Assessment of insecticides residues from some ruminants' tissues in Sulaimani province

Thesis submitted to the College of Veterinary Medicine, University of Sulaimani, in partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Veterinary Medicine/ Meat inspection and Hygiene

By

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Dedications

- **To my great family**
- **To assistant professor Dr Hazhaow Omer**
- **To my best friends**

To those who:

- **Helped me in this project**
- **Supported me during the hard times**

Abstract

The wide spectrum properties and benefits of some pesticides compounds have made them to be used in agriculture and livestock extensively. This has caused increasing the chances of accumulation in animal tissues and its products and raises in turn their negative health effects on consumers. Regarding animal product such as meat, the risk assessments of pesticides residues are based on residues levels in uncooked meat; even though, a large proportion of meat is either cooked or processed before consumption. Detection and quantification of pesticide multiresidues in complex matrices like meat and fat samples require a multistep procedure and should be validated in accordance of European Commission regulatory for analytical quality control and analysis of food and food products.

This project was designed to develop a method for detection and quantification of six pesticides in the muscle and fat tissues of cattle, sheep, and goats. Meat and fat samples were collected in Sulaimani slaughterhouse from carcasses of animals raised from five different districts including Darbandikhan, Said Sadiq, Arbat, Bazian, and Piramagrun.

The analysis was performed based on developed QuEChERS extraction method, including liquid-liquid partition (LLP) and dispersive solid-phase extraction (d-SPE).The detection was performed by using high performed liquid chromatography coupled with ultraviolet detector (HPLC–UV), and gas chromatography coupled with mass spectrometry (GC–MS).

In this study, the effect of boiling (100°C, 30 min) and broiling (176°C, 20 min) were also tested on the reducing level of pesticides in meat and fat samples, and the results were compared statistically. Finally, HPLC-UV and GC-MS performances were compared.

In HPLC and GC analysis, acceptable recovery of all analytes were obtained at concentration levels from 0.01 to 0.1 mg/kg for four different spiking levels in meat and fat

samples. In HPLC analysis, responses were validated in correlation coefficients (r^2) of ≥ 0.9998 for meat and fat samples. Limits of detection values ranged from 0.003 to 0.013 and 0.003 to 0.016 mg/kg for meat and fat samples, respectively, and the limits of quantification values ranged from 0.011 to 0.039 and 0.010 to 0.048 mg/ kg for meat and fat samples respectively. The recoveries values that obtained for all spiked levels of the studied pesticides ranged between 78.08 to 101% and 77.3 to 106.2 % for meat and fat samples respectively, with relative standard deviation (RSD) of 0.5 to 15.7 % for meat and 0.2 to 12.9 % for fat samples.

In GC analysis the responses were validated at correlation coefficients (r^2) of ≥ 0.9997 for meat and fat samples. Limits of detection and quantification values ranged from 0.004 to 0.014 and 0.012 to 0.043 mg/kg for meat and 0.0052 to 0.014, and 0.015 to 0.044 mg/ kg for fat samples, respectively. The obtained recoveries values ranged between 79.2 to 104.3% with acceptable RSD of 0.32 to14.6% for meat samples. Similarly, for fat samples the recoveries values ranged from 81.5 to 98.6 % with acceptable RSD of 0.3 to 9.3 %.

The methods developed in this study were applied successfully for 300 meat and fat samples. The dominant pesticide residue found in cattle muscle and fat samples was hexachlorobenzene, while deltamethrin was abundant in sheep and goats meat and fat samples. In comparison between meat and fat samples, all pesticide residue concentrations were higher in fat samples of cattle, sheep and goat than in meat sample of the same animals. About the deference in the level of the studied pesticide between districts of Sulamani, the highest pesticide residual levels found in samples of animal carcasses raised from Bazian and Piramagrun, while the lowest residual levels found in samples collected from carcasses of animals raised in Darbandikhan.

According to the effect of heat treatment in reducing the level of pesticide in meat and fat samples, the effect of boiling in reducing the level of pesticides was significant ($P < 0.05$), whereas broiling showed much less efficient in reducing the level of pesticides residues. In both heating method, the highest reduction level noticed in pyrethroids, while the lowest reduction percentages presented in organophosphorus pesticides.

In comparison between HPLC and GC analysis, statistically there were no significant differences between the found pesticides concentrations in HPLC and GC for both meat and fat samples; however, HPLC presented a little higher sensitivity than GC.

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Declaration Form

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I certify that a Thesis Examination Committee has met on 10 / 10 / 2020 to conduct the final examination of (Ahmed Yaseen Hamadamin) on her thesis entitled "Assessment of insecticide residues from some ruminant's tissues in Sulaimani province". The Committee recommends that the student be awarded the Degree of Doctor of Philosophy.

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Date:

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Name: **Professor Dr. Faraidoon Abdul Sattar Mohammed**

Date:

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Material
BARCC	Brazilian Agricultural Research Cooperation Centre
BDL	Below detection limit
C18	Octadecylsilane
CAC	Codex Alimentarius Commission
CADs	Chloroacetamide
CARs	Carbamates
CMT	Cypermethrin
CPS	Chlorpyrifos
DDT	Dichlorodiphenyltrichloroethane
DMT	Deltamethrin
d-SPE	dispersive solid-phase extraction
EC	European Commission
ECA	European Cooperation for Accreditation
ECD	Electron capture detector
ECD*	Electrolytic conductivity detection
ECPA	European Crop Protection Agency
ESI	Electrospray Ionization
ELISA	Enzyme Linked Immunosorbant Assay
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
EU	European Union
EURL	European Union Reference Laboratory

FAO	Food and Agricultural Organization
FID	Flame ionization detector
Florisil	Synthetic magnesium silicate $MgSiO_3$
FP	Free from Pesticides
FPD	Flame photometric detector
FSIS	Food Safety and Inspection Service
FTN	Fenitrothion
GC	Gas Chromatography
GPC	Gel permeation chromatography
HCB	Hexachlorobenzene
HPLC	High Performance Liquid Chromatography
I.D	Internal Diameter
IQ	Intelligence quotient
IS	Internal Standard
ISO	International Organization for Standardization
JMPR	Joint FAO/WHO meeting on pesticide residue
LLE	Liquid – Liquid extraction
LLP	Liquid -Liquid Partitioning
LOD	Limit of Detection
LOQ	Limit of Quantification
m/z	Mass to charge ratio
MAE	Microwave-assisted extraction
mAU	milli Absorbance Unit
MeCN	Acetonitrile
MMC	Matrix Matched Calibration
MRL	Maximum residue limit
MS	Mass spectrometry

MS/MS	Tandem mass spectrometry
MSPD	Matrix solid-phase dispersion
NCCP	National Cancer Control Policy
NPD	Nitrogen phosphorus detector
MRM	Multiresidue method
NTN	National Toxics Network
OCPs	Organochlorines pesticides
OCs	Organochlorines
OPPs	Organophosphorus pesticides
OPs	Organophosphorus
P.S	Particle Size
PAHs	Polycyclic Aromatic Hydrocarbons
PID	Photoionization detector
PSA	Primary and Secondary Amine
PTN	Parathion
PYRs	Pyrethroids
PYZPs	Pyrazolopyrimidines
QqQ MS/MS	Triple quadrupole tandem mass spectrometry
QuEChERS	Quick, easy, cheap, effective, rugged and safe
R%	Reduction percentages
R.T	Retention time
R^2	Correlation Coefficient
SBSE	Stir-Bar Sorptive Extraction
SD	Standard deviation
SDI	Standard Deviation of intercept
SEM	Standard error of the mean
SFE	Supercritical fluid extraction

SPE	Solid Phase Extraction
SPME	Solid-phase microextraction
TCD	Thermal conductivity detector
TZN	Triazine
USDA	United States Department of Agriculture
USEPA	United states Environmental Protection Agency
UV	Ultraviolet
UV/Vis	Ultraviolet Visible
WHO	World Health Organization
WSS	Working standard solution
α-HCH	Alpha-Hexachlorocyclohexane

Chapter One

INTRODUCTION

A pesticide is any substance, intended for destroying or controlling any pest, including vectors of human or animal disease, nuisance pests, and unwanted species of plants or animals causing harm or interfering with the production, processing, storage, transport, or marketing of food, wood or animal feeding stuffs (FAO/WHO, 2016).

