4$)**	**R^2**	**ILOD (µg L^{-1})**	**ILOQ (µg L^{-1})**	**%RSD_r**
Cyfluthrin Isomer 1	0.594	0.064	-0.08	0.12	0.97	65	196	7.3	Paraoxon-methyl	71.2	1.1	6.6	1.8	0.999	6.1	19	0.70
Cyfluthrin Isomer 2	0.675	0.056	-0.16	0.10	0.98	50	152	8.8	Parathion	8.83	0.37	-0.27	0.61	0.993	3.1	9.4	6.9
Cyhalofop butyl	1.061	0.082	0.15	0.14	0.98	42	128	10	Parathion-methyl	1.063	0.036	0.054	0.060	0.995	17	50	3.5
Cyhalothrin (lambda-)	17.01	0.44	-1.08	0.73	0.997	0.31	0.94	1.4	Penconazole	25.66	0.83	4.2	1.4	0.996	10	30	4.6
Cypermethrin Isomer 1	0.152	0.014	-0.062	0.028	0.98	56	170	6.7	Permethrin (cis-)	1.445	0.057	-0.099	0.095	0.994	0.8	2.3	3.6
Cypermethrin Isomer 2	0.345	0.059	0.05	0.12	0.95	156	472	43	Permethrin Isomer 1	1.432	0.053	-0.089	0.089	0.994	0.8	2.3	4.1
Cypermethrin Isomer 3	0.043	0.010	0.169	0.021	0.94	162	492	4.6	Permethrin Isomer 2	0.634	0.047	0.117	0.077	0.98	16	48	7.4
Cyproconazole Isomer 1	9.35	0.22	1.01	0.37	0.998	10	31	3.9	Permethrin Isomer 3	11.42	0.50	-0.80	0.83	0.992	8.9	27	4.5
Cyproconazole Isomer 2	8.22	0.17	-0.01	0.29	0.998	1.9	5.7	1.4	Phosalone	18.86	0.42	0.44	0.69	0.998	10	30	6.6
Cyprodinil	201	11	25	17	0.99	9.4	28	3.1	Phosmet	30.1	2.6	0.2	4.4	0.97	10	30	6.7
Cyromazine	114.8	4.6	18.2	7.6	0.994	10	30	6.0	Phosphamidon Isomer 1	27.72	0.65	0.1	1.1	0.998	1.6	4.8	2.2
DEET (Diethyltoluamide)	179.1	4.0	12.9	6.6	0.998	8.5	26	5.0	Phosphamidon Isomer 2	26.5	1.6	-0.5	2.6	0.99	7.7	23	2.9
Deltamethrin Isomer 1	6.10	0.24	-0.61	0.40	0.994	13	40	4.5	Phoxim	102.6	2.2	9.4	3.6	0.998	14	42	2.8
Deltamethrin Isomer 2	5.94	0.23	-0.59	0.38	0.994	11	35	4.8	Picoxystrobin	61.3	1.4	5.9	2.2	0.998	11	34	3.9
Demeton-S-methylsulfone	128.9	1.8	13.8	3.0	0.999	11	33	4.0	Pinoxaden	119.0	4.9	6.3	8.1	0.993	13	38	3.6
Diazinon	240	33	123	55	0.93	8.4	25	8.5	Pirimicarb	245	14	33	23	0.99	13	38	4.5
Dichlofluanid	11.49	0.42	1.09	0.69	0.995	7.9	24	3.7	Pirimiphos-ethyl	221	38	157	62	0.9	9.1	28	5.7
Dichlorvos	31.7	1.5	5.3	2.5	0.991	5.1	16	4.3	Pirimiphos-methyl	209	13	23	21	0.98	9.5	29	5.2
Difenacoum Isomer 1	7.22	0.11	0.82	0.18	0.9991	11	34	3.8	Procymidone	0.615	0.075	0.29	0.12	0.94	1.2	3.7	31
Difenacoum Isomer 2	8.47	0.25	-0.97	0.42	0.996	5.3	16	4.9	Prometryn (Caparol)	182.0	5.8	13.4	9.7	0.996	10	31	5.6
Dimethoate	103.1	2.8	14.7	4.6	0.997	12	36	3.0	Propazine	26.27	0.67	3.2	1.1	0.997	8.7	26	1.8
Diniconazole	7.29	0.45	0.14	0.74	0.99	12	37	6.1	Propham	29.38	0.49	-0.16	0.81	0.999	4.8	15	1.5
Dinitramine	1.235	0.082	0.04	0.14	0.98	8.5	26	5.4	Propoxycarbazone	7.29	0.23	-0.39	0.38	0.996	10	30	2.0
Disulfoton	53.5	2.2	-2.0	3.6	0.993	8.3	25	3.2	Prothiofos (Tokuthion)	4.27	0.16	0.00	0.26	0.995	11	32	4.9
Ethiofencarb	76.5	1.3	9.7	2.2	0.999	9.3	28	2.5	Pyridaben	51.3	1.1	0.8	1.9	0.998	12	36	1.5
Ethion	143.9	6.8	18	11	0.991	15	47	5.2	Pyridalyl	3.17	0.15	-0.33	0.24	0.992	12	38	7.0
Ethoprophos. Ethoprop	54.77	0.84	3.7	1.4	0.9991	5.7	17	2.2	Pyrifenox Isomer 1	27.68	0.66	3.3	1.1	0.998	11	34	2.9
Etrimfos	180.1	4.6	26.8	7.6	0.997	13	38	3.1	Pyrifenox Isomer 2	86.8	1.4	11.8	2.3	0.9990	8.9	27	3.8
Fenamiphos	78.2	1.4	5.9	2.4	0.999	9.4	29	5.1	Pyroxsulam	8.20	0.13	0.07	0.21	0.9991	5.8	18	4.7
Fenarimol	3.17	0.11	0.08	0.19	0.995	10	31	2.6	Quinalphos	65.21	0.92	7.3	1.5	0.9992	7.7	23	0.38

Analyte	b (×10^2)	S_b (×10^2)	a (×10^4)	S_a (×10^4)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r
Fenitrothion	2.333	0.093	0.00	0.15	0.994	11	35	6.4
Fenoxaprop-ethyl	58.4	3.1	13.9	5.1	0.99	6.7	20	1.1
Fenoxycarb	45.5	1.2	6.1	1.9	0.997	11	34	3.9
Fenthion	31.7	1.5	10.9	2.5	0.991	8.0	24	4.3
Fenthion-sulfoxide	99.8	2.7	6.3	4.5	0.997	11	34	2.3
Fenvalerate	3.45	0.11	-0.14	0.18	0.996	2.9	8.9	4.9
Flamprop	22	13	0.02682	0.00078	0.997	5.6	17	2.4
Fluazuron	3.300	0.058	-0.098	0.096	0.999	7.8	24	1.1
Flubendiamide	8.06	0.14	0.11	0.24	0.999	8.0	24	4.8
Fludioxonil	26.47	0.16	1.27	0.27	0.9999	3.3	10	7.2
Flufenoxuron	1.608	0.095	-0.17	0.16	0.99	16	48	1.8
Fluthiacet-methyl	19.08	0.55	-0.90	0.91	0.997	10	31	2.5
Foramsulfuron	1.169	0.027	0.014	0.044	0.998	7.0	21	2.4
Halosulfuron-methyl	6.73	0.10	-0.17	0.17	0.9991	8.3	25	1.2
Imidacloprid	26.89	0.84	1.1	1.4	0.996	4.8	15	1.5
Inabenfide	2.77	0.10	0.01	0.17	0.995	9.5	29	1.6
Sulfentrazone	0.338	0.018	0.022	0.032	0.992	31	95	20.3
Isoprothiolane	87.4	1.2	10.6	1.9	0.9993	6.4	19	1.6
Isoproturon	161.0	4.3	13.5	7.1	0.997	4.8	14	3.8
Isopyrazam	102.5	5.4	4.1	9.0	0.99	6.3	19	1.2
Lufenuron	0.132	0.012	-0.030	0.024	0.98	61	184	13
Quinoxyphen	50.8	1.8	1.2	3.0	0.995	10	29	3.4
Simazine	15.76	0.77	3.6	1.3	0.990	10	29	4.7
Simeconazole	17.74	0.30	1.06	0.49	0.999	9.2	28	4.0
Spinosad A (Spinosyn A)	45.1	2.9	0.9	4.8	0.98	10	30	3.6
Spinosad D	8.09	0.30	-0.08	0.49	0.995	10	30	3.7
Spirotetramate-enol	42.5	1.7	5.8	2.8	0.994	10	29	4.8
Spirotetramate-keto-hydroxy	6.58	0.21	-0.33	0.35	0.996	9.3	28	3.7
Spirotetramate-mono-hydroxy	23.47	0.29	0.99	0.48	0.9994	5.6	17	0.41
Spiroxamine Isomer 1	87.4	2.2	0.6	3.6	0.998	9.3	28	1.9
Spiroxamine Isomer 2	126.3	2.9	5.6	4.7	0.998	10	31	4.2
TCMTB	8.20	0.16	0.14	0.26	0.999	7	20	3.4
Tebuconazole	17.23	0.44	1.22	0.74	0.997	6.0	18	2.9
Tepraloxydim Isomer 1	11.40	0.27	-0.96	0.45	0.998	7.8	24	0.9
Tepraloxydim Isomer 2	8.39	0.26	-0.10	0.43	0.996	0.8	2.5	3.8
Terbufos	12.20	0.65	-0.9	1.1	0.99	7.6	23	4.6
Terbuthylazine	104.89	0.87	13.2	1.4	0.9997	4.5	14	2.0
Tetrachlorvinphos (Stirofos)	27.78	0.70	2.2	1.2	0.997	9.0	27	15
Tetradifon	0.274	0.020	0.024	0.041	0.99	41	125	12
Thiacloprid	99.3	1.4	8.3	2.3	0.9992	7.6	23	3.6
Triadimefon	16.44	0.93	2.0	1.6	0.99	2.4	7.1	13
Trichlorfon (Dylox)	20.09	0.49	2.05	0.82	0.998	5.5	17	4.2
Tritosulfuron	3.17	0.13	-0.14	0.21	0.994	22	67	3.5

Table AI.2: Calibration curves for 20-300 µg L^{-1}, ILODs, ILOQs and instrumental RSD_r for negative ionization with LC-ESI-QTOF.

Analyte	b (×10)	S_b (×10)	a (×10^3)	S_a (×10^3)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r
Acephate	9.54	0.31	-0.69	0.51	0.996	1.6	4.8	6.4
Benodanil	15.38	0.79	-1.1	1.3	0.99	5.3	16	8.2
Boscalid	21.0	1.8	2.4	3.0	0.97	12	35	3.9
Bromoxynil	187	15	48	25	0.98	6.6	20	1.4
Climbazole	32.3	2.0	-0.2	3.3	0.99	6.1	19	8.2
Cyanazine	2.52	0.13	0.15	0.21	0.990	19	58	17
Cyproconazole Isomer 1	1.07	0.35	-0.05	0.70	0.83	215	650	21
Cyproconazole Isomer 2	1.126	0.091	0.38	0.18	0.99	54	162	5.2
Difenacoum	427	35	182	58	0.97	9.6	29	3.3
Dinoseb	499	19	103	31	0.995	6.4	19	3.6
Dinoterb	529	19	101	31	0.995	4.6	14	3.5
Endosulfan-sulfate	91.3	6.8	7	11	0.98	1.5	4.6	0.62
Fenarimol	9.54	0.40	-1.46	0.67	0.993	23	70	7.6
Flamprop	13.04	0.28	-1.74	0.47	0.998	4.0	12	5.8
Fluazuron	136	11	41	19	0.97	5.4	16	1.9
Flubendiamide	298	25	74	42	0.97	7.3	22	1.7
Fludioxonil	657	13	68	22	0.998	10	31	1.8
Flufenoxuron	123	11	49	19	0.97	5.2	16	4.1
Halosulfuron-methyl	69.7	3.1	-3.0	5.1	0.992	4.2	13	2.5
Imidacloprid	14.72	0.82	0.3	1.4	0.99	6.5	20	5.0
Inabenfide	138	12	31	19	0.97	7.4	22	1.9
Isopyrazam	240	15	43	25	0.98	7.5	23	1.9
Lufenuron	116	11	42	17	0.97	8.7	26	3.7
Mesotrione	11.99	0.63	-0.7	1.1	0.99	6.7	20	4.5
Metaflumizone e-isomere	269	19	64	32	0.98	7.9	24	1.0
Methoxyfenozide	127.3	5.6	7.4	9.2	0.992	3.5	11	2.7
Procymidone	3.07	0.52	0.88	0.86	0.90	3.5	11	5.3
Pyroxsulam	3.74	0.26	-0.75	0.48	0.99	19	57	6.4
Spirotetramate-enol	237	11	14	19	0.991	8.1	24	1.9
Spirotetramate-keto-hydroxy	0.60	0.30	-0.10	0.68	0.8	224	680	10
Sulfentrazone	69.4	6.0	9.8	9.9	0.97	3.4	10.3	3.3
Tebuconazole	2.46	0.33	0.19	0.59	0.95	45	137	15
Tepraloxydim Isomer 1	165	12	-1	19	0.98	1.0	3.0	1.4
Tepraloxydim Isomer 2	42.5	4.8	2.5	8.0	0.95	12	37	2.6
Thiacloprid	1.37	0.14	0.80	0.25	0.97	38	116	1.5

Table AI.3: Calibration curves for 20-300 μg L^{-1}, ILODs, ILOQs and instrumental RSD_r for positive ionization with GC-APCI-QTOF.

Analyte	b ($\times10^{-3}$)	S_b ($\times10^{-3}$)	a ($\times10^{-1}$)	S_a ($\times10^{-1}$)	R^2	ILOD (μg L^{-1})	ILOQ (μg L^{-1})	%RSD_r	Analyte	b ($\times10^{-3}$)	S_b ($\times10^{-3}$)	a ($\times10^{-1}$)	S_a ($\times10^{-1}$)	R^2	ILOD (μg L^{-1})	ILOQ (μg L^{-1})	%RSD_r
2.4-DDT	65.0	1.8	-8.2	2.9	0.997	4.5	14	2.9	Folpet	16.7	2.1	-11.2	3.8	0.95	33	99	7.5
4.4-DDD	60.0	2.2	1.6	3.7	0.995	2.9	8.8	4.1	Heptachlor Epoxide	30.98	0.50	-2.91	0.83	0.9990	5.6	17	0.69
4.4-DDE	65.8	1.5	-9.2	2.5	0.998	7.0	21	2.0	Heptachlor	7.62	0.64	-1.9	1.1	0.97	7.6	23	2.5
4.4-DDT	30.76	0.75	-4.6	1.2	0.998	8.3	25	5.2	Hexachlorobenzene	4.2	1.0	-1.7	1.8	0.9	142	429	8.7
Acephate	25.0	2.2	-9.2	3.7	0.97	1.4	4.3	37	Hexachlorobutadiene								
Acrinathrin	25.3	1.2	-3.0	2.0	0.991	3.2	9.8	10	Iprodione	5.05	0.31	-0.46	0.51	0.99	3.4	10	2.2
Aldrin	4.78	0.38	-1.46	0.63	0.98	18	53	6.6	Isodrin	18.0	1.0	-5.5	1.7	0.99	3.9	12	0.53
Amitraz	19.69	0.95	1.2	1.6	0.991	0.6	1.8	1.5	Malaoxon	71.7	1.5	-9.6	2.6	0.998	5.2	16	11
Atrazine	74.8	1.8	1.8	3.0	0.998	4.2	13	2.6	Malathion	46.9	2.6	1.4	4.3	0.99	3.7	11	1.3
Azinphos Ethyl	29.2	1.7	-6.1	2.8	0.99	0.15	0.46	5.8	Mercarbam	74.7	3.3	4.9	5.5	0.992	4.8	15	6.1
Azinphos Methyl	11.1	1.5	-5.2	2.6	0.93	26	78	13	Metalaxyl	67.3	6.5	17	11	0.96	3.6	11	0.19
Benfuracarb	20.06	0.86	-0.2	1.4	0.993	4.9	15	7.3	Methacrifos	45.9	5.1	-11.1	8.4	0.95	4.0	12	9.2
Bifenthrin	91.9	8.7	28	14	0.97	3.7	11	2.9	Methamidophos	29.0	1.8	-0.3	3.0	0.98	69	210	5.5
Bromophos Ethyl	46.9	1.9	-0.4	3.1	0.994	5.3	16	5.0	Methidathion	19.90	0.64	0.3	1.1	0.996	1.2	3.8	5.7
Bromophos Methyl	47.1	2.9	-14.0	4.9	0.98	7.0	21	2.1	Methomyl	16.9	1.0	-1.7	1.7	0.99	3.3	10	8.7
Carbaryl Isomer 1	14.03	0.92	-1.4	1.5	0.98	5.6	17	16	Methoprotryne	64.7	1.9	3.2	3.1	0.997	2.1	6.3	1.9
Carbaryl Isomer 2	5.01	0.28	-0.70	0.46	0.99	6.7	20	15	Methoxychlor (DMTD)	9.59	0.97	4.3	1.6	0.96	1.7	5.1	3.9
Chlorfenvinphos mix of Z&E isomer 1	6.51	0.30	-0.68	0.50	0.991	3.4	10	4.7	Metolachlor	48.0	1.6	-0.3	2.6	0.996	3.8	11	3.8
Chlorfenvinphos mix of Z&E isomer 2	44.6	3.1	10.8	5.2	0.98	3.6	11	1.0	Metribuzin	61.0	2.3	4.4	3.9	0.994	3.9	12	2.1
Chlorothalonil	9.84	0.95	-5.3	1.7	0.97	92	280	13	Monocrotophos	41.8	2.9	-8.7	4.9	0.98	13	39	13
Chlorpyrifos Ethyl	77.0	7.2	6	12	0.97	7.0	21	1.5	Myclobutanil	73.4	4.5	12.2	7.5	0.99	2.9	8.9	5.4
Chlorpyrifos Methyl	43.8	2.7	-13.4	4.5	0.99	5.7	17	3.6	Omethoate	37.6	3.6	-12.7	6.1	0.96	2.5	7.6	31
Climbazole	44.64	0.74	0.1	1.2	0.999	3.5	11	3.2	Paraoxon Ethyl	112.0	6.6	4	11	0.99	3.8	12	5.2
Cyanazine	45.0	2.4	8.3	3.9	0.99	1.0	3.1	2.0	Parathion-Ethyl	122	20	63	33	0.90	5.1	15	3.4
Cycloate	43.2	5.4	-9.7	8.9	0.94	12	37	5.2	Parathion-Methyl	94.2	2.6	-17.6	4.2	0.997	5.1	15	4.4
Cyfluthrin Isomer 1	0.370	0.030	0.068	0.050	0.97	36	109	20	Penconazole	40.2	1.8	2.1	2.9	0.992	1.5	4.7	2.2
Cyfluthrin Isomer 2	0.449	0.048	0.327	0.079	0.96	8.7	27	12	Pentabromobenzyl acrylate	14.61	0.68	-2.4	1.1	0.991	17	50	1.3
Cyfluthrin Isomer 3	3.49	0.21	0.21	0.35	0.99	4.9	15	1.5	Pentabromoethylbenzene	16.6	1.1	-4.9	1.8	0.98	7.7	23	4.1
Cyfluthrin Isomer 4	12.45	0.98	1.4	1.6	0.98	17	52	3.0	Pentachlorobenzene	1.28	0.26	-0.70	0.49	0.92	127	384	16

Analyte	b ($\times10^{-3}$)	S_b ($\times10^{-3}$)	a ($\times10^{-1}$)	S_a ($\times10^{-1}$)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r	Analyte	b ($\times10^{-3}$)	S_b ($\times10^{-3}$)	a ($\times10^{-1}$)	S_a ($\times10^{-1}$)	R^2	ILOD (µg L^{-1})	ILOQ (µg L^{-1})	%RSD_r
Cyhalothrin-lambda Isomer 1	48.9	2.7	4.0	4.5	0.99	2.3	6.9	2.8	Permethrin Isomer 1	36.4	2.1	6.7	3.6	0.99	2.3	7.0	0.78
Cyhalothrin-lambda Isomer 2	0.76	0.15	0.68	0.25	0.9	109	330	22	Permethrin Isomer 2	92	11	43	19	0.94	2.7	8.1	4.6
Cypermethrin Isomer 1	10.78	0.42	0.07	0.69	0.994	10	31	1.7	Phosalone	33.6	1.7	-6.4	2.9	0.990	2.0	6.1	5.7
Cypermethrin Isomer 2	9.19	0.49	0.11	0.81	0.99	10	29	1.9	Phosmet	38.6	3.3	-13.8	5.4	0.97	11	33	12
Cypermethrin Isomer 3	9.45	0.27	0.14	0.45	0.997	8.0	24	3.8	Phosphamidon isomer 1	7.71	0.47	-1.18	0.78	0.99	1.3	3.8	6.0
Cypermethrin Isomer 4	8.22	0.70	1.0	1.2	0.97	12	36	2.8	Phosphamidon isomer 2	12.20	0.77	-2.5	1.3	0.98	1.6	4.8	0.84
Cyproconazole	44.3	2.0	-0.7	3.3	0.992	1.0	2.9	1.6	Pirimicarb	102.7	3.5	3.7	5.8	0.995	2.6	8.0	3.7
Cyprodinil	120	11	21	19	0.97	2.2	6.6	1.1	Pirimiphos Methyl	76.9	2.1	-3.0	3.5	0.997	3.2	10	2.2
DEET	51.5	4.8	-13.1	8.0	0.97	4.2	13	2.3	Pirimiphos ethyl	88	11	36	18	0.94	4.3	13	1.2
Deltamethrin	6.24	0.39	-0.31	0.65	0.98	7.1	21	6.1	Procymidone	86.1	6.0	11.5	9.9	0.98	3.5	10	2.4
Diazinon	119	13	12	22	0.95	1.7	5.2	4.7	Profenophos	66.5	3.6	5.1	6.0	0.99	3.8	12	2.2
Dichlofluanid	17.93	0.21	-2.38	0.35	0.9995	6.9	21	4.5	Propazine	49.6	1.7	-3.6	2.8	0.995	3.5	11	1.9
Dichlorvos	5.7	1.2	0.9	2.0	0.9	12	35	3.9	Propham	34.2	2.9	-8.3	4.7	0.97	8.4	26	3.3
Dicofol	37.5	2.7	-4.8	4.5	0.98	1.6	4.8	2.5	Prothiophos	64.3	2.4	1.7	4.0	0.994	5.3	16	2.4
Dieldrin	13.40	0.53	-2.28	0.88	0.994	4.3	13	2.0	Quinalphos	70.9	1.8	1.1	2.9	0.998	3.8	11	2.8
Dimethoate	43.2	2.7	-9.9	4.5	0.98	4.8	15	6.2	Quinoxyfen	58.4	1.3	0.1	2.2	0.998	1.6	4.9	2.6
Diniconazole	26.90	0.94	-0.7	1.6	0.995	2.8	8.6	1.0	Quintozene (Pentachloronitrobenzene)	9.2	1.1	-3.7	1.8	0.95	7.0	21	2.6
Dinitramine	51.2	2.2	-2.8	3.7	0.993	5.5	17	4.1	Simazine	15.49	0.49	-1.07	0.82	0.996	1.5	4.4	3.7
Disulfoton	5.82	0.35	-1.53	0.59	0.99	9.0	27	3.9	Spiroxamine Isomer 1	23.32	0.35	-1.88	0.59	0.9991	2.0	6.1	3.5
Endosulfan alpha	10.53	0.66	-2.9	1.1	0.98	10	31	2.2	Spiroxamine Isomer 2	30.61	0.34	-2.10	0.57	0.9995	2.5	7.5	3.0
Endosulfan sulphate	34.4	1.2	-4.2	2.0	0.995	3.4	10	3.4	Tebuconazole	51.4	2.5	4.0	4.1	0.991	2.4	7.3	1.7
Endrin	19.56	0.85	-4.1	1.4	0.993	4.8	15	1.6	Terbufos	4.20	0.40	-1.65	0.66	0.97	4.9	15	3.3
Ethalfluralin	37.0	2.7	-11.1	4.5	0.98	10	31	3.9	Terbuthylazine	84.8	5.1	7.6	8.4	0.99	3.6	11	2.4
Ethiofencarb	9.24	0.64	-1.9	1.1	0.98	6.8	21	6.3	Tetrachlorvinphos	47.2	1.6	-5.0	2.7	0.995	5.7	17	4.1
Ethion	72.0	4.1	8.5	6.8	0.99	4.0	12	2.8	Tetradifon	36.47	0.76	-3.0	1.3	0.998	0.6	1.7	3.4
Ethoprophos	16.1	1.6	-5.4	2.7	0.96	6.8	21	1.9	Tetrasul	14.99	0.51	-2.12	0.85	0.995	4.3	13	3.9
Etrimphos	118.0	5.7	-12.2	9.4	0.991	2.0	6.2	1.3	Triadimefon	57.8	2.4	1.0	4.1	0.993	2.8	8.5	2.4
Fenamiphos	105.0	7.3	16	12	0.98	2.7	8.1	2.5	Trifluralin	42.1	2.9	-12.2	4.8	0.98	3.8	11	0.94
Fenarimol Isomer 1	46.8	2.6	1.2	4.4	0.99	3.1	10	1.2	a-HCH	7.79	0.69	-2.7	1.1	0.97	4.2	13	5.4
Fenarimol Isomer 2	25.3	1.1	2.2	1.9	0.992	0.047	0.14	2.5	b-HCH	13.16	0.88	-3.5	1.5	0.98	15	47	3.5