Pesticides have been classified based on numerous criteria, considering their targeted pest including insecticides, acaricides, bactericides, herbicides, fungicides, rodenticides, nematicides, molluscicides, avicides and algaecides (USEPA, 2018, Zhang et al., 2015); however, the most common classification are based on their chemical structure (i.e., pyrethroids, organochlorines, organophosphorus, carbamates, etc).

Since the discovery of the high efficient properties pesticides in the early 20^{th} century, the worldwide utilisation of pesticides has incredibly increased in agriculture and in veterinary medicine, and reached to more than 5 million tons per year (FAO, 2017), and due to this intensive use of pesticides, they reach a destination other than their target species, including non-target species such as, air, water, as well as residues in animals tissues which might be transferred to humans and cause toxicological risk that strongly impacts on human health and become a major public health problem worldwide (Gyenwali et al., 2017).

Pesticides of Organochlorines (OCPs), Oranophosphoruses (OPPs) are used in agriculture and Pyrethroids (PYRs) used in veterinary medicine and agriculture. Thus, fruit and vegetables, and animal products such as, meat, fish and dairy product have been identified at the primary immediate intake route of pesticides in general population, and since humans occupy the peak of food pyramid, the residue accumulation is more in them (Singh et al., 2017).

It has been announced that most of pesticides are potential carcinogenic compounds (NCCP, 2015), endocrine disruptors, and cause of birth defects, reproductive failure and sterility, deformations in fetuses, allergies, acute intoxications and even death (Ventura et al., 2016, Upadhayay et al., 2020). Therefore, to ensure food safety the European Community established Maximum Residue Levels (MRLs), which is the highest level of any pesticide and its derivatives and metabolites residue that is legally tolerated in food or feed (Codex Alimentarius, 2015).

Since red meat is one of the main components in human diet and it is an excellent source of protein and zinc, it is commonly used by consumers in very different types of food. Lean meat has a different level of fat according to the type of animal (cattle, sheep, goat) which is considered as 10-15% (Williamson et al., 2005); therefore, there is need for regular screening of meat and meat products for pesticide residues which is being felt in the trade and also consumers' level.

Due to the low levels of pesticides in food samples, several techniques with a high selectivity and detectability been developed for pesticides determination such as, gas chromatography (GC), liquid chromatography (LC), ultra-high-performance liquid chromatography, etc. (Zamora-Sequeira et al., 2019).

To ensure food safety, it is also significant to evaluate simple, cost effective strategies to reduce pesticide residue concentration in the food commodities. Understandably, food processing such as heat treatment considered as the best method at domestic and industry level to tackle the current scenario of unsafe food.

1.1. The aims of the study

1. To detect and quantify of six highly used pesticides in sulaimani province including, cypermethrin, deltamethrin, hexachlorobenzene, α-hexachlorocyclohexane, chlorpyrifos and fenitrothion in muscle and fat tissues of cattle, sheep and goats.
2. To develop a multiresidue method (MRM) by optimization of extraction processes of QuEChERS method and combined with HPLC-UV and GC-MS techniques.
3. To compare the residue levels in tissues between species (cattle, sheep, and goats), matrices type (meat and fat tissues) and districts (Darbandikhan, Said Sadiq, Arbat, Bazian, and Piramagrun).
4. To study the effects of boiling at 100°C for 30 min and broiling at 176°C for 20 min on the pesticides residue levels in meat and fat tissues.

Chapter Two

LITERATURE REVIEW

2.1. Pesticides

Pesticides are any compound or mixture of substances used to kill or control pests, including insects, rodents, fungi, unwanted plants, etc. (USEPA, 2018). Hence, pesticides can be insecticides, rodenticides, fungicides, herbicides, etc (Jayaraj et al., 2016). By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed properly (FAO/ WHO, 2016). The broad use of pesticides leads to its accumulation in ecosystem, which increases the possibility of their entering the food chain (Castillo et al., 2012).

There are over 350,000 current and historic pesticide products have been registered, while only over of 20700 pesticide items have enlisted for usage (USEPA, 2019).

Pesticides are not a modern invention, in 2500 BC; Sumerians used foul smelling sulfur compounds on their bodies to control insects. Later, inorganic compounds (mercury and arsenic), fumigants, oil sprays and sulfur ointments were used by Rome to control body lice (Davis, 2014).

The widespread use of natural pesticides such as nicotine, mercuric oxide, and copper sulfate as a fungicide began in the 17^{th} –18^{th} centuries in Europe (Kinkela, 2016). In the 19^{th} century, some researches were performed on natural compounds extracted from the roots of some tropical vegetables, perennial plants and flowers such as Chrysanthemums, which was coincident with rapid growth in the use of inorganic compounds (Matthews, 2018). Since that time to the present day, tremendous activities to develop of synthetic and biological based pesticides are continued (Lamberth et al., 2013).

2.2. Classification of pesticides

Recently, most popular classifications of pesticides are those based on the target pest organism, application and the chemical nature of the pesticide (Yadav and Devi, 2017). Pesticides have been classified based on target organism. The classification also based on application requirement such as pesticides are used in agriculture, veterinary medicine, public health, and domestic. The classification can also be based on chemical nature such as organic and inorganic compounds. Organic compounds also include natural and synthetic compounds. Inorganic compounds are simpler compounds, crystalline or salt-like appearance, and mostly stable environmentally such as, ferrous sulfate, copper sulfate, mercury, etc. (Tadeo et al., 2008). Natural pesticides are usually those derived from vegetable plants, bacteria or fungi such as pyrethrum, rotenone, spinosad, etc, while synthetic compounds are produced artificially which was mostly produced post-World War II such as DDT, malation, permethrin, etc. (Mossa et al., 2018).

Pesticides have also been classified according to their toxicity ranking, which ordered from the lowest to highest toxicity in numbers including I through IV, being extremely toxic, highly toxic, moderately toxic, slightly toxic, respectively (Yadav and Devi, 2017).

Researchers classified pesticides based on chemical nature as represented in table 2.1 (Kafkas et al., 2019, Lushchak et al., 2018, Jayaraj et al., 2016).

Table 2.1. Pesticides classification based on their chemical structure and sub-groups.

Groups		Chemical names
1	Pyethroids	Allethrin, Bonthrin, Dimethrin, Tetramethrin, Permethrin, Cyclethrin Furethrin, Fenevelerate, Alphamethrin, Cypermethrin, Deltamethrrin
2	Organochlorines	DDT, DDD, Dicofol, Eldrin, Dieldrin, Chlorobenziate, Lindane, Hexachlorocyclohexane, Hexachlorobenzene, Methoxychlor, Aldrin, Chlordane, Heptaclor, Endosulfan, Isodrin, Isobenzan, Toxaphene, Chloropropylate
3	Organophosphorus	Dimefox, Mipafox, Parathion, Ronnel, Enitrothion, Bidrin, Phorate, Fenthion, Fenirothion Caumphos, Temofos, Dichlorovas, Diptrex, Phosphomidon, Demetox, Oxydemeton-methyl, Malathion, Dimethoate, Trichlorofan, Chlorphyrifos
4	Carbamates	**Methyl:** Carbaryl, Carbanolate, Prupoxur, Dimethan, Dimetilan, Isolan, Carbofuran, Pyrolan, Aminocarb, Aldicarb **Thio:** Vernolate, Pebulate, Diallate, Monilate, Butylate, Cycloate, Trillate, Thiourea **Dithio:** Methan, Thiram, Ferban, Amoban, Ziram Polyran, Dithane M- 45
5	Trazines	Atrazine, Simazine, Ametryn, Atratone, Chlorazine, Cynazine, Cyprazine, Metribuzin, Propazine, Turbutryn, Simetryn
6	Phenyl amides	**Carbanilates:** Barban, Carbetamide, Chlororprofan, Prophan, Phenyl **Urea derivatives:** Fenuron, Monuron, Diuron, Flumeturon, Chloroxuron, Neburon, Bromuron **Acylanalide:** Propanil, Solan, Dicryl, Karsil, Propachlor, Alachlor, Butachlor **Toluidines:**Trifluralin, Dipropanil, Benefin, Oryzalin, Isopropanil, Nitralin **Acetamide:** Diphenamid
7	Phenoxy	2,4-D (2,4 Dichloro phenoxy acetic acid), 2,4 5 T(2,4 5 Trichloro Phenoxy acetic acid), Dichloroprop, Mecoprop, Sesone
8	Benzoic acid group	Dicamba, Dichlorobenil, Chloroambin, Tricamba, Bromoxynil
9	Heterocyclic compounds	Benzimidazole, Triazole derivatives
10	Phtalimides	Captan, Diflotan, Folpet
11	Dipyrids	Paraquat, Diaquat
12	Hydrocarbons, ketones, aldehydes derivatives	Benzene, Toluene, Cerenox
13	Fluorine-containing compounds	Cryolite, Acetoprole, Dichlofluanid
14	Copper-containing compounds	Champion WP, Caocobre, Macc 80
15	Others	Pentachlorophenol, Floroacetate, Phenyl mercuric acetate, Ethylmercuric Phosphate, Methyl mercuric chloride, Calcium arsenate, Lead arsenate, Cacodylic acid, Aluminium phosphide, Zinc phosphide, Phenol and nitrophenol derivatives. e.g., dinocap, dinoseb