Analyte	**b (×10^{-3})**	**S_b (×10^{-3})**	**a (×10^{-1})**	**S_a (×10^{-1})**	**R^2**	**ILOD (μg L^{-1})**	**ILOQ (μg L^{-1})**	**%RSD$_r$**	**Analyte**	**b (×10^{-3})**	**S_b (×10^{-3})**	**a (×10^{-1})**	**S_a (×10^{-1})**	**R^2**	**ILOD (μg L^{-1})**	**ILOQ (μg L^{-1})**	**%RSD$_r$**
Fenitrothion	114.1	8.5	0	14	0.98	5.9	18	2.6	c-HCH (Lindane)	11.72	0.72	-3.1	1.2	0.99	24	73	2.1
Fenoxycarb	55.3	2.5	-4.5	4.2	0.992	1.0	3.0	8.9	cis Chlordane	10.41	0.74	-3.1	1.2	0.98	5.0	15	2.9
Fenthion	130	23	65	39	0.9	5.4	16	4.8	d-HCH	16.9	1.1	-4.6	1.8	0.98	7.8	24	3.5
Fludioxonil	98.4	4.3	11.1	7.1	0.993	2.0	5.9	3.6	trans Chlordane	5.71	0.21	-0.98	0.35	0.994	8.2	25	6.3

Table AI.4: Validation Parameters for trueness, precision and matrix effect for positive ionization mode LC-ESI-QTOF.

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME	Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	$\%RSD_r$	$\%RSD_{wR}$	%R	%RSDr	$\%RSD_{wR}$			%R	$\%RSD_r$	RSDrw	%R	%RSDr	$\%RSD_{wR}$	
Acephate	72.84	0.90	2.4	73.59	0.97	2.1	-35	Malaoxon	70.7	1.7	25	81.1	1.3	21	-67
Ametoctradin	37.1	9.3	14	47.8	7.2	12	-51	Malathion	80.4	3.3	27	102.8	5.5	25	-97
Amitraz							-100	Mandipropamid	64.4	4.6	5.1	82.1	2.9	4.7	-61
Atrazine	79.7	2.5	15	89.3	3.4	13	-90	Mecarbam	65.6	9.3	9.4	73	19	8.3	-74
Atrazine-desethyl	69.4	9.6	13	74.7	8.6	10	-86	Mephosfolan	71.6	4.7	4.0	84.0	2.4	3.2	-65
Azinphos-ethyl	60.6	6.9	8	71	16	5.0	-47	Mesosulfuron-methyl	70.79	0.64	8.7	80.7	1.8	8.0	-39
Azinphos-methyl (Guthion)	81	25	53	85.6	3.2	25	-92	Mesotrione							-100
Benfuracarb	6.0	58	61				-57	Metaflumizone							-31
Benodanil	66.4	4.0	3.8	79.4	1.9	1.6	-73	Metalaxyl	78.7	2.6	15	88.0	3.3	12	-69
Bifenthrin							-66	Methabenzthiazuron	71.8	4.2	16	77.8	4.1	15	-75
Bitertanol	55.0	8.2	10	63.9	3.0	6.8	-39	Methacrifos Isomer 2	196	15		154	16		
Boscalid	79	15	22	84.4	7.6	19	-81	Methacrifos Isomer 1	311	3.5	60	224.9	3.0	35	-98
Bromophos-ethyl							-39	Methamidophos	95.8	3.9	22	90.3	2.9	21	-56
Butocarboxim	119.2	2.4	7.9	100.7	3.0	6.4	-80	Methidathion	70.7	1.5	17	87.3	2.7	15	-79
Butoxycarboxim	90.5	4.0		95.2	1.5		-72	Methomyl	89.67	0.36	1.0	98.1	3.9	1.5	-85
Carbaryl	75.4	1.5	16	81.1	5.6	12	-50	Methoprotryne	67.6	3.6	13	67.3	7.9	12	-91
Carbendazim							-87	Methoxyfenozide	83.9	2.6	21	89.64	0.43	18	-70
Chlorfenvinphos (E/Z) Isomer 1	61.7	4.7	15	74.8	2.5	11	-49	Metolachlor	78.2	5.8	30	92.0	7.2	29	-72
Chlorfenvinphos (E/Z) Isomer 2	66.1	1.7	30	71.30	0.33	22	-75	Metribuzin	43.4	7.2	35	53.9	9.1	28	-81
Chlormequat	28.5	5.9	17	30.76	3.0	13	-79	Monocrotophos	71.8	2.4	2.1	74.8	2.5	1.9	-72
Chlorpyriphos	69.5	9.8	39	66.0	12	19	-51	Myclobutanil	82.30	0.15	3.7	68	27	2.8	-94
Chlorpyriphos-methyl	65.0	13	37	65.5	6.1	21	-66	Nitralin							-100
Cyanazine	74.5	1.9	8.4	78.6	5.4	7.5	-57	Omethoate	67.4	2.9	3.2	74.4	4.6	2.7	-35
Cycloate	183	6.7	35	149.6	4.5	25	-82	Oxamyl	67.3	2.6	5.3	63.6	12	6.2	-62

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME	Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSDr	%RSD$_{wR}$			%R	%RSD$_r$	RSDrw	%R	%RSDr	%RSD$_{wR}$	
Cyflufenamid	48.6	5.2	4.2	58.3	3.2	2.6	-40	Paraoxon	76.9	4.5	22	83.6	2.8	21	-72
Cyfluthrin Isomer 1							-50	Paraoxon-methyl	81.4	3.4	9.2	84.2	6.4	8.5	-80
Cyfluthrin Isomer 2							-49	Parathion	64.9	2.0	22	84.0	8.5	22	-72
Cyhalofop butyl	61	37	49	54.6	18	34	-52	Parathion-methyl							-100
Cyhalothrin (lambda-)	3.4	30	29	3.41	28	23	-24	Penconazole	57.2	6.3	9.1	65.5	6.1	11	-70
Cypermethrin Isomer 1							26	Permethrin (cis-)							-34
Cypermethrin Isomer 2							-37	Permethrin Isomer 1							-34
Cypermethrin Isomer 3							324	Permethrin Isomer 2							-21
Cyproconazole Isomer 1	88	30	25	61.2	15	22	-97	Permethrin Isomer 3							-39
Cyproconazole Isomer 2	68	38	32	76.6	11	29	-81	Phosalone	39.2	12	20	46.09	2.0	17	-62
Cyprodinil	21.4	13	11	30.3	9.6	10	-50	Phosmet	70.7	7.1	42	74.0	4.8	33	-77
Cyromazine	42.2	2.9	9.1	46.4	5.1	8.3	-50	Phosphamidon Isomer 1	79.9	2.5	5.3	85.1	8.2	4.3	-68
DEET (Diethyltoluamide)	149.6	3.6	42	151.3	2.7	28	-85	Phosphamidon Isomer 2	77.0	3.9	11	89.3	5.7	10	-66
Deltamethrin Isomer 1							-28	Phoxim	48.3	8.8	25	57.0	3.2	22	-58
Deltamethrin Isomer 2							-100	Picoxystrobin	60.8	3.6	5.9	68.4	1.5	5.1	-89
Demeton-S-methylsulfone	73.8	1.6	13	78.8	4.4	10	-81	Pinoxaden	61.4	1.7	3.7	72.9	5.2	3.2	-39
Diazinon	107.3	3.7	36	116.1	3.1	32	-55	Pirimicarb	85.3	4.7	18	96.9	1.3	14	-68
Dichlofluanid	69.6	6.3	28	71.4	1.5	21	-81	Pirimiphos-ethyl	37.7	10	18	48.0	6.6	13	-18
Dichlorvos	322.6	1.2	52	227.7	1.4	40	-94	Pirimiphos-methyl	62.6	6.3	19	77.1	6.1	15	-50
Difenacoum Isomer 1	15.6	27	26	15.6	19	21	-26	Procymidone							-100
Difenacoum Isomer 2	14.3	12	28	16.6	26	21	-23	Prometryn (Caparol)	56.9	1.9	5.7	52.4	7.6	3.9	-75
Dimethoate	83.84	0.9	14	88.2	2.1	12	-84	Propazine	75.1	2.7	31	75.6	10	28	-90
Diniconazole	50.4	12	10	64.5	2.5	9.5	-50	Propham	251	4.7	48	166.8	1.3	30	-78
Dinitramine	96	17	19	63.7	5.0	14	-71	Propoxycarbazone	62.2	9.5	12	71.0	2.6	10	-41
Disulfoton	98.3	8.2	25	83.1	8.9	20	-73	Prothiofos (Tokuthion)							-33
Ethiofencarb	53.0	6.9	35	52.6	1.8	19	-74	Pyridaben	7.1	26	25	8.9	13	22	-22