This classification is the most common way to allocate pesticides based on their chemical structure and sub-group or exemplifying active ingredient. The commonly applied pesticides are in group of synthetic organic pesticides namely pyrethroids (PYRs), organochlorines (OCs), organophosphorus (OPs), and carbamates (CARs) (Jayaraj et al., 2016), which are mostly used in agriculture, veterinary medicine, and public health, etc. (Castillo et al., 2012).

2.2.1. Pyrethroids (PYRs)

Pyrethroids were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which extracted from flowers of pyrethrums (*Chrysanthemum cinerarriaefolium* and *Chrysanthemum coccinceum*), and modified to increase their stability in the environment (Gammon et al., 2019). They range from non-polar to low-polarity lipophilic compounds. Owing to their hydrophobicity, they tend to bio-accumulate in lipid compartments, and becoming a potential source of human exposure through foodstuffs (Ledoux, 2011).

The toxic effects of PYRs are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. Hence, PYRs change the dynamics of the Na^+ channels in the membrane of the nerve cell, and increase the opening time causes prolonging sodium current across the membrane in both insects and vertebrates, and causing disrupting nervous system (Dong et al., 2014). Other targets, particularly voltage-gated calcium and chloride channels, have been implicated as alternative or secondary sites of action for a subset of PYRs, which alters the activity of glutamate and acetylcholine receptors and adenosine triphosphatases and induces DNA damage and oxidative stress in the neuronal cells (Soderlund, 2012).

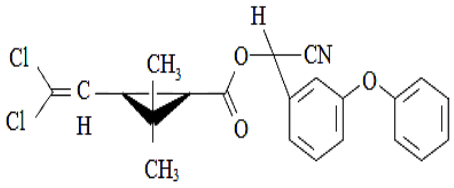
PYRs are reported to be 2250 times more toxic to insect than mammals due to insects' smaller size, lower body temperature and more sensitive sodium channels (Chrustek et al.,

2018). PYRs are mainly used to control insect pests of agriculture, horticulture, forestry and household. PYRs are considered comparatively safe but their extensive use makes them harmful for humans and animals (Bordoni et al., 2019).

2.2.1.1. Cypermethrin (CMT)

Cypermethrin is a highly active pyrethroid insecticide has forms of viscous yellow liquid to a semi-solid colorless crystalline mass at ambient temperatures (Laskowski, 2002). It dissolves in cyclohexane, ethanol, acetone, chloroform, and insoluble in water. CMT is a common use highly active type II synthetic pyrethroid insecticide that is chemically modified via the addition of α-cyano group at the phenoxybenzyl alcohol moiety resulting in improving of its photo-stability and potentiating of its toxicity (Habotta et al., 2018).

IUPAC Name	(RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis, trans-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylate
Synonym	Cypermethrin cis:trans/40:60
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Molecular weight	416.3 g/mol
Stability	Relatively stable in neutral and weakly acidic media, hydrolyzed in alkaline media. Relatively not very stable to light in field situations.



CMT is widely used in many tropical countries, and have become more popular on the area most severely affected by malaria (Moiroux et al., 2018). In agriculture, it is used to control many pests, including moth and pests of cotton, fruit and vegetable crops, while in veterinary medicine it is used as an anti-parasitic active ingredient against external parasites such as

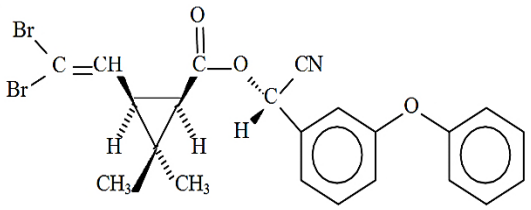
lice, mites, fleas, flies and ticks (Akre, 2016). It is also used in public health for crack, crevice and spot treatment to control pests in stores, warehouses, industrial buildings, houses, apartment buildings, and in non-food areas such as schools, hospitals, and hotels (Lainsbury, 2018). When CMT is exposed, it is poorly absorbed through the skin (Ensley, 2018). Compared to humans, CMT is absorbed very slowly in animals due to their hair coat (Larsen et al., 2019).

CMT toxic effect is on the nervous system, which disrupts sodium ion transport through the cell membrane and causes continuous opening of the sodium channel, which leads to the continuous depolarization of the membrane and blocking the generation of action potentials which strongly disrupts the transmission of nervous impulses (Nasuti et al., 2007).

2.2.1.2. Deltamethrin (DMT)

Deltamethrin is a colorless, white and light beige crystal in ambient temperature (Stenersen, 2004). It was described for the first time in 1974 and registered by the United States Environmental Protection Agency (USEPA) in 1994 (Ding et al., 2017).

IUPAC Name	(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate
Synonym	Decamethrin
Molecular formula	$C_{22}H_{19}Br_2NO_3$
Molecular weight	505.2 g /mol
Stability	Stable on exposure to air, and stable < 190^{o}C. More stable in acidic media than in alkaline media.



In veterinary medicine, DMT is used to control lice, fleas, ticks, etc. (Arsenopoulos et al., 2017), and to control vectors of malaria, such as *Aedes aegyptii* and *Anopheles gambiae* (Kongmee et al., 2019). In agriculture, it is used to control pests in open filed area and greenhouses such as aphids, cutworms, leafminers, etc, while in households it is used to control spiders, ants, bed bugs, cockroaches, etc. (Sands et al., 2018). DMT can get into animals body, through the skin, digestive and respiratory systems and, a part of it metabolized in the liver and a part excreted in the urine (Hedges et al., 2019).

2.2.2. Organochlorines (OCs)

Organochlorines are chlorinated hydrocarbon compounds including at least a single covalent bond of chlorine which affects the chemical behavior of the molecule (Kaushik and Kaushik, 2007). The chloralkaline class (Alkanes with one or more hydrogens substituted by chlorine) gives plenty structural variation and different chemical properties of OCs, which provides a wide range of names and usage (Afful et al., 2010). The commonly used OCs compounds in this group are DDT, methoxychlor, chlordane, dieldrin, mirex, toxaphene, lindane, hexachlorocyclohexane and hexachlorobenzene (MacBean, 2015).

OCs are too persistent in the environment, and remains in water, soil and air for ages; therefore, most of OCPs have been banned already (Jayaraj et al., 2016). The main way of OCs exposure is direct contact and ingestion (Waliszewski et al., 2003). In general, the mechanism of action of OCs insecticides is not yet fully understood, but it has been known that the mode of action is two types; namely, DDT type and chlorinated alicyclis type. The DDT-type insecticides inhibit the activation of sodium channels and the activation of potassium conductance, resulting in an increased negative after-potential and prolonged action potentials. As a result, repetitive firing and a spontaneous train of action potentials occur. The chlorinated mode of action

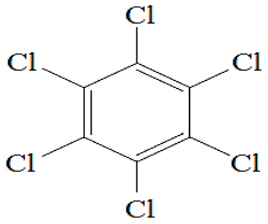
involving the binding at the picrotoxinin site in the gamma-aminobutyric acid (GABA) chloride ionophore complex, and this binding inhibits chloride flux into the nerve (Jayaraj et al., 2016).

OCs insecticides were commonly using in past, but many OCs compounds were removed from the markets due to their health and environmental effects (Pardio et al., 2012). Two of the commonly used OCs in agriculture are hexachlorobenzene and hexachlorocyclohexane.