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME	Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSDr	%RSD$_{wR}$			%R	%RSD$_r$	RSDrw	%R	%RSDr	%RSD$_{wR}$	
Ethion	20.0	11	10	25.9	5.5	10	-19	Pyridalyl							5
Ethoprophos. Ethoprop	145.3	6.7	19	135	20	16	-87	Pyrifenox Isomer 1	59.7	9.1	10	67.3	14	9.1	-77
Etrimfos	101.4	3.9	39	109.4	7.2	30	-64	Pyrifenox Isomer 2	56.1	2.3	6.1	62.8	1.8	5.5	-91
Fenamiphos	64.8	3.5	8.1	78.9	4.0	11	-91	Pyroxsulam	72.1	1.6	1.4	81.9	2.1	1.2	-30
Fenarimol	66.2	14	13	88.8	3.5	11	-62	Quinalphos	60.9	4.9	10	72.7	2.8	8.8	-65
Fenitrothion							-100	Quinoxyphen	14.6	13	11	17.1	7.4	10	-47
Fenoxaprop-ethyl	36.3	9.0	11	45.6	7.1	10	-24	Simazine	75.9	7.1	21	81.1	4.0	21	-80
Fenoxycarb	56.0	8.8	12	61.9	3.5	11	-78	Simeconazole	68.7	4.5	12	82.8	5.8	10	-66
Fenthion	58.9	5.2	16	63.04	1.2	14	-43	Spinosad A (Spinosyn A)	30.9	8.1	8.8	43.6	4.3	7.2	-51
Fenthion-sulfoxide	84.2	5.0	4.4	92.5	4.7	2.9	-65	Spinosad D	21.7	12	20	35.2	14	15	-53
Fenvalerate							-31	Spirotetramate-enol	70.7	6.0	5.1	74.5	7.6	4.6	-52
Flamprop	45.0	12	14	63.5	4.9	12	-51	Spirotetramate-keto-hydroxy	68.5	4.3	3.6	79.3	2.3	3.2	-50
Fluazuron	16.0	24	24	18.1	19	21	-16	Spirotetramate-mono-hydroxy	74.2	2.8	4.6	81.1	3.6	3.9	-38
Flubendiamide	58.9	11.0	17	68.0	6.2	16	-83	Spiroxamine Isomer 1	58.2	1.9	24	97.3	3.2	21	-87
Fludioxonil	64.7	1.7	30	79.4	7.1	26	-93	Spiroxamine Isomer 2	88.2	9.6	30	92	13	26	-95
Flufenoxuron	7.9	16	21	9.8	24	18	9	TCMTB	64.8	4.9	15	74.5	10	12	-84
Fluthiacet-methyl	54.60	0.61	10	64.3	2.7	11	-57	Tebuconazole	55.54	1.7	1.4	66.3	3.8	1.1	-58
Foramsulfuron	49	35	30	63.0	11	24	-17	Tepraloxydim Isomer 1	73.6	7.3	9.0	83.4	3.0	10	-81
Halosulfuron-methyl	58	18	38	74.0	2.3	28	-77	Tepraloxydim Isomer 2	58.2	6.8	28	67.0	7.0	25	-83
Imidacloprid	80.0	4.4	40	77.3	3.8	32	-85	Terbufos	98.6	9.6	35	74.6	11	24	-56
Inabenfide	73.3	4.0	7.3	81.0	12.0	4.5	-55	Terbuthylazine	62.2	4.2	12	70.4	5.0	11	-76
Sulfentrazone	93	43	46	89	15	22	-6	Tetrachlorvinphos (Stirofos)	62.1	6.9	27	74.5	2.2	23	-78
Isoprothiolane	79.2	3.0	12	93.6	5.6	11	-98	Tetradifon							-51
Isoproturon	74.0	1.5	10	92.6	2.5	10	-70	Thiacloprid	65.0	14	13	71.4	6.5	12	-93

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSDr	%RSD$_{wR}$	
Isopyrazam	46.9	4.9	4.8	59.0	3.8	3.5	-42
Lufenuron							-100

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	RSDrw	%R	%RSDr	%RSD$_{wR}$	
Triadimefon	108	25	30	76	30	22	-98
Trichlorfon (Dylox)	83.6	2.9	25	89.4	1.8	21	-85
Tritosulfuron	72	21	20	68.7	12	17	-75

Table AI.6: Validation Parameters for trueness, precision and matrix effect for negative ionization mode LC-ESI-QTOF.

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$	
Acephate	92.0	6.2	14	67.6	11	10	-26
Benodanil	81.1	8.1	6.7	75.2	11	9.2	-48
Boscalid	83.0	2.1	18	80.8	8.5	15	-42.3
Bromoxynil	54.1	3.9	5.4	50.86	1.5	3.9	-59.2
Climbazole	66.5	6.4	32	63.8	4.7	20	-6.8
Cyanazine	125	64	57	59	30	19	-74
Cyproconazole Isomer 1	▨	41	38	143	56	15	44
Cyproconazole Isomer 2	▨	52	41	81	14	12	143
Difenacoum	15.7	35	29	18.0	23	18	-31
Dinoseb	71.6	3.7	13	55.5	5.7	10	20.6
Dinoterb	67.8	7.7	8.9	51.9	6.7	5.7	19.7
Endosulfan-sulfate	47.5	14	12	47.4	11	10	35
Fenarimol	76	13	15	68.1	11	11	-11
Flamprop	58.4	13	12	57.0	6.1	9.2	22.5
Fluazuron	13.5	34	28	19.1	16	20	-1
Flubendiamide	71.0	3.3	2.8	76.9	4.8	4.2	-42.4
Fludioxonil	81.5	5.0	9.4	71.2	2.6	7.4	15
Flufenoxuron	6.0	46	48	6.62	13	22	-26
Halosulfuron-methyl	79.9	8.8	44	72.2	6.7	26	29
Imidacloprid	95.6	6.5	7.5	71	15	6.8	-84
Inabenfide	76.0	7.5	26	76.5	3.9	22	-38
Isopyrazam	61.8	7.7	13	71.3	2.2	11.0	62
Lufenuron	15.1	33	38	18.1	7.8	30	-25
Mesotrione	45.9	13	59	53.7	7.5	32	-2
Metaflumizone e-isomere	6.6	45	41	9.1	22	38	-10
Methoxyfenozide	82.37	0.89	6.4	78.0	1.4	5.2	-44
Procymidone	108	15	21	80	38	18	-74
Pyroxsulam	▨	▨	▨	▨	▨	▨	-68
Spirotetramate-enol	85.5	6.0	32	61.4	4.8	20	-35
Spirotetramate-keto-hydroxy	▨	▨	▨	▨	▨	▨	-100
Sulfentrazone	81.7	6.0	19	74.7	2.6	16	21
Tebuconazole	46.0	15	48	68	16	40	-14
Tepraloxydim Isomer 1	61.6	2.5	2.2	51.1	12	1.9	-89
Tepraloxydim Isomer 2	75.1	2.2	16	54.2	13	1.7	-28
Thiacloprid	▨	▨	▨	▨	▨	▨	-100

Table AI.5: Validation Parameters for trueness, precision and matrix effect for positive ionization mode GC-APCI-QTOF.

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME	Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$			%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$	
2.4-DDT	96	20	12	107.5	11	9.2	-35	Folpet	198	17		213	16		-71
4.4-DDD	63.5	13	15	68.5	18	11	68	Heptachlor Epoxide	112	8.2	10	77.3	14	9.1	-27
4.4-DDE	121	10	9.1	35.6	27	8.3	-29	Heptachlor	293	18	7.1	255	9.5	6.3	58
4.4-DDT	107	21	5.1	60.6	11	4.5	-86	Hexachlorobenzene	651	25	6.4	721	18	2.5	32
Acephate	57.1	23.5	4.7	33.8	20	4.2	140	Hexachlorobutadiene							
Acrinathrin	53.0	4.4	13	112	22	11	-8	Iprodione	39.0	14	23	43.3	2.0	19.0	-48
Aldrin	239	22	10	227	17	8.7	-7	Isodrin	203	18	9.0	149	12	10	-6
Amitraz								Malaoxon	61.4	9.1	14	69.8	16	12	41
Atrazine	63.8	13	15	54.4	14	12	213	Malathion	48.3	15	25	35.6	9.0	21.0	-6
Azinphos Ethyl	60	15	30	36.8	17	25	76	Mercarbam	59.7	8.8	11	52.7	5.9	10.0	55
Azinphos Methyl	82.8	7.5	21	75.2	21	20	7	Metalaxyl	38.5	5.1	23	23.9	15	21	141
Benfuracarb							-80	Methacrifos	273	19	18	147	6.6	16.0	152
Bifenthrin	67.9	12	15	65.2	13	14	62	Methamidophos	80.1	29	5.8	39.0	15	5.4	257
Bromophos Ethyl	78	18	15	70.5	11	11	48	Methidathion	70.7	11	14	46.4	17	13	137
Bromophos Methyl	111	12	10	93.2	12	8.7	9	Methomyl							-50
Carbaryl Isomer 1	61.5	11	15	49.1	10	9.2	127	Methoprotryne	45.1	6.8	24	28.3	3.3	22.0	38
Carbaryl Isomer 2	78.5	32	10	60.8	15	7.8	20	Methoxychlor (DMTD)	62	15	16	29.2	9.4	15.0	95
Chlorfenvinphos mix of Z&E isomer 1	57.5	8.5	22	46	15	21	31	Metolachlor	51.0	20	8.3	27.9	7.9	7.2	-5
Chlorfenvinphos mix of Z&E isomer 2	51.7	3.9	17	40	15	12	96	Metribuzin	42.9	12	21	47.9	13	20	29
Chlorothalonil	77.8	24	3.5	160	5.9	2.6	-30	Monocrotophos	66.5	31	6.2	67.8	20	5.1	-8
Chlorpyrifos Ethyl	203	11	34	67.4	13	26	129	Myclobutanil	43.8	8.2	22	20.7	1.8	21.0	31
Chlorpyrifos Methyl	138	8.2	11	142	14	5.7	78	Omethoate	63.5	26	2.7	75	26	2.8	116
Climbazole	37.0	3.6		15	14		-68	Paraoxon Ethyl	71.2	21	3.6	69	16	3	47
Cyanazine	32.2	37	16	112	13	8.2	22	Parathion-Ethyl	80.5	13	10	66.5	6.5	9.2	103
Cycloate	251	18	24	193	14	22	188	Parathion-Methyl	110.0	19	4.0	137	12	2.5	66

Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME	Analyte	10 µg Kg^{-1}			20 µg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$			%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$	
Cyfluthrin Isomer 1	67.1	21	18	68	26	11	376	Penconazole	45.1	4.2	16	30.2	10	15	27
Cyfluthrin Isomer 2	131	37	51	308	17	29	716	Pentabromobenzyl acrylate	37.0	18	7.2	72.6	15	6.6	-12
Cyfluthrin Isomer 3	66.0	20	12	89	20	10	-49	Pentabromoethylbenzene	72	19	12	64	26	11	44
Cyfluthrin Isomer 4	86.8	18	14	104	5.8	16	-18	Pentachlorobenzene	1452	43		2178	15		194
Cyhalothrin-lambda Isomer 1	54.5	8.8	15	77.8	10	13	-27	Permethrin Isomer 1	62.7	16	17	65.0	15	16	-8
Cyhalothrin-lambda Isomer 2	95	34	58	64.2	5.3	30	1667	Permethrin Isomer 2	55.5	22	20	50.7	14	18	80
Cypermethrin Isomer 1	62.1	19	24	59.3	13	18	18	Phosalone	51.7	2.1	20	66.7	4.8	17.0	-13
Cypermethrin Isomer 2	59.9	11	19	71.9	7.9	17	18	Phosmet	63.2	9.3	17	70.9	18	15	9
Cypermethrin Isomer 3	58.8	10	21	61.4	15	11	37	Phosphamidon isomer 1	58.5	7.1	16	40.2	18	12	49
Cypermethrin Isomer 4	55.2	24	25	56.2	11	22	48	Phosphamidon isomer 2	52.7	6.3	16	41.2	25	13	126
Cyproconazole	45.6	0.33	23	25.7	4.0	21	52	Pirimicarb	69.4	19.0	4.8	70.5	18	4.2	31
Cyprodinil	62.9	8.5	10	53.2	17	7.9	81	Pirimiphos Methyl	72	15	20	48.0	10	17	67
DEET	121	15	11	80.5	19	10	111	Pirimiphos ethyl	73.9	7.5	13	53.9	8.0	12.0	133
Deltamethrin	40.1	36	26	59	8.4	26	-20	Procymidone	56.5	2.1	17	45.3	13	16	76
Diazinon	116	8.7	13	84.2	14	12	91	Profenophos	60.1	6.8	18	102	7.2	12.0	17
Dichlofluanid	102	4.7	13	42.0	18	12	-44	Propazine	60	13	14	42.8	12	13	70
Dichlorvos	326	23	7.3	202	10	6.6	472	Propham	221	27	3.5	182	12	3.6	20
Dicofol	136	23	2.3	96.2	8.3	1.8	10	Prothiophos	94	64	93	403.8	9.4	50.0	-19
Dieldrin	92.4	13	8.9	71	11	8.8	-37	Quinalphos	88	10	17	50.4	14	15	67
Dimethoate	60.1	18	27	38.0	24	21	33	Quinoxyfen	58.9	12	13	49.5	16	12	29
Diniconazole	42.0	7.2	20	27	16	18	17	Quintozene (Pentachloronitrobenzene)	380	15	10	448	7.3	9.2	66
Dinitramine	57.4	8.9	13	56.5	12	12	73	Simazine	63.1	11	16	49.4	10	12	107
Disulfoton	140	17	5	166	13	4.8	33	Spiroxamine Isomer 1	56.7	14	12	31.2	25	11	68
Endosulfan alpha							-96	Spiroxamine Isomer 2	42.4	18	7	34.9	8.5	6.3	1
Endosulfan sulphate	51.2	4.6	11	63.4	5.7	10	-45	Tebuconazole	47.9	13	23	27.1	12	16	10
Endrin	83.9	10	10	64.1	11	9.2	-11	Terbufos	109	21		103	8.2		52
Ethalfluralin	180	11	12	197	10	11	52	Terbuthylazine	61.4	14	15	54.9	10	12	150
Ethiofencarb	62.3	25.4	6.7	48.6	12	6.3	-4	Tetrachlorvinphos	59.8	10	12	67.8	14	11	-2
Ethion	58.7	10	18	55.6	9.2	9.0	56	Tetradifon	54.3	12	17	50.6	17	15	9
Ethoprophos	128	11	21	97.7	15	21	144	Tetrasul	67.9	21	13	66.6	15	11	52

Analyte	10 μg Kg^{-1}			20 μg Kg^{-1}			%ME	Analyte	10 μg Kg^{-1}			20 μg Kg^{-1}			%ME
	%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$			%R	%RSD$_r$	%RSD$_{wR}$	%R	%RSD$_r$	%RSD$_{wR}$	
Etrimphos	106	11	10	87.4	11	5.8	55	Triadimefon	49.9	5.3	22	33.4	16	21	96
Fenamiphos	40.3	23	32	34.8	8.1	29	-26	Trifluralin	187	16	8.7	230	14	8.8	59
Fenarimol Isomer 1	46	22	31	35.6	8.6	28	-6	a-HCH	236	13	13	227	7.0	12	-11
Fenarimol Isomer 2	39.4	4.8	24	25.5	20	16	89	b-HCH	83.9	11	8.7	94.7	11	9.1	6
Fenitrothion	93	48	33	166	16	30	-44	c-HCH (Lindane)	153.6	16	6.0	153.4	7.7	5.5	-3
Fenoxycarb	50.1	11	13	33.7	18	11	50	cis Chlordane	104	12	12	84.1	13	10	-11
Fenthion	86	16.7	7.5	77.3	8.4	6.4	102	d-HCH	96.6	13	3.0	242.0	12	11	-34
Fludioxonil	51.3	20		40.6	18		-35	trans Chlordane	115	25	1.6	49.2	2.3	1.2	
Fenitrothion	93	48	33	166	16	28	-44	c-HCH (Lindane)	154	16	6.0	153	7.7	6.2	-3
Fenoxycarb	50.1	11	13	33.7	18	11	50	cis Chlordane	104	12	12	84.1	13	11	-11
Fenthion	86.0	17	7.5	77.3	8.4	6.7	102	d-HCH	97	13	3.0	242	12	10	-34
Fludioxonil	51.3	20		40.6	18		-35	trans Chlordane	115	25	1.6	49.2	2.3	1.5	

ANNEX II

Table AII.1: Procedural Standard Calibration curves for 2-30 μg kg^{-1}, MLODs, MLOQs for positive ionization with LC-ESI-QTOF.

Analyte	**b (×10^3)**	**S$_b$ (×10^3)**	**a (×10^4)**	**S$_a$ (×10^4)**	**R^2**	**MLOD (μg kg^{-1})**	**MLOQ (μg kg^{-1})**	**Analyte**	**b (×10^3)**	**S$_b$ (×10^3)**	**a (×10^4)**	**S$_a$ (×10^4)**	**R^2**	**MLOD (μg kg^{-1})**	**MLOQ (μg kg^{-1})**
Acephate	36.07	0.80	0.1	1.3	0.998	0.028	0.084	Malaoxon	3.028	0.055	0.027	0.091	0.999	0.22	0.67
Ametoctradin	28.6	1.2	-3.7	1.9	0.993	0.66	2.0	Malathion	12.33	0.18	0.04	0.30	0.9992	0.51	1.6
Amitraz								Mandipropamid	12.96	0.35	-0.87	0.58	0.997	1.5	4.5
Atrazine	6.86	0.13	0.12	0.21	0.999	0.66	2.0	Mecarbam	35.04	0.73	-1.2	1.2	0.998	0.48	1.5
Atrazine-desethyl	1.96	0.10	0.34	0.17	0.99	2.9	8.7	Mephosfolan	4.423	0.056	0.085	0.093	0.9994	0.27	0.81
Azinphos-ethyl	2.288	0.041	0.231	0.068	0.999	0.49	1.5	Mesosulfuron-methyl							
Azinphos-methyl (Guthion)	0.638	0.047	0.116	0.077	0.98	2.2	6.5	Mesotrione							
Benfuracarb	0.01	0.41	1.22	0.68	0.0002	187	568	Metaflumizone	40.17	0.95	-0.5	1.6	0.998	0.46	1.4
Benodanil	11.64	0.19	0.15	0.32	0.999	0.34	1.0	Metalaxyl	12.19	0.32	0.46	0.54	0.997	1.6	4.8
Bifenthrin								Methabenzthiazuron	0.506	0.032	0.089	0.053	0.98	0.29	0.86
Bitertanol	1.981	0.031	-0.025	0.051	0.9990	0.37	1.1	Methacrifos Isomer 2	1.518	0.038	0.057	0.063	0.997	1.4	4.2
Boscalid	1.291	0.083	0.03	0.14	0.98	0.69	2.1	Methacrifos Isomer 1	33.61	0.84	2.8	1.4	0.998	0.17	0.53
Bromophos-ethyl								Methamidophos	13.83	0.15	-0.46	0.24	0.9996	0.85	2.6
Butocarboxim	4.20	0.11	0.69	0.19	0.997	0.53	1.6	Methidathion	6.10	0.17	0.73	0.29	0.997	0.081	0.25
Butoxycarboxim	4.325	0.080	0.32	0.13	0.999	0.35	1.1	Methomyl	9.63	0.34	0.15	0.62	0.996	2.4	7.1
Carbaryl	27.94	0.79	2.0	1.3	0.997	0.35	1.1	Methoprotryne	2.469	0.054	0.657	0.090	0.998	0.11	0.32
Carbendazim								Methoxyfenozide	19.62	0.38	-0.40	0.63	0.999	1.1	3.2
Chlorfenvinphos (E/Z) Isomer 1	31.6	1.0	2.8	1.7	0.996	0.78	2.4	Metolachlor	7.02	0.40	-0.53	0.66	0.99	3.1	9.4
Chlorfenvinphos (E/Z) Isomer 2	1.93	0.10	0.06	0.17	0.99	0.71	2.1	Metribuzin	18.33	0.42	1.10	0.70	0.998	0.96	2.9
Chlormequat	3.92	0.29	1.44	0.48	0.98	0.052	0.16	Monocrotophos	0.687	0.016	0.142	0.029	0.998	0.36	1.1
Chlorpyriphos	9.9	3.3	3.5	5.9	0.8	0.99	3.0	Myclobutanil							
Chlorpyriphos-methyl	0.551	0.024	-0.002	0.039	0.993	4.4	13.4	Nitralin	58.17	0.88	2.2	1.5	0.9991	0.0010	0.0030
Cyanazine	5.19	0.10	0.40	0.17	0.998	0.055	0.17	Omethoate	6.49	0.54	1.49	0.89	0.97	1.9	5.7
Cycloate	22.57	0.59	-1.00	0.99	0.997	1.7	5.0	Oxamyl	21.51	0.25	0.31	0.42	0.9995	0.29	0.88
Cyflufenamid	12.66	0.28	0.25	0.47	0.998	0.98	3.0	Paraoxon	9.34	0.53	0.96	0.89	0.99	0.72	2.2

Analyte	**b ($\times10^3$)**	**S_b ($\times10^3$)**	**a ($\times10^4$)**	**S_a ($\times10^4$)**	**R^2**	**MLOD (μg kg^{-1})**	**MLOQ (μg kg^{-1})**	**Analyte**	**b ($\times10^3$)**	**S_b ($\times10^3$)**	**a ($\times10^4$)**	**S_a ($\times10^4$)**	**R^2**	**MLOD (μg kg^{-1})**	**MLOQ (μg kg^{-1})**
Cyfluthrin Isomer 1								Paraoxon-methyl	1.370	0.051	0.1370	0.0051	0.994	0.21	0.63
Cyfluthrin Isomer 2								Parathion							
Cyhalofop butyl	0.117	0.040	0.318	0.073	0.7	49	149	Parathion-methyl	4.50	0.12	-0.08	0.20	0.997	0.77	2.3
Cyhalothrin (lambda-)	0.39	0.13	0.07	0.21	0.7	12	37	Penconazole							
Cypermethrin Isomer 1								Permethrin (cis-)							
Cypermethrin Isomer 2								Permethrin Isomer 1							
Cypermethrin Isomer 3								Permethrin Isomer 2							
Cyproconazole Isomer 1	0.137	0.015	0.074	0.028	0.96	15	47	Permethrin Isomer 3	2.71	0.10	0.19	0.17	0.994	0.62	1.9
Cyproconazole Isomer 2	1.116	0.032	0.317	0.054	0.997	0.42	1.3	Phosalone	4.065	0.066	0.26	0.11	0.9990	1.3	4.1
Cyprodinil	23.76	0.71	-0.8	1.2	0.996	2.0	6.0	Phosmet	7.21	0.14	-0.23	0.24	0.998	0.20	0.61
Cyromazine	25.5	1.1	1.4	1.8	0.992	0.21	0.65	Phosphamidon Isomer 1	6.800	0.097	0.09	0.16	0.9992	0.018	0.053
DEET (Diethyltoluamide)	39.1	1.3	-2.7	2.1	0.996	0.64	1.9	Phosphamidon Isomer 2	19.50	0.37	0.24	0.62	0.999	0.37	1.1
Deltamethrin Isomer 1								Phoxim	4.09	0.13	-0.21	0.21	0.996	4.0	12.2
Deltamethrin Isomer 2								Picoxystrobin	43.01	0.94	1.4	1.6	0.998	0.088	0.27
Demeton-S-methylsulfone	17.31	0.33	0.07	0.54	0.999	0.32	0.97	Pinoxaden	69.6	2.6	-6.9	4.3	0.994	0.83	2.5
Diazinon	117.2	7.5	8	12	0.98	0.93	2.8	Pirimicarb	103.6	4.4	0.3	7.4	0.993	1.8	5.3
Dichlofluanid	1.442	0.089	0.12	0.15	0.99	3.4	10	Pirimiphos-ethyl	67.9	1.7	-4.4	2.8	0.998	0.37	1.1
Dichlorvos	3.265	0.085	0.35	0.14	0.997	0.66	2.0	Pirimiphos-methyl							
Difenacoum Isomer 1	0.83	0.11	0.15	0.21	0.95	8.3	25	Procymidone	23.28	0.68	6.2	1.1	0.997	1.6	4.9
Difenacoum Isomer 2	1.01	0.19	0.04	0.35	0.90	7.1	22	Prometryn (Caparol)	1.813	0.040	0.179	0.067	0.998	0.13	0.40
Dimethoate	12.97	0.22	0.12	0.36	0.999	0.31	0.93	Propazine	9.70	0.15	0.07	0.24	0.9991	0.38	1.2
Diniconazole	1.927	0.026	0.042	0.043	0.9993	0.067	0.20	Propham	2.83	0.12	-0.17	0.20	0.993	1.2	3.6
Dinitramine	0.156	0.026	0.085	0.047	0.92	0.98	3.0	Propoxycarbazone							
Disulfoton	9.39	0.25	0.76	0.41	0.997	0.42	1.3	Prothiofos (Tokuthion)	2.85	0.38	0.29	0.63	0.93	1.0	3.1
Ethiofencarb	8.95	0.32	0.60	0.54	0.995	0.60	1.8	Pyridaben							
Ethion	25.9	1.8	1.3	3.0	0.98	0.84	2.6	Pyridalyl	4.153	0.072	-0.01	0.12	0.999	0.71	2.1
Ethoprophos. Ethoprop	9.61	0.23	-0.27	0.38	0.998	1.7	5.2	Pyrifenox Isomer 1	4.138	0.027	-0.175	0.045	0.9998	0.40	1.2
Etrimfos	61.9	2.4	-4.3	3.9	0.994	0.070	0.21	Pyrifenox Isomer 2	4.195	0.091	-0.22	0.15	0.998	1.2	3.6
Fenamiphos	4.88	0.14	-0.20	0.23	0.997	1.4	4.1	Pyroxsulam	12.958	0.075	0.02	0.12	0.9999	0.28	0.84
Fenarimol	0.935	0.048	0.041	0.080	0.99	2.8	8.6	Quinalphos	3.78	0.16	0.11	0.26	0.993	1.9	5.7