2.2.2.1. Hexachlorobenzene (HCB)

Hexachlorobenzene is a white crystalline solid, sparingly dissolves in organic and halogenated solvents such as chloroform and less soluble in esters, short chain alcohols, and insoluble in water (Matthews, 2015). HCB was introduced as an agricultural pesticide in 1945, and was banned for agricultural purposes in the European Union (EU) from 1981 (Matthews, 2018). It is still used as an industrial chemical and is released to the environment; hence, it has been found in many crops, fruit and vegetables from contaminated areas (Owago et al., 2009). HCB is distributed globally by long-range and is highly bio-accumulated in lipid-rich tissues (Panseri et al., 2013).

IUPAC Name	1,2,3,4,5,6-hexachlorobenzene
Synonym	Hexachlorbenzol, Perchlorobenzene, Amatin
Molecular formula	C_6Cl_6
Molecular weight	284.8 g/mol
Stability	Very stable in environment, even to acids and alkalines



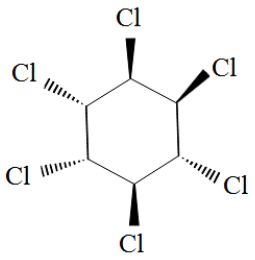
The major toxic action of HCB is on the nervous system, both central and peripheral. As this is a reversible process, HCB effects vary considerably in toxicity and have little or no obvious effect when dosages are small.

In agriculture, HCB is mostly applied as a seed dressing and crops such as wheat, barley, and oats to prevent growth of fungi, and used to prevent insects in grapes, lettuce, tomato, corn, rice, sorghum and cotton (Beltiz et al., 2004).

2.2.2.2. Hexachlorocyclohexane (HCH)

Hexachlorocyclohexane is a white needles, (brown to white in α isomer) in color, synthetic chemical exists in eight isomers (Matthews, 2015). It is a synthetic chemical that exists in chemical forms called isomers such as α, β, γ, δ, ε ζ, η, and θ, which are named according to the position of the hydrogen and chlorine atoms in the structure, that differ in the spatial orientation of the carbon atoms (Bradley et al., 2016). The γ-HCH has been banned, however it is still used in developing countries and available as an insecticide; as well as, available as a prescription medicine (lotion, cream, or shampoo) to treat and/or control scabies and head lice in humans (Lainsbury, 2018).

IUPAC Name	α-1,2,3,4,5,6-hexachlorocyclohexane
Synonym	Cyclohexane, 1,2,3,4,5,6, Cyclohexane, alpha-1,2,3,4,5,6-hexachloro
Molecular formula	$C_6H_6Cl_6$
Molecular weight	290.83 g/mol
Stability	Stable to light, heat, air strong acids. Unstable in alkaline condition.



Technical HCH including about 53–70% of α-isomer (Matthews, 2015), and commonly used as effective insecticide on fruit, vegetables, forest crops, and animals premises (Laquitaine et al., 2016). Technical HCH mostly is a mixture of α, β, γ and δ isomers, and been banned in the United States for many years; however, α, β, and γ-HCH continuously detected in environmental media because of the long environmental persistence of these compounds. The isomers properties and effects are different, for instance from the eight isomers, α and γ isomers are central nervous stimulants and β and δ are depressants (Davis, 2014).

Technical HCH is mostly used in agriculture to treat wheat, rice, maize, cotton, soybean, sorghum, orchards, and some vegetables to control the fungal diseases and bunt (Pereira et al., 2010). The α-HCH is among the used isomers in agricultural as insecticide (Kiranmayi et al., 2016), and a pharmaceutical treatment for lice and scabs (Bradley et al., 2016), with respect to their environmental fate and effects due to its potent insecticidal properties.

2.2.3. Organophosphorus (OPs)

Among pesticides groups, OPs compounds were first developed shortly before and during the Second World War which were used as an agricultural insecticides (Kazemi et al., 2012).

Organophosphorus pesticides (OPPs) binds to the active site of the acetylcholinesterase (ACh) and other cholinesterase (ChE) enzymes through formation of inactive phosphoryl esterases and inhibit them in the central and peripheral nervous system. The resulting accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death (Sogorb and Vilanova, 2002).

OPPs are nowadays hugely applied in agriculture after prohibition on many OCPs; hence, they created several health complications (Chawla et al., 2018). In agriculture, OPPs is used to protect vegetable crops, fruit trees, grains, cotton, sugarcane, and treat stored cereal such as

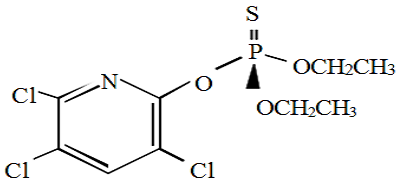
wheat, barley, oats, and rice; as well as, used to control a number of ectoparasites in domestic animals (Eto, 2018, Kavvalakis and Tsatsakis, 2012).

OPPs have a wide variety of physicochemical properties of polarity and water solubility. They are less persistent than OCPs, and they provide efficacious, safe and cost effective, control of wide range pests; therefore, they used to be the first choice for treatment. OPPs can be absorbed by inhalation, ingestion, and dermal absorption (Castillo et al., 2012). Among the OPPs, chlorpyrifos and fenitrothion are commonly used especially in agriculture (Eto, 2018).

2.2.3.1. Chlopyrifos (CPS)

Chlorpyrifos is a colorless to white crystalline, irregularly flaked solid, has a very faint mercaptan odor. It dissolves in methanol and isooctane but insoluble in water (Matthews, 2015). It is available in market as variety of formulations, including granules, wettable powder, dustable powder (crop-dusting), and emulsifiable concentrate (Davis, 2014). Among the various kinds of OPPs, chlorpyrifos is one of the most widely used in developing countries by the relevant plant protection organizations (Ghavidel et al., 2014).

IUPAC Name	Diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ^5-phosphane
Synonym	Dursban, Lorsban
Molecular formula	$C_9H_{11}C_{l3}NO_3PS$
Molecular weight	350.6 g/mol
Stability	Relatively stable in environment, and stable under 160^{o}C, but decomposed above 160^{o}C.

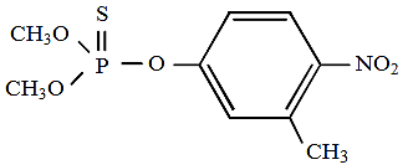


In agriculture, it is used as insecticide, acaricide and nematicide for the purposes of killing insects, worm and variety of other pests in horticulture. In forestry, it is used on a wide range of crops, and for the control of different types of pests including termites, mosquitoes, cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, fire ants, and lice (Rathod and Garg, 2017). CPS is used in the treatment of lawns /ornamentals pasture and farmsteads, indoor crack and spot treatment. It is also used in animal practice as ectoparasitic (pet collars, cattle ear tags) (Karabasanavar et al., 2012). In residential applications, it is also used to control cutworms, corn root worms, cockroaches, grubs, flies, termites, fire ants, and lice (Davis, 2014).

2.2.3.2. Fenitrothion (FTN)

Fenitrothion is an organic thiophosphate, brownish-yellow oily, broad-spectrum OPs, originally found in1969 as toxic substances (Matthews, 2015). CPS is a lipophilic pesticide dissolves in alcohols, esters, ketones, aromatic hydrocarbons, hexane, and insoluble in water (MacBean, 2015). Hence, it used against of the insects listed by the World Health Organization as effective vectors of malaria such as anopheles mosquitos (WHO, 2016).

IUPAC Name	Dimethoxy-(3-methyl-4-nitrophenoxy)-sulfanylidene-λ^5-phosphane
Synonym	Fenitrothion 122-14-5, Phenitrothion
Molecular formula	$C_9H_{12}NO_5PS$
Molecular weight	277. 24 g/mol
Stability	Relatively stable in environment, stable under 145^oC.



FTN is far less toxic than CPS and Parathion (PTN) with a wide range of insecticidal activity, and the difference in precursor chemicals make it somewhat more expensive (Eto, 2018). Hence, it is heavily used in countries where PTN has been banned such as Japan (MacBean, 2015). FTN in markets comes in flowable, fogging concentrate, granules, oil-based liquid spray.

FTN is an important component of quality assurance and resistance management programs used by the grain industry. FTN also serves a secondary purpose as a structure and equipment treatment, to remove residual infestations and prevent the establishment of new infestations in grain storages, and control of pasture pests. It is used in agriculture, horticulture, forestry and public health against chewing and sucking insects on rice, cereals, fruits, vegetables, stored grains, cotton, etc.; as well as, used for flies, mosquitoes and cockroaches in public health.

2.2.4. Carbamates (CARs)

Carbamates are also organic compounds derived from carbamic acid (NH_2COOH) include carbamate ester, ethyl carbamate, and carbamic acids (Roberts, 2008). CARs act through a similar mechanism as OPs, by blocking an enzyme essential for the transmission of nerve impulses (Immig and NTN, 2010). CARs are used as insecticides, herbicides, fungicides and nematicides, and they are less persistent than OCs and OPs (Zamora-Sequeira et al., 2019). Because of CARs class is not generally persistent in the environment; they are the mostly home used, indoors and on gardens and lawns against cockroaches, ants, fleas, crickets, scale, whitefly, lace bugs and mealy bugs (Hamey, 2003).

2.3. The use of pesticides

The world use for pesticides has reached to over 5 million tons per year, for different biological reasons and target organisms (FAO, 2017). According to the world ministry of health reports, about 80% of reported pesticide poisoning cases each year worldwide occur in developed countries (MacBean, 2015), and in many developing countries programs to control exposures are limited or non-existent (Matthews, 2019). Among countries, China is the major consumer of the pesticides and over 1,807000 tons of active ingredients used per year, followed by Argentina i. e. 207,706 tons of active ingredients, then USA, India, and Mexico, were in the line with 125,677, 110,100, and 98,814 tons of active ingredients used per year, respectively (FAO, 2017, Carvalho, 2017). European countries are the highest producers and traders of the pesticides worldwide, followed by USA, China, Brazil, Japan and India, respectively (Rani et al., 2017).

Agriculture is the largest user of pesticides compounds, consuming 85% of world pesticide production, to chemically control the various pests (Deguine et al., 2017). Overall 44% of used pesticides in the world are insecticides, 30% is herbicides, 21% is fungicides, and the 5% is others (Aktar et al., 2009). Crops that apply the bulk of these products are corn, cotton, potatoes, peppers, tomatoes, beans, cucumber, cabbage, cauliflower, wheat and barley (Deguine et al., 2017). Some pesticides, like herbicides, are applied to clear roadside weeds, trees, and shrubs and are commonly applied in ponds and lakes to control unwanted aquatic plants (Lushchak et al., 2018).The second most pesticides user is public health activities which consume about 10% of the total used pesticides to control vector-borne diseases such as malaria, control rodents, water purification, control pests in large structures such as malls, buildings, airplanes, trains and boats, in ornamental landscaping, recreational parks and gardens to control the proliferation of

insects, fungi and growth of grass and weeds (Lainsbury, 2018). A part of pesticides are also used in veterinary medicine and domestic care for treatment of external parasites (flea, mites, mange, lice, ticks, etc.) via the use of pool of pesticides, spray on the animals, ear tags, injectable, pour on, etc. Pesticides are also incorporated in products such as cosmetics, shampoos, soaps and insect repellents, washing and drying of carpets. Household disinfectants and care products for pets and plants could also be insecticides (Edwards, 2013).

Herbicides are mostly used at summer and autumn, while in winter and spring, fungicides, and insecticides compounds are more used; hence, pesticides case reports and residues in food products could be different between the seasons (MacBean, 2015).

2.4. Health risks of pesticides

Pesticides lead to over three million poisoning cases annually and up to 220,000 deaths, at least 50% of the intoxicated and 75% of those who die are agricultural workers; the rest is due to poisoning by using contaminated food (Singh et al., 2018).

In developing nations, many non-patented, more toxic, environmentally persistent, and inexpensive types of pesticides are used extensively, creating significant acute health problems and also local environmental contamination (Zhang et al., 2015).

Although the general population is exposed to these compounds, farmers are in a high risk group and therefore require bio monitoring studies to evaluate acute and chronic diseases caused by exposure to pesticides (Damalas and Koutrroubas, 2016, Zare et al., 2015). Farm workers who work in agricultural projects, in the pesticide industry, and exterminators of house pests are more susceptible for pesticide exposure; as well as, consumers who are on contaminated fruit, vegetables, and animal origin products (Soares and de souza porto, 2009).

Workers who mix, load, transport and apply formulated pesticides are normally considered to be the group that will receive the greatest exposure (Dhananjayan and Ravichandran, 2018).

Exposure of the general population to pesticides occurs mainly through eating food and drinking water contaminated with pesticides, or through inhalation of residual air concentrations or exposure to residues found on surfaces, clothing, or application equipment whereas substantial exposure to pesticides can also occur when living close to a workplace that uses pesticides or even when farm workers bring home contaminated articles (Kim et al., 2017).

Generally, the risks found to be associated with pesticides classified based on whether they had short-term effects such as, diarrhea, abdominal pain, headaches, nausea, vomiting, etc. or had the long-term effects such as, immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities, genetic disorders, diabetes, cancer, etc. (Aidoo et al., 2019).

Evidences exist on the possible role of pesticide exposures in the elevated incidence of human diseases such as carcinogenicity, neurotoxicity, pulmonotoxicity, reproductive toxicity, developmental toxicity, and metabolic toxicity, alzheimer, parkinson, amyotrophic lateral sclerosis, asthma, bronchitis, infertility, birth defects, attention deficit, hyperactivity disorder, autism, and diabetes (Mostafalou and Abdollahi, 2017). The possible association between exposure to pesticide and various types of cancers in humans, including prostate, bladder, lymphoma and multiple myeloma, and lung cancer have also been noticed extensively (Sabarwal et al., 2018, Silva et al., 2016, Jones et al., 2015). Some pesticides are also associated with gastric, skin, kidney, liver, testis cancer (Reji et al., 2016). Studies have also demonstrated that most of commonly used pesticides are carcinogenic (Costa, 2018), cytotoxic (Gogoi et al., 2016), genotoxic (Nada and Saleha, 2016), teratogenic (Yu et al., 2017), and immunotoxic (Medina-

Buelvas et al., 2019). Symptoms of severe poisoning include death due to respiratory paralysis and neurological complications have also noticed due to pesticides exposures (Changsheng et al., 2019).

More studies noticed possible link between pesticide exposure and respiratory diseases such as rhinitis (Slager et al., 2010); a well as, allergies and hypersensitivity (Sarwar, 2015), mutagenicity, reproductive and hormonal effects (Garg, 2016).

Although PYRs are thought to be safe for humans, but toxicological tests have shown that an excessive exposure to PYRs can cause serious health effects, such as paraesthesia, headache, dizziness, nausea, and skin irritation. For these reasons, pyrethroids have been included in the Group B substances (Veterinary drugs and contaminants) (Nardelli et al., 2018).

CMT has caused many health hazards including neurotoxicity, reproductive toxicity, and molecular toxicity especially in long-term exposure (Sharma et al., 2018). It has produced oxidative stress and enhances inflammatory damage of liver (Abdou and Sayed, 2019). Consuming the food under the effect of used CMT may cause impairments of the structure of seminiferous tubules (Hu et al., 2013), with decrease sperm count and pathological alterations in testes and epididymis (Ahmad et al., 2012).

DMT has also showed several health issues in human and animals. Long-term and low dose exposure to DMT has caused prolong headaches, lacrimation, abdominal pain, nausea, diarrhea, vomiting, apathy, ataxia, limb spasms, convulsions, allergic reactions and hypersensitivity to sound and touch (Kumar et al., 2011). Symptoms such as blurred vision and a burning sensation have been also observed (Khalatbary et al., 2015). DMT administered orally or through the skin may accumulate in brain neurons, and if exposed in pregnancy period may result in changes in fetal central nervous system (Kim et al., 2017, Viel et al., 2015).

HCB is also classified as a possible human carcinogen (Group B) compound based on tumor development studies on experimental animals (Starek-Świechowicz et al., 2017).

In humans, the symptoms described are general weakness, skin lesions, porphyria, hyposomia, osteoporosis, and arthritis (Daugaard-Petersen et al., 2018). Chronic exposure of humans to HCB leads to a number of effects, such as triggering of porphyria, microsomal enzyme induction, thyroid dysfunctions, neurological symptoms, and immunological disorders (Starek-Świechowicz et al., 2017).