Analyte	b (×10^3)	S$_b$ (×10^3)	a (×10^4)	S$_a$ (×10^4)	R^2	MLOD (μg kg^{-1})	MLOQ (μg kg^{-1})
Fenitrothion							
Fenoxaprop-ethyl	18.50	0.63	1.1	1.1	0.995	0.71	2.2
Fenoxycarb	5.341	0.098	0.24	0.16	0.999	0.40	1.2
Fenthion	10.13	0.49	1.08	0.81	0.991	0.010	0.030
Fenthion-sulfoxide	28.02	0.72	0.3	1.2	0.997	0.75	2.3
Fenvalerate							
Flamprop	0.760	0.031	-0.019	0.051	0.994	2.2	6.7
Fluazuron	0.405	0.078	0.12	0.14	0.9	6.7	20.2
Flubendiamide	0.901	0.041	-0.162	0.074	0.994	2.7	8.2
Fludioxonil	1.249	0.040	-0.032	0.066	0.996	1.3	4.1
Flufenoxuron	0.122	0.051	0.07	0.10	0.7	22	66
Fluthiacet-methyl	4.529	0.096	-0.22	0.16	0.998	2.0	5.9
Foramsulfuron	0.477	0.045	0.002	0.081	0.97	1.9	5.6
Halosulfuron-methyl	1.020	0.072	-0.05	0.13	0.99	4.2	12.8
Imidacloprid	2.64	0.18	-0.08	0.33	0.99	4.1	13
Inabenfide	0.819	0.054	0.027	0.098	0.99	3.6	11
Sulfentrazone	1.427	0.058	0.024	0.096	0.993	0.075	0.23
Isoprothiolane	38.9	1.6	-3.2	2.7	0.993	0.58	1.8
Isoproturon	31.70	0.27	-1.11	0.45	0.9997	0.037	0.11
Isopyrazam							
Lufenuron	39.74	0.90	-1.4	1.5	0.998	0.61	1.8
Quinoxyphen	2.253	0.023	0.354	0.039	0.9996	0.65	2.0
Simazine	4.709	0.035	-0.181	0.057	0.9998	0.34	1.0
Simeconazole	9.14	0.30	-0.79	0.50	0.996	0.76	2.3
Spinosad A (Spinosyn A)	1.15	0.10	-0.17	0.19	0.98	5.4	17
Spinosad D	13.95	0.63	1.3	1.0	0.992	0.035	0.11
Spirotetramate-enol	2.266	0.033	0.130	0.055	0.9992	0.79	2.4
Spirotetramate-keto-hydroxy	9.91	0.30	0.82	0.49	0.996	0.43	1.3
Spirotetramate-mono-hydroxy	7.174	0.088	0.02	0.15	0.9994	0.67	2.0
Spiroxamine Isomer 1	5.34	0.17	-0.01	0.28	0.996	1.5	4.5
Spiroxamine Isomer 2	0.295	0.043	-0.101	0.086	0.96	9.6	29
TCMTB	0.802	0.036	0.066	0.060	0.992	1.8	5.5
Tebuconazole	4.32	0.14	0.07	0.24	0.996	0.24	0.73
Tepraloxydim Isomer 1	1.519	0.021	0.137	0.034	0.9993	0.75	2.3
Tepraloxydim Isomer 2	0.789	0.044	0.145	0.080	0.991	3.4	10
Terbufos	2.85	0.31	0.93	0.51	0.96	3.2	9.8
Terbuthylazine	14.85	0.47	0.49	0.79	0.996	0.12	0.37
Tetrachlorvinphos (Stirofos)	4.116	0.088	-0.15	0.15	0.998	1.0	3.1
Tetradifon							
Thiacloprid	4.19	0.15	0.31	0.24	0.995	1.9	5.8
Triadimefon	0.315	0.047	0.032	0.086	0.94	9.0	27
Trichlorfon (Dylox)	2.346	0.054	0.229	0.089	0.998	0.59	1.8
Tritosulfuron	0.580	0.086	-0.01	0.16	0.94	4.4	13

Table AII.2: : Procedural Standard Calibration curves for 2-30 µg kg^{-1}, MLODs, MLOQs for negative ionization with LC-ESI-QTOF.

Analyte	b (×10^{2})	S_b (×10^{2})	a (×10^{3})	S_a (×10^{3})	R^2	MLOD (µg kg^{-1})	MLOQ (µg kg^{-1})	Analyte	b (×10^{2})	S_b (×10^{2})	a (×10^{3})	S_a (×10^{3})	R^2	MLOD (µg kg^{-1})	MLOQ (µg kg^{-1})
Acephate	4.023	0.059	0.422	0.098	0.9991	0.20	0.62	Halosulfuron-methyl	61.5	1.9	-10.5	3.1	0.996	0.33	1.0
Benodanil	5.18	0.27	-0.78	0.44	0.990	2.6	7.8	Imidacloprid	1.73	0.26	0.33	0.53	0.96	10	31
Boscalid	9.96	0.31	-0.99	0.51	0.996	1.7	5.1	Inabenfide	66.5	1.0	-6.6	1.7	0.9991	0.84	2.5
Bromoxynil	42.4	1.8	-7.6	3.0	0.993	2.4	7.2	Isopyrazam	269	13	10	22	0.991	0.42	1.3
Climbazole	18.37	0.65	-2.5	1.1	0.995	1.5	4.6	Lufenuron	20.9	1.8	-2.4	3.0	0.97	1.9	5.6
Cyanazine	0.31	0.17	0.26	0.29	0.4	10	32	Mesotrione	6.02	0.45	-1.26	0.81	0.98	4.5	14
Cyproconazole Isomer 1	0.226	0.055	0.58	0.11	0.9	9.2	28	Metaflumizone e-isomere	30.3	5.1	-2.5	8.4	0.9	1.2	3.7
Cyproconazole Isomer 2	0.84	0.10	-0.26	0.20	0.97	7.9	24	Methoxyfenozide	55.3	1.8	-6.6	3.1	0.996	1.3	4.0
Difenacoum	75.9	9.2	-4	15	0.94	5.0	15	Procymidone	0.77	0.17	-0.27	0.33	0.91	14	44
Dinoseb	350.3	9.8	-30	16	0.997	0.90	2.7	Pyroxsulam							
Dinoterb	336.7	7.1	-32	12	0.998	0.21	0.62	Spirotetramate-enol	90.5	3.5	4.8	5.8	0.994	1.1	3.3
Endosulfan-sulfate	62.3	2.1	-7.8	3.5	0.995	1.2	3.5	Spirotetramate-keto-hydroxy							
Fenarimol	5.23	0.24	-0.76	0.39	0.992	0.95	2.9	Sulfentrazone	57.3	2.5	2.4	4.2	0.992	0.35	1.1
Flamprop	9.00	0.50	-1.61	0.83	0.99	3.0	9.2	Tebuconazole	1.46	0.11	-0.21	0.23	0.99	3.6	11
Fluazuron	30.4	2.1	-3.1	3.5	0.98	1.2	3.6	Tepraloxydim Isomer 1	7.11	0.15	0.49	0.27	0.999	1.3	3.9
Flubendiamide	132.5	1.9	-10.0	3.1	0.9992	0.77	2.3	Tepraloxydim Isomer 2	16.00	0.63	0.3	1.0	0.994	1.6	4.8
Fludioxonil	478	18	45	30	0.994	0.24	0.71	Thiacloprid							
Flufenoxuron	9.2	1.0	-1.4	1.7	0.95	0.74	2.2								

Table AII.3: Procedural Standard Calibration curves for 2-30 µg kg^{-1}, MLODs, MLOQs for positive ionization with GC-APCI-QTOF.

Analyte	b (×10^{-2})	S$_b$ (×10^{-2})	a	S$_a$	R^2	MLOD (µg kg^{-1})	MLOQ (µg kg^{-1})	Analyte	b (×10^{-2})	S$_b$ (×10^{-2})	a	S$_a$	R^2	MLOD (µg kg^{-1})	MLOQ (µg kg^{-1})
2.4-DDT	63.9	5.7	-0.41	0.94	0.97	1.4	4.4	Folpet	6.24	0.87	0.06	0.14	0.93	2.6	7.8
4.4-DDD	137.1	8.0	-2.3	1.3	0.99	0.94	2.8	Heptachlor Epoxide	34.2	1.6	-0.32	0.26	0.99	2.2	6.7
4.4-DDE	50.3	4.9	-1.64	0.81	0.96	0.10	0.29	Heptachlor	36.1	4.5	-0.19	0.75	0.94	0.045	0.14
4.4-DDT	6.13	0.22	-0.176	0.041	0.996	2.2	6.7	Hexachlorobenzene	70.5	9.5	-1.7	1.2	0.95	0.80	2.4
Acephate	37.9	3.7	0.19	0.61	0.96	5.2	16	Hexachlorobutadiene	4.02	0.46	-0.065	0.076	0.95	6.2	19
Acrinathrin	16.7	1.2	-0.15	0.21	0.98	2.5	7.6	Iprodione	3.22	0.30	-0.128	0.060	0.98	6.2	19
Aldrin	16.6	2.1	-0.29	0.26	0.95	3.7	11	Isodrin	36.8	3.6	-0.28	0.61	0.96	1.6	4.8
Amitraz								Malaoxon	83.9	8.8	1.6	1.5	0.96	5.2	16
Atrazine	311	24	-4.0	4.0	0.98	0.9	2.9	Malathion	43.8	6.3	-1.6	1.0	0.92	0.18	0.53
Azinphos Ethyl	50.1	4.0	-0.72	0.67	0.97	2.2	6.5	Mercarbam	122.8	6.7	-1.0	1.1	0.99	3.0	9.1
Azinphos Methyl	11.80	0.88	-0.18	0.14	0.994	3.8	12	Metalaxyl	104.7	6.4	-1.2	1.1	0.993	0.11	0.33
Benfuracarb								Methacrifos	344	18	-3.6	2.9	0.990	0.77	2.3
Bifenthrin	239	18	-5.6	3.0	0.98	1.5	4.6	Methamidophos	109.3	7.9	-1.8	1.3	0.98	2.7	8.1
Bromophos Ethyl	124.5	9.8	-1.1	1.6	0.98	0.14	0.41	Methidathion	53.6	4.2	-0.81	0.70	0.98	3.2	9.7
Bromophos Methyl	87.0	3.6	-0.99	0.59	0.993	0.44	1.3	Methomyl							
Carbaryl Isomer 1	30.4	1.7	-0.53	0.28	0.99	1.5	4.6	Methoprotryne	74.2	9.4	-1.4	1.6	0.94	1.2	3.8
Carbaryl Isomer 2	5.5	1.0	0.13	0.19	0.90	11	35	Methoxychlor (DMTD)	23.9	4.5	-0.87	0.81	0.90	0.90	2.7
Chlorfenvinphos mix of Z&E isomer 1	7.17	0.41	-0.007	0.069	0.99	3.2	9.6	Metolachlor	39.8	7.4	-1.2	1.2	0.9	10	31
Chlorfenvinphos mix of Z&E isomer 2	85.4	4.4	-0.64	0.72	0.990	2.8	8.5	Metribuzin	68.4	1.8	-0.32	0.31	0.997	0.32	1.0
Chlorothalonil	7.2	1.2	0.13	0.20	0.90	9.0	27	Monocrotophos	54.5	3.3	1.00	0.42	0.993	17	52
Chlorpyrifos Ethyl	246	60	52	10	0.8	7.6	23	Myclobutanil	57.5	7.7	-0.9	1.3	0.93	3.4	10
Chlorpyrifos Methyl	133	11	-0.4	1.8	0.97	1.9	5.7	Omethoate	70.4	8.3	-0.3	1.0	0.96	4.8	14
Climbazole	8.8	1.2	-0.61	0.24	0.96	4.9	15	Paraoxon Ethyl	155	11	1.3	1.8	0.98	3.8	11
Cyanazine	23.6	5.4	-0.49	0.74	0.91	10	31	Parathion-Ethyl	336	13	0.1	2.1	0.994	2.1	6.4
Cycloate	440	19	-7.6	3.2	0.992	0.38	1.2	Parathion-Methyl	222	17	0.0	2.9	0.98	3.5	11
Cyfluthrin Isomer 1	5.69	0.55	-0.180	0.085	0.991	4.9	15	Penconazole	41.0	3.5	-0.95	0.59	0.97	1.1	3.2
Cyfluthrin Isomer 2	9.52	0.11	-0.203	0.021	0.9996	0.72	2.2	Pentabromobenzyl acrylate	10.1	1.7	-0.36	0.24	0.94	7.8	24
Cyfluthrin Isomer 3	4.57	0.64	-0.05	0.12	0.94	8.4	25	Pentabromoethylbenzene	29.2	3.5	-0.73	0.43	0.96	1.1	3.4
Cyfluthrin Isomer 4	11.87	0.70	-0.07	0.13	0.990	3.6	11	Pentachlorobenzene	67	13	-1.9	1.5	0.91	7.5	23