HCH, is another probable carcinogenic OCPs, regarding the reports of US Environmental Protection Agency (USEPA) (USEPA, 2015a). The USEPA has recently included α-HCH on its fourth Draft Contaminant Candidate List (CCL 4) (USEPA, 2015b). Hence, it is proposed that occurrence HCH in public water supplies it is monitored as part of the 4th Unregulated Contaminant Monitoring Rule (UCMR) program (USEPA, 2015a).

Regarding isomers, USEPA classifies α-HCH as a probable human carcinogen (Bradley et al., 2016). The α-HCH has showed teratogenic, mutagenic and genotoxic effects and the main symptoms been recorded are vomiting, faintness, tremor, restlessness, muscle spasms, ataxia and clonic and tonic convulsions (Nayyar et al., 2014).

CPS is also very toxic when consumed by humans because of its property of acetyl-cholinesterase inhibition (Ghavidel et al., 2014). CPS is associated with paresthesia, tachycardia, seizure-like, and exposure to high dose leads to coma and finally death (Rathod and Garg, 2017). Several studies have indicated that CPS targets neurotransmitter systems and exposure in low-dose, causes neurochemical and neurobehavioural changes (Mie et al., 2018), even very low dose changes brain acetylcholinesterase (AChE) (Greer et al., 2019), and it is totally associated with reducing intelligence quotient (IQ) at school age children (Grandjean and Landrigan, 2014).

FTN is also toxic to vertebrates and invertebrates as cholinesterase inhibitors leading to a permanent overlay of acetylcholine neurotransmitter across a synapse. As a result, nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles, and leading to paralysis and death (Elhalwagy et al., 2008). General symptoms of FTN toxicity are, fatigue, headache, loss of memory and ability to concentrate, anorexia, nausea, thirst, loss of weight, cramps, muscular weakness and tremors (Stenersen, 2004).

2.5. Sources of contaminations and routes of entry

Animal feed that contaminated with pesticides could be the main source for accumulation of these residues in tissues of animals, which is directly proportional to their concentration in feed (Ledoux, 2011). Routes entries of pesticides in to body include direct entry by direct application on animals, while, indirect routes of entry include feeding and/or from environment (Lainsbury, 2018, Khan and Rahman, 2017). Further sources are drinking water contaminated through spraying field of crops, run off pollution, misapplication of pesticide to animals or their housing (Anju et al., 2010). Hence, pesticide residues in livestock accumulate by two ways; either pesticide are applied to animals through insecticide-impregnated ear tag, spray, dipping, self-treatment back rubber, dust bags and injectable or through pesticides application on agricultural crops and fodder and application in livestock areas (Khan and Rahman, 2017). The major source of residue in animal tissues and/or products is by presence pesticides residues in their feed stuffs; other factors that could be minor contribute to this sort of contamination included the application of pesticides on farm animals, environmental contamination and accidental spills (Kan and Meijer, 2007).

Animals such as cattle, sheep and goats are frequently raised on grass and/or left over crops, although supplementation with concentrates feed used and varies between farms and

districts. Thus, the differences in pesticide residual levels could be related to the farming system (Tsiplakou et al., 2010). Rainfall/ irrigation, vegetation and slope are environmental condition and affects pesticide runoff which finally affects the pesticides residual levels (Lushchak et al., 2018). Most of pesticides used in agriculture, animal husbandry, even poultry farms have specific affinity for adipose tissue, and all these ultimately lead to their accumulation in livestock products including milk, meat, internal organs, and poultry products; hence, fat, meat, animal organs, milk and other fat-rich substances are the key items for pesticides accumulation (Pardio et al., 2012).

2.6. Fate of pesticides

Researches have estimated only 1% of applied pesticides reach the target and remaining 99% goes waste and entered the environment as pollutant and remain for ages (Rathore and Nollet, 2012). Pesticide sprays directly hit non-target vegetation, or can drift or volatilize from the treated area and contaminate air, soil, and non-target plants. Drift can account for a loss of 2 to 25% of the chemical being applied, which can spread over a distance of a few yards to several hundred miles (Deguine et al., 2017). As much as 80– 90% of an applied pesticide can be volatilised within a few days of application, the rest remains for a long depends on the types of pesticides (Vanloon and Duffy, 2017).

Some fractions of the pesticide may be deposited on crop surface and transported by wind currents or deposited from the atmosphere and transferred to humans and animals via contaminated corps or directly used on animals; consequently, a part of pesticides store in the animal body, and the stored level depends on animals weight (Chevrier et al., 2000), age (Risher et al., 2010), route of contamination (EFSA, 2011), physical and chemical property of pesticides (Castillo et al., 2012).

2.7. Maximum residue limits (MRL) and pesticides regulations

The MRLs is the maximum amount of the pesticide residue which if found less in food substances may not to be a concern to human health (EC, 2019a). MRL builds-in a safety margin100x that of the actual safety level for a pesticide residue; hence, consumed pesticides little above MRL may not definitely cause health issues some times. While, food products that exceed a MRL are not allowed on the market totally, and finding exceeded pesticides than MRL, usually indicates that pesticide has been used incorrectly (ECPA, 2019).

MRLs for pesticides are set in European Union(EU), Canada, New Zealand, United States, etc., while their established levels occasionally are not same (FAO and WHO, 2019).

Beside EU, three federal government agencies shared the responsibility for the regulation of pesticides including Environmental Protection Agency (EPA), Food Safety and Inspection Service (FSIS) and United States Department of Agriculture (USDA) that approve the use of pesticides and established and the maximum amounts of residues that are permitted in a food (Lydy et al., 2004).

The Joint FAO/WHO meeting on pesticide residues (JMPR) is an international expert scientific group administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). JMPR has met regularly since 1963 and responsible for the risk assessment of pesticide residues in food and feed (Singh, 2017).

The Codex Alimentarius Commission (CAC) is another intergovernmental body established by FAO and WHO in 1963 to set international food quality and safety standards to protect consumers' health and to ensure fair practices use in the food trade (Singh, 2017).

Table 2.2. Established MRL of studied pesticides in animal meat and fat tissues (EC, 2019a).

Pesticides	**Commodity**	**MRL, mg/Kg**	**Reference**
Cypermethrin	Meat	0.05	(EU) R No.396/2005
		0.05	(EU) R No.2377/90, 2004
	Fat	0.2	(EU) R No.2377/90, 2004
		2.0	(EU) R No.396/2005
Deltamethrin	Meat	0.03	(EU) R No.396/2005
		0.05	(EU) R No.2377/90, 2004
	Fat	0.05	(EU) R No.396/2005
Hexachlorobenzene	Meat	0.02	(EU) R No.149/2008
		0.2	(EU) R No.396/2005
		0.01	(EU)R No.(EU) 978/2017
	Fat	0.2	(EU) R No.396/2005
α-hexachlorocyclohexane	Meat	0.02	(EU) R No.149/2008
		0.1	(EU) R No.396/2005
		0.01	(EU)R No.(EU) 978/2017
	Fat	0.2	(EU) R No.396/2005
Chlorpyrifos	Meat	0.05	(EU) R No 396/2005
	Fat	0.05	(EU) R No.396/2005
Fenitrohion	Meat	0.05	(EU) R No 396/2005
	Fat	0.05	(EU) R No.396/2005

2.8. Detection and quantification of pesticides

Pesticides could be in soil (Tor et al., 2006), air (Yusa et al., 2014), food (Fernandez-Gozales et al., 2013) and ground water (Lari et al., 2014); therefore, the detection method must be satisfied for all these kinds of matrices.

European Union Reference Laboratory (EURL) validates the methods used to monitor pesticide residue by verifying their sensitivity, specificity, accuracy and reliability (EC, 2019b).The agencies set methods that can identify as many pesticides as possible in a range of food commodities, and the method must be sensitive, use instruments associated soft and hardware, the reagents should be readily available (Khan and Rahman, 2017), as well as, it must be successfully validated through an inter-laboratory study (Pal et al., 2014).

Several procedures have been used to quantify of pesticide multiresidues in animal derived foods, but the key techniques are: firstly, how several varieties of residues among pesticides groups can be efficiently extracted from the complex matrixes; secondly, how abundance of cleaning up performed and interfering matters co-extraction with the pesticides; and thirdly, what analytical method should be proposed for the quantification. Therefore, the extraction and cleanup analytes before detection and quantification is a key factor to success the analysis technique, which must be able to extract target analytes and minimize co-extracts (Lehotay, 2011).

2.9. Multiresidue detection methods

Multiresidue methods (MRMs) come as closest methods required by the regulatory agencies for detection of contaminants (Tuzimski, 2016). MRMs contain the steps of preparation, extraction, cleanup, separation, and detection. They are designed to identify and quantify a number of pesticides and their toxicologically significant metabolites simultaneously in a range of foods (Vu-Duc et al., 2019).

MRMs usefulness is based on a combination of three factors (Khan and Rahman, 2017).

1. Detection abroad spectrum of pesticides and their metabolites in an array of food
2. Highly sensitive, precise, and accurate to be acceptable to the scientific community.
3. Economically suitable or at least affordable for laboratories.

The primary weakness of existing MRMs is that they cannot detect every pesticide in a single injection sometimes. A second weakness is that some MRMs require a great deal of time to perform the analysis.

Besides the use of MRMs, many laboratories develop and validate their own method for pesticide residues analysis. Using the same technique, but different equipment or equipment settings, also make difficulties to reach an accepted analytical method. Hence, the European Commission's Directorate for Health and Food Safety (DG SANTE and DG SANCO) provided guidance to laboratories for the validation of methods for pesticide residues analysis in food and feed. This to ensure a consistent and reliable approach with the use of quality control measures like certified reference materials and participation in proficiency tests. Almost all MRMs methods and validations established by European Commission (EC) are based upon chromatography either LC or GC as the determinative step (Vu-Duc et al., 2019).

2.10. Extraction techniques

Extraction means separation of compounds residues from matrices by using solvents. The extraction procedures for pesticides should be such that quantitatively extract pesticides form matrix (high efficiency), does not cause chemical change in pesticide, easily performed and use inexpensive apparatus (McMurry, 2014).

An inherent problem with a multiresidue approach is that matrix co-extractives as the method includes a wider polarity range of analytes; hence, no current method is suited for extracting all pesticides from all types of matrices (Picó et al., 2006).

Introduction of solventless techniques have become a benchmark in analytical perspective (Puri, 2014). Hence, it is preferable that sample preparation be achieved in minimal possible steps and solvents, to decrease the possibility of contaminations and/or losses and during sample preparation and handlings (Puri, 2014). Traditional extraction methods include solid phase extraction (SPE) and liquid-liquid extraction (LLE), which are multistage, consuming solvents and require a long time to execute (Lehotay, 2011). Recent approaches also includes multistep procedure for example supercritical fluid extraction (SFE) (Gallo et al., 2017), pressurized-liquid extraction (PLE) (Zhou et al., 2019), microwave-assisted extraction (MAE) (Wang et al., 2016), matrix solid-phase dispersion (MSPD) (Chatzimitakos et al., 2018), solid-phase microextraction (SPME) (Gomez-Rios et al., 2017), and stir-bar sorptive extraction (SBSE) (Xiao et al., 2016). However, the extraction method which have proved successful for extraction of several kinds of pesticides is QuEChERS “quick, easy, cheap, effective, rugged, and safe” (Kim et al., 2019).

2.10.1. QuEChERS method

The QuEChERS method was originally introduced for the extraction of multiresidue pesticides in various agricultural products with high water content (Anastassiades et al., 2003).

Later, the QuEChERS approach was continuously modified for extraction wide range of pesticides, including polar, non-polar, and planar pesticides in food and environmental matrices (González-Curbelo et al., 2015, Lee et al., 2017). The QuEChERS method aims are to simplify and streamline the extraction and purification procedures, minimize cost, with providing miniaturization and automation to make the analysis be easier. It has been more utilized with the chromatography detection techniques, due to their high sensitivity and specificity with the method (Lehotay, 2011). For analysis of multiresidue pesticides, the QuEChERS method has been steadily extended for the extract and analysis of various pollutants in different kinds of food and environmental matrices (Kalachova et al., 2013, Surma et al., 2017).

QuEChERS method has several advantages which have made it to be applied in most of laboratories for extraction of many kinds of compounds in different matrices. Firstly, the method steps are all relatively straightforward and can be easily modified. Secondly, it is user-friendly method because the extraction solvent, lab space, and dishwashing requirements are considerably lower than those for other approaches, which making it possible to reduce the time and costs associated with routine laboratory analysis (O'Sullivan, 2013).Third, it allows for the extraction of target compounds from a homogenized sample and purification effectively in less than 30 min (González-Curbelo et al., 2015, Grimalt and Dehouck, 2016). Hence, the method have been extensively developed and applied to extract many components in different kinds of matrices.

A literature research found that there were 2,087 articles that featured the term “QuEChERS” in their title, abstract, and/or keywords and 1,896 articles that featured both “pesticides” and “QuEChERS” together since 2009 (Kim et al., 2019).

In QuEChERS method, the initial step is liquid- liquid partioning (LLP), in which single-phase extraction of samples is performed with MeCN, for low fat samples and double-phase

extraction with MeCN and hexane are performed for high fat samples. In this step, anhydrous $MgSO_4$ and/or NaCl are also added to the solution. Later, eliminating residual water and cleanup are carried out by a procedure called dispersive solid-phase extraction (d-SPE), in which anhydrous magnesium sulfate $(MgSO)_4$, primary and secondary amine (PSA), and/or (octadecylsilane) C18 sorbent are simply added with the single or double- phase extract. Cleanup process also refers to a step or series of steps in the analytical procedure in which the bulk of the potentially interfering co-extractives are removed by physical or chemical methods. LLP extracts analytes and co-extractives in to the solution, and cleanup process minimizes matrix interferences. Both processes of LLP and d-SPE provide suitable recovery for LC and GC detected amenable analytes, give high reproducibility and less costly than many typical sample preparation approaches (Anastassiades et al., 2003, Wilkowska and Biziuk, 2011).

Several approaches have been attempted to eliminate co-extracted interference from extracts, including freeze centrifugation, gel permeation chromatography (GPC) (Frenich et al., 2006), adsorption chromatography on different adsorbents such as silica, florisil (Synthetic magnesium silicate $MgSiO_3$) or alumina on ready to use catridges (Pagliuca et al., 2006, Stefanelli et al., 2009).

The use of MeCN, hexane, PSA, C18, $MgSO_4$ and NaCL in the QuEChERS method proved successful for extraction of several pesticides classes from animal tissues such as, 16 PYRs, OCPs, OPPs, in meat and fat and animal organs (Castillo et al., 2012), 37 PYRs, OCPs, OPPs in fat tissue (Castillo et al., 2011), 45 PYRs, OCPs and OPPs, in meat and liver tissue (Meligy et al., 2019), 15 OCPs and 11 PYRs in meat (Paramasivam et al., 2011), 152 PYRs, OCPs and OPPs in meat (Oliveira et al., 2018), 24 PYRs, OCPs, OPPs in fish (Sahu and

Nelapati, 2018). The method has also used for extraction of veterinary drugs (Eprinomectin, Abamectin, Doramectin, and Ivermectin) in meat (Bandeira et al., 2017).

2.11. Detection and quantification methods

2.11.1. Chromatography

Initially chromatography was discovered as an analytical technique in the early 20th century in Russia. The name chromatography referring *chroma*, which means color, and *graphy* means writing, this is because the technique was firstly used for separating colored compounds (Vitha, 2016). Later, chromatography developed during the 1930s and 1940s and used for separating variety kinds of chemical compounds (Smith, 2013).

Chromatography separation techniques based on the principle that the compounds separated are dispersed between two phases; one of them is mobile while the other is stationary. Depending on the method, the mobile phase can either be a liquid or a gas, inspiring the names liquid (LC) and gas chromatography (GC) (Panseri et al., 2013).

Chromatography is a method by which mixtures are separated by distributing their components between two phases. The stationary phase remains fixed in place, while the mobile phase carries the components of the mixture through the medium (Vitha, 2016).

The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases. Because of the differences in factors such as the solubility and volatility of certain components in the mobile phase, and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture (McMurry, 2014).

There are different types of chromatography, and most commonly used chromatography methods for detection of pesticides are liquid and gas chromatography (Vitha, 2016).