Analyte	b ($\times10^{-2}$)	S_b ($\times10^{-2}$)	a	S_a	R^2	MLOD (μg kg^{-1})	MLOQ (μg kg^{-1})	Analyte	b ($\times10^{-2}$)	S_b ($\times10^{-2}$)	a	S_a	R^2	MLOD (μg kg^{-1})	MLOQ (μg kg^{-1})
Cyhalothrin-lambda Isomer 1	56.0	2.6	-1.08	0.43	0.992	0.062	0.19	Permethrin Isomer 1	39.5	5.1	-0.18	0.84	0.94	1.4	4.3
Cyhalothrin-lambda Isomer 2	25.6	3.0	-0.80	0.50	0.95	0.89	2.7	Permethrin Isomer 2	207	13	-3.7	2.2	0.98	0.39	1.2
Cypermethrin Isomer 1	18.9	1.1	-0.33	0.19	0.99	0.65	2.0	Phosalone	28.3	1.1	-0.18	0.18	0.994	2.1	6.4
Cypermethrin Isomer 2	17.6	1.3	-0.40	0.21	0.98	0.75	2.3	Phosmet	23.3	2.3	0.14	0.38	0.96	0.17	0.51
Cypermethrin Isomer 3	18.9	1.2	-0.38	0.19	0.99	3.4	10	Phosphamidon isomer 1	8.03	0.41	0.121	0.069	0.990	2.8	8.5
Cypermethrin Isomer 4	17.5	1.5	-0.38	0.25	0.97	2.2	6.7	Phosphamidon isomer 2	20.3	1.0	0.01	0.17	0.99	2.8	8.6
Cyproconazole	40.1	4.0	-0.12	0.67	0.96	2.4	7.3	Pirimicarb	135	11	-0.4	1.9	0.97	4.6	14
Cyprodinil	255	22	-1.7	3.7	0.97	3.3	10	Pirimiphos Methyl	176	18	-4.9	3.0	0.96	0.66	2.0
DEET	219	11	-3.1	1.9	0.99	1.3	4.0	Pirimiphos ethyl	321	24	-5.5	3.9	0.98	2.1	6.3
Deltamethrin	6.7	1.1	-0.13	0.18	0.90	8.9	27	Procymidone	160.6	7.8	-1.5	1.3	0.991	2.3	6.8
Diazinon	503	43	1.0	7.1	0.97	0.43	1.3	Profenophos	75.5	5.0	-0.63	0.62	0.99	2.7	8.2
Dichlofluanid	10.83	0.84	0.32	0.14	0.98	3.0	8.9	Propazine	112.4	8.5	-3.1	1.4	0.98	0.10	0.29
Dichlorvos	173	10	-3.0	1.7	0.99	0.77	2.3	Propham	134	10	-1.2	1.7	0.98	4.1	12
Dicofol	57.9	3.9	-0.12	0.64	0.98	3.7	11	Prothiophos	87	22	-2.6	2.1	0.9	7.8	24
Dieldrin	7.2	1.1	0.27	0.18	0.92	5.5	17	Quinalphos	142.4	5.7	1.26	0.94	0.994	0.39	1.2
Dimethoate	66.4	5.4	-1.50	0.89	0.97	2.0	6.0	Quinoxyfen	81.5	5.2	-1.16	0.86	0.98	1.3	3.8
Diniconazole	26.7	2.1	-0.59	0.34	0.98	2.4	7.3	Quintozene (Pentachloronitrobenzene)	57.1	6.6	-0.1	1.1	0.95	1.6	5.0
Dinitramine	105.7	6.2	-2.1	1.0	0.99	1.6	4.8	Simazine	30.4	1.6	-0.51	0.26	0.99	1.5	4.6
Disulfoton	15.5	1.3	-0.10	0.21	0.97	1.5	4.5	Spiroxamine Isomer 1	19.6	2.2	0.78	0.36	0.95	6.0	18
Endosulfan alpha								Spiroxamine Isomer 2	14.28	0.92	0.58	0.15	0.98	3.5	11
Endosulfan sulphate	19.9	1.5	-0.22	0.24	0.98	4.0	12	Tebuconazole	42.3	1.4	-0.40	0.23	0.996	0.82	2.5
Endrin	20.5	1.2	-0.23	0.20	0.99	1.4	4.3	Terbufos	17.0	1.6	-0.18	0.27	0.96	3.3	10
Ethalfluralin	146	12	-0.7	1.9	0.98	0.61	1.9	Terbuthylazine	300	20	-6.4	3.3	0.98	0.23	0.69
Ethiofencarb	5.41	0.40	0.103	0.066	0.98	3.5	11	Tetrachlorvinphos	43.1	4.8	0.44	0.80	0.95	6.1	19
Ethion	142.8	5.4	-2.15	0.90	0.994	0.025	0.076	Tetradifon	40.2	2.0	-0.57	0.33	0.990	2.5	7.7
Ethoprophos	86.9	6.3	-1.5	1.0	0.98	3.1	9.4	Tetrasul	23.6	3.2	-0.14	0.54	0.93	2.2	6.8
Etrimphos	334	19	-6.7	3.1	0.99	1.9	5.7	Triadimefon	83.1	6.2	-1.2	1.0	0.98	1.2	3.5
Fenamiphos	42.4	2.3	0.00	0.39	0.99	2.5	7.5	Trifluralin	175.5	8.1	-3.1	1.3	0.992	0.70	2.1
Fenarimol Isomer 1	43.9	6.4	-1.6	1.1	0.92	0.21	0.64	a-HCH	24.7	1.7	-0.12	0.28	0.98	1.6	4.9
Fenarimol Isomer 2	35.8	2.7	-0.66	0.45	0.98	0.54	1.6	b-HCH	16.42	0.89	-0.06	0.15	0.99	2.4	7.3

Analyte	b ($\times 10^{-2}$)	S_b ($\times 10^{-2}$)	a	S_a	R^2	MLOD (μg kg^{-1})	MLOQ (μg kg^{-1})	Analyte	b ($\times 10^{-2}$)	S_b ($\times 10^{-2}$)	a	S_a	R^2	MLOD (μg kg^{-1})	MLOQ (μg kg^{-1})
Fenitrothion	74	17	2.7	2.1	0.91	9.5	29	c-HCH (Lindane)	24.3	1.7	-0.08	0.28	0.98	3.8	11
Fenoxycarb	82.0	5.5	-1.45	0.91	0.98	3.2	9.6	cis Chlordane	17.36	0.91	-0.23	0.15	0.99	1.0	3.2
Fenthion	421	43	-2.2	5.3	0.97	4.1	13	d-HCH	10.2	1.5	0.03	0.25	0.92	1.2	3.5
Fludioxonil	63.4	6.6	-1.13	0.90	0.98	4.7	14	trans Chlordane							

REFERENCES

[1] United,Nations, International Code of Conduct on the Distribution and Use of Pesticides (Revised Version) (adopted by the Hundred and Twenty-third Session of the FAO Council in November 2002), Organization, 2002.

[2] European Council, Regulation 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, Official Journal of the European Union, vol. L 309/1, 2009, pp. 1–50. http://data.europa.eu/eli/reg/2009/1107/oj.

[3] D.J. Ecobichon, Pesticide use in developing countries, vol. 160, 2001, pp. 27–33.

[4] M.W. Aktar, D. Sengupta, A. Chowdhury, Impact of pesticides use in agriculture: their benefits and hazards., Interdisciplinary Toxicology, vol. 2, 2009, pp. 1–12.

[5] P. Schreinemachers, P. Tipraqsa, Agricultural pesticides and land use intensification in high , middle and low income countries, Food Policy, vol. 37, 2012, pp. 616–626.

[6] A. Masiá, M.M. Suarez-varela, A. Llopis-gonzalez, Y. Picó, Pesticides and veterinary drug residues determination in food by liquid chromatography-mass spectrometry: A review, Analytica Chimica Acta, 2016, pp. 1-69.

[7] T. Banaszkiewicz, Chapter 1 Evolution of Pesticide Use, Contemporary Problems of Management and Environmental Protection, vol. 5, 2010, pp. 7–18. http://www.uwm.edu.pl/environ/vol05/vol_05_chapter01.pdf.

[8] K. S. Delaplane, Human, Pesticide Usage in the United States : History , Benefits , Risks , and Trends, 1996.

[9] https://agrochemicals.iupac.org/index.php?option=c (Accessed 27 May 2019).

[10] https://www.epa.gov/ingredients-used-pesticide-pro (Accessed 27 May 2019).

[11] T. Tuzimski, Herbicides and Pesticides, 3rd ed., Elsevier Inc., 2018, pp. 1-9.

[12] T.C. Sparks, R. Nauen, IRAC: Mode of action classification and insecticide resistance management, Pesticide Biochemistry and Physiology, vol. 121, 2015, pp. 122–128.

[13] World Health Organization, The WHO Recommended Classification of pesticides by hazard 2009, Wissenchaftliche Verlagsgesellschaft mbH, 2010, pp. 1-78.

[14] C. Bolognesi, F.D. Merlo, Pesticides : Human Health Effects, 2nd ed., Elsevier Inc., 2019, pp 1-16.

[15] N. Kumar, A.K. Pathera, M. Kumar, The effects of pesticides on human health, General Pharmacology: The Vascular System, vol. 22, 2005, pp. 764.

[16] W. Mnif, A.I.H. Hassine, A. Bouaziz, A. Bartegi, O. Thomas, B. Roig, Effect of endocrine disruptor pesticides: A review, International Journal of Environmental Research and Public Health, vol. 8, 2011, pp. 2265–2303.

[17] K.R. Hakeem, M.S. Akhtar, S.N.A. Abdullah, Plant, soil and microbes: Implications in crop science, Plant, vol. I, 2016, pp. 1–366.

[18] H.K. Gill, H. Garg, Pesticides : Environmental Impacts and Management Strategies, 2014, pp. 187-230.

[19] J. Socorro, A. Durand, B. Temime-Roussel, S. Gligorovski, H. Wortham, E. Quivet, The persistence of pesticides in atmospheric particulate phase: An emerging air quality issue, Scientific Reports, 2016, pp 1-7.

[20] G.W. Ware, Effects of pesticides on nontarget organisms, Residue Reviews, vol. 76, 2012, pp. 173–201.

[21] C.E. Handford, C.T. Elliott, K. Campbell, A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards, Integrated Environmental Assessment and Management, vol. 11, 2015, pp. 525–536.

[22] A. Agüera, A.B. Martínez-Piernas, M.C. Campos-Mañas, Analytical Strategies Used in HRMS, 2017, pp. 59-82.

[23] http://www.fao.org/fao-who-codexalimentarius/codex (Accessed 27 May 2019).

[24] Food and Agriculture Union of United Nations, Submission and evaluation of pesticide residues data for the estimation of maximum residues limits in food and feed, 2016, pp. 1-286.

[25] European Commission, New rules on pesticide residues in food, 2008.

[26] https://ec.europa.eu/food/plant/pesticides/max_r (Accessed 27 May 2019).

[27] https://www.mpi.govt.nz/growing-and-harvesting/pla (Accessed 27 May 2019).

[28] Hellenic Republic, Ministry of Rural Development and Food, Law 4036/2012 “ Pesticide marketing , sustainable use and other provisions”, 2012, pp. 1-29.

[29] Ministry of the Reconstruction of Production, Energy and Environment, Implementation of Sud in Greece, 2009.

[30] http://www.minagric.gr/index.php/el/for-farmer-2/c (Accessed 27 May 2019).

[31] http://www.eurl-pesticides.eu/docs/public/tmplt (Accessed 27 May 2019).

[32] http://www.eurl-pesticides.eu/userfiles/file/SANTE (Accessed 27 May 2019).

[33] https://sisu.ut.ee/lcms_method_validation/31-li (Accessed 27 May 2019).

[34] European Comimission, Directorate of General Health and Consumer Protection, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Document no SANTE/2015/11945, 2015.

[35] Euroopean Commission, Directorate General for Health and Food Safety, Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed., 2017, pp. 1-42.

[36] M. S. Ladaniya, Commercial Fresh Citrus Cultivars and Producing Countries, Fruits, 2008, pp. 13-65.

[37] M. Ladanyia, Citrus Fruit, Elsevier Science, 2010, pp.103-124.

[38] http://www.fao.org/waicent/faoinfo/economic/fao (Accessed 27 May 2019).

[39] E. Cotton, M. Cotton, H. Gulchard, Citrus Fruits, Encyclopedia of Food and Health, 2015, pp. 119–128.

[40] https://www.medicalnewstoday.com/articles/27278 (Accessed 27 May 2019).

[41] https://orangebook.tetrapak.com/chapter/orange (Accessed 27 May 2019).

[42] J. Clever, C. Valverde, Expected Rebound in EU-28 Citrus Production may Reduce Imports for MY 2018 / 19, 2018, pp. 1-29.

[43] T. Tuzimski, Methods of Pesticide Residue Analysis, Journal of AOA C International, vol. 95, No. 5, 2012, pp. 1256–1257.

[44] Organisation for Economic Co-operation and Development, OECD, Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment, vol. 72, 2007, pp. 34.

[45] D.A. Lambropoulou, T.A. Albanis, Methods of sample preparation for determination of pesticide residues in food matrices by chromatography – mass spectrometry-based techniques : a review, 2007, pp. 1663–

1683.

[46] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and, Journal of AOAC International, vol. 86, 2003, pp. 412–431.

[47] A. Balinova, Strategies for chromatographic analysis of pesticide residues in water, vol. 754, 1996, pp. 125–135.

[48] E.A. Hogendoorn, High-Performance Liquid Chromatography Methods in Pesticide Residue Analysis, 2006, pp. 1-36

[49] https://www.austinpublishinggroup.com/analytical-p (Accessed 27 May 2019).

[50] K. Vekey, A. Telekes, A. Vertes, Medical Applications of Mass Spectrometry. Elsevier Science, 2008, pp.61-92.

[51] https://books.google.gr/books?hl=el&lr=&id=_WVDqDI (Accessed 27 May 2019).

[52] F.J. Santos, M.T. Galceran, The application of gas chromatography to environmental analysis, Trends in Analytical Chemistry, vol. 21, 2002, pp. 672-685

[53] M.S. Tswett, Mass Spectrometry and Gas Chromatography, Journal of the Mass Spectrometry Society of Japan, vol. 1959, 2012, pp. 3.

[54] https://teaching.shu.ac.uk/hwb/chemistry/tutor (Accessed 27 May 2019).

[55] J.R.J. Pare, V. Yaylayan, Instrumental Methods of Food Analysis, Chapter 7 Mass Spectrometry : Principles and Applications, Elsevier Science, 1997, pp. 239-266.

[56] F. G. Kitson, B. S. Larsen, C. N. McEwen, What is GC-MS, Gas Chromatography and Mass Spectrometry, 1952, pp. 3-23.

[57] A. Harrison, Chemical ionization, Journal de Chimie Physique, vol. 90, 1993, pp. 1411–1432.

[58] A.P. Bruins, W.M.A. Niessen, Atmospheric Pressure Ionization Techniques, Mass Spectrometry, Third Edition, Elsevier, 2019, pp. 299-305

[59] http://www.chm.bris.ac.uk/ms/apci-ionisation.xhtml (Accessed 27 May 2019).

[60] V. Desfontaine, J.L. Veuthey, D. Guillarme, Hyphenated Detectors, Supercritical fluid chromatography, 2017, pp. 214-244.

[61] D. Tsipi, H. Botitsi, A. Economou, Mass spectrometry for the analysis of pesticide residues and their metabolites, John Wiley & Sons, 2015, pp.131-160.

[62] F A Mellon, Principles and Instrumentation, Mass spectrometry, 2003, pp 3739-3749.

[63] R.A. Zubarev, A. Makarov, Orbitrap Mass Spectrometry, Analytical Chemistry, ACS publications, 2013, pp. 5288-5296.

[64] V.C. Fernandes, V.F. Domingues, N. Mateus, C. Delerue-Matos, Determination of pesticides in fruit and fruit juices by chromatographic methods. An overview, Journal of Chromatographic Science, vol. 49, 2011, pp. 715–730.

[65] T. Schwarz, T.A. Snow, C.J. Santee, C.C. Mulligan, T. Class, M.P. Wadsley, S.C. Nanita, QuEChERS Multiresidue Method Validation and Mass Spectrometric Assessment for the Novel Anthranilic Diamide Insecticides Chlorantraniliprole and Cyantraniliprole, Agricultural and food chemistry, 2011, pp. 814–821.

[66] A. Uclés, S.H. López, B. Reichert, A. Lozano, M. Dolores, H. Guil, A.R. Fernández-alba, Microflow liquid chromatography coupled to mass spectrometry - an approach to significantly increase sensitivity , decrease matrix effects and reduce organic solvent usage in pesticide residues analysis, Analytical Chemistry, 2014, pp. 1-13.

[67] P. Sivaperumal, P. Anand, L. Riddhi, Rapid determination of pesticide residues in fruits and vegetables , using ultra-high-performance liquid

chromatography / time-of-flight mass spectrometry, Food Chemistry, vol. 168, 2015, pp. 356–365.

[68] M. García, R.J. Fussell, S.L. Stead, D. Roberts, M. Mccullagh, R. Rao, Evaluation and validation of an accurate mass screening method for the analysis of pesticides in fruits and vegetables using liquid chromatography – quadrupole-time of flight – mass spectrometry with automated detection, Journal of Chromatography A, vol. 1373, 2014, pp. 40–50.

[69] C. Soler, B. Hamilton, A. Furey, K.J. James, J. Ma, Y. Pic, Optimization of LC – MS / MS using triple quadrupole mass analyzer for the simultaneous analysis of carbosulfan and its main metabolites in oranges, Analytica Chimica Acta, vol. 571, 2006, pp. 1–11.

[70] C. Soler, J. Ma, Y. Pic, Routine application using single quadrupole liquid chromatography – mass spectrometry to pesticides analysis in citrus fruits, Journal of Chromatography A, vol. 1088, 2005, pp. 224–233.

[71] C. Soler, J. Ma, Y. Pic, Determination of carbosulfan and its metabolites in oranges by liquid chromatography ion-trap triple-stage mass spectrometry, Journal of Chromatography A, vol. 1109, 2006, pp. 228–241.

[72] F. Hern, O.J. Pozo, J. V Sancho, L. Bijlsma, M. Barreda, E. Pitarch, Multiresidue liquid chromatography tandem mass spectrometry determination of 52 non gas chromatography-amenable pesticides and metabolites in different food commodities, Journal of Chromatography A, vol. 1109, 2006, pp. 242–252.

[73] E. Regional, D. Food, R.N. West, P.A. Group, Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection, Journal of AOAC international, vol. 88, 2005, pp. 595–614.

[74] F.J. Arrebola, A.G. Frenich, Validation of a Gas Chromatographic – Tandem Mass Spectrometric Method for Analysis of Pesticide Residues in Six Food Commodities. Selection of a Reference Matrix for Calibration, Chromatographia, 2004, pp. 321–327.

[75] D. Štajnbaher, L. Zupan, Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry, Journal of Chromatography A, vol. 1015, 2003, pp. 185–198.

[76] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, Determination of Neonicotinoid Pesticide Residues in Chromatography Mass Spectrometry, Journal of Agricultural and Food Chemistry, 2003, pp. 2501–2505.

[77] M. I. Cervera, C. Medina, T. Portoles, E. Pitarch, J. Beltran, E. Serrahima, L. Pinneda, G. Munoz, F. Centrich, F. Hernandez, Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry, Analytical Bioanalytical Chemistry 2010, pp. 2873–2891.

[78] European Commission, LC-QTOF-MS/MS method for the simultaneous full scan and MS /MS analysis of pesticides in fruit and vegetables, pp. 1-10

[79] P. Taylor, P. Aysal, Á. Ambrus, S.J. Lehotay, A. Cannavan, Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction, Journal of Environmental Science and Health, 2007, pp. 481-490.

[80] D. Ortelli, P. Edder, C. Corvi, Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography – electrospray – tandem mass spectrometry, Analytica Chimica Acta, vol. 520, 2004, pp. 33–45.

[81] L. Cherta, T. Portolés, J. Beltran, E. Pitarch, J.G.J. Mol, F. Hernández, Application of gas chromatography–(triple quadrupole) mass

spectrometry with atmospheric pressure chemical ionization for the determination of multiclass pesticides in fruits and vegetables, Journal of Chromatography A, vol. 1314, 2013, pp. 224–240.

[82] F. Hernández, S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, B. Kasprzyk-Hordern, C. Ort, M. Reid, J. V. Sancho, K. V. Thomas, A. L. N. van Nuijs, E. Zuccato, L. Bijlsma, Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater, Mass Spectrometry Reviews, 2016 (online publication ahead of print)

[83] M. Krauss, H. Singer, J. Hollender, LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns, Analytical and Bioanalytical Chemistry, vol. 397, 2010, pp. 943-951

[84] A. A. Bletsou, D. E. Damalas, P. Gago Ferrero, E. L. Schymanski, H. P. Singer, J. Hollender, N. S. Thomaidis, Wide-scope quantitative target screening of 2327 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS

[85] J. Aceña, S. Stampachiacchiere, S. Pérez, Damià Barceló, Advances in liquid chromatography–high-resolution mass spectrometry for quantitative and qualitative environmental analysis, Analytical and Bioanalytical Chemistry, vol. 407, 2015, pp. 6289-6299

[86] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 17.8.2002

[87] I. González-Mariño, E. Gracia-Lor, R. Bagnati, C. P. B. Martins, E. Zuccato, S. Castiglioni, Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry, Analytical and Bioanalytical Chemistry, vol. 408, 2016, pp. 4297-4309