In Liquid chromatography (LC) is an analytical chromatographic technique is based on separating ions or molecules that are dissolved in a solvent (Vitha, 2016). The LC methods are used for the separation and quantitative determination of non-volatile target compounds in medium to large range of molecular mass (Kromidas, 2017). LC technologies can be easily applied for the analysis of both water-soluble and water-insoluble analytes but the analysis of different types of materials needs different types of liquid chromatography. There are several types of liquid chromatography namely; high-performance liquid chromatography, liquid-solid chromatography, ion chromatography, affinity chromatography, etc. (Snyder et al., 2011). The most commonly used LC is high performance liquid chromatography (HPLC), which is recommended by European Directives in European Commission Decision, 2002/657 as an official control program of contaminant chromatographic methods for quantification of pesticides (Karageorgou and Samanidou, 2011).

2.11.1.1. High performance liquid chromatography

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is the most common technique of LC, officially invented in the late 1960s (Kromidas, 2017). Despite some concerns regarding the complexity of sample preparation, disposal of potentially hazardous effluents, and the possible unsuitability for high-throughput analyses, HPLC methods are still widely used separation techniques for determination of pesticides in a variety of food, water and biological settings (Velkoska-Markovska and Petanovska-Ilievska, 2019, Rajput et al., 2018, Shurubor et al., 2017, Harshit, 2017). It is known by different names such as high-pressure liquid chromatography, because of the high pressures required to force the mobile phase or solvent through the stationary

phase, and high-resolution liquid chromatography, because of the good resolution achieved using this technique (Giddings, 2017).

In HPLC system, the sample is injected into the column as the mobile phase, which flows over the stationary phase. These components leave the column at different time and reach the detector which detects the components and gives the signal to the recorder, which shows the chromatogram (Adamovics, 2017).

HPLC has been used for detection and quantification of many kinds of pesticides in different types of food and food products (Ledoux, 2011). It has been used for detection of 29 pesticides (CARs and OPPs) in liver tissue (Luzardo et al., 2014), 10 pesticides (PYRs, OCPs, and OPPs) in spiked minced pork and beef (Picó et al., 2006), 5 pesticides compounds (PYRs, OCPs, and OPPs) in cattle, meat, liver, kidney, and lung tissue (Muhammad et al., 2010), DMT in beef (Khashan, 2016), DMT in sheep and goat meat(Abdulrahman, 2016), 9 pesticide compounds (PYRs, OCPs, and OPs) in beef (Kiranmayi et al., 2016), CPS, α-endosulfan and β-endosulfan sulfate in buffalo meat (Kumar et al., 2008), detection of 171 pesticides (PYRs and OCPs) in beef (Oliveira et al., 2018). HPLC has also used for quantification of several OPPs in beef and cattle fat (Ioerger and Smith, 1993), atrazine and simazine in rabbit meat and fat (Baranowska et al., 2006).

Researchers have also used HPLC for identification of pesticides in fruits and vegetables such OPPs in cucumber, tomatoes (Peng et al., 2016) and PYRs, OCPs and OPPs, in lettuce and orange (Lehotay et al., 2005a).Therefore, HPLC technology has been described as an excellent tool for the identification and structural elucidation of metabolites and transformation products of pesticides in foodstuffs (Komidas, 2017). In HPLC system, there is no universal individual detector to monitor all types of compounds in food commodities; hence, several detectors have

been designed and applied for monitoring namely; ultraviolet (UV), ultraviolet visible (UV/Vis), photo diode array (PDA), evaporative light scattering (ELS), mass spectrometer (MS), conductivity (CD), fluorescence (FL), electro chemical detector (EC) and tandem mass spectrometry (MS\ MS) (Vitha, 2016).

Commonly used detector coupled with HPLC is UV detector which read a plenty range compounds in single run, since many compounds of interest absorb in the UV from plenty nanometer (Snyder et al., 2011). During the analysis, sample goes through a clear color-less glass cell, called flow cell in where sample absorbs a part of UV light. Thus, the intensity of UV light observed for the mobile phase (without sample) and the eluent containing sample will differ. By measuring this difference, the amount of sample can be determined. A standard UV detector allows user to choose wavelength between 195 to 370 nm, and most commonly used wavelength is 254-260 nm. Compared to a UV detector, a Vis detector uses longer wavelength (400 to 700 nm). There are detectors that provide wider wavelength selection, covering both UV and Vis ranges (195 to 700 nm) called UV/Vis detector (Snyder et al., 2011). HPLC-UV detectors can be used by any lab to analyse variety kinds of samples including pesticides in food samples (Peng et al., 2016) proteins (Sturaro et al., 2016), therapeutic drug testing (Luciani-Giacobbe et al., 2018), coenzyme A (CoA) and acetyl-coenzyme A (acetyl-CoA) in a variety of biological samples, including cells in culture, and plasma, liver, kidney, and brain tissues (Shurubor et al., 2017).

2. 11.1.2. Gas chromatography

Gas chromatography (GC) is another most common type of chromatography based method used for detection of pesticides. GC is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase such as nitrogen or helium

(McNair et al., 2019). GC firstly invented in 1952, since that time until now it has been used for detection and quantification the most types of compounds in foods and environment matrices. In GC, the components of a sample are separated based on difference in partition coefficients between a liquid stationary phase (silicone grease or wax) and a gaseous mobile phase (Hubschmann, 2015). In GC, the mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column, while stationary phase composed of a liquid or particulate solid in which the mixture is separated into its component compounds according to their affinity for the stationary phase. The column which the gas phase passes is located in an oven where the temperature can be controlled (McNair et al., 2019).

GC can be combined with different kind of detectors namely; mass spectrometry (MS), tandem mass spectrometry (MS/MS), photoionization detector (PID), flame photometric detector (FPD), thermal conductivity detector (TCD), electron capture detector (ECD), nitrogen phosphorus detector (NPD), electrolytic conductivity detection (ECD*), and flame ionization detector (FID) (Hubschmann, 2015).

GC has been used to quantify different kinds of pesticides in different matrices, such as the use of GC-MS for detection of 8 OCPs in beef, liver, and kidney (Letta and Attah, 2013), and 37 PYRs, OCPs, and OPPs in fat (Castillo et al., 2011). GC-ECD used for detection of 7 PYRs in beef (Niewiadowska et al., 2010), 8 PYRs in bovine fat (Akre and MacNeil, 2006), 15 OCPs in meat (Dimitrova et al., 2018), and 6 PYRs in meat and egg (Nardelli, et al., 2018), GC-MS/MS was used for detection 24 PYRs, OCPs, and OPPs in fish (Sahu and Nelapati, 2018), and 20 OCPs, OPPs and PAHs (Polycyclic Aromatic Hydrocarbons) in beef (Arioli et al., 2019).

Some detectors respond to any solute eluting from the column, while others respond only to solutes with specific structures, functional groups or atoms. The use of MS/MS may overcome

the problems arising from chromatographic interference that occurred with GC-MS and ECD (McNair et al., 2019), because it provides better sensitivity and able to determine pesticides from different classes and to identify their metabolites and degradation products in the same acquisition run (Giddings, 2017); while, it is still less used because of its high cost. MS detector is one of the most preferred by laboratories, due to its high sensitivity, suitability for halogen and non-halogen containing compounds (Grimalt and Dehouck, 2016). Compared to other detectors, MS more suffered from sample matrix interferences, due to the impossibility of monitoring all potential co-eluted compounds (Meligy et al., 2019). This has made difficulties to optimize a method reliably screen several groups of pesticides in a complex tissue. Hence, it has to be adapted with a sensitive analytical procedure during the analysis (McNair et al., 2019).

2. 11.2. Other methods

There are several other methods have been used for detection of pesticides include:

Biosensors, which are methods, have also been described as novel strategy and analytical machines (Lima et al., 2018). These devices have several advantages over traditional methods, for example, in their simplicity, sensitivity, selectivity and capacity to be deployed in the field, which is still highly desirable for the monitoring of pesticide contamination (Zamora-sequeira, 2019).They are coupled with bio recognition elements with various detection techniques and used for detection of biological components including, antibiotics (Majdinasab et al., 2019), and microorganisms (Kuss et al., 2018). It is also used for detection of heavy metals, illegal additives, pesticide residues, veterinary drug residues, biological toxins, and foodborne pathogen (Zeng et al., 2018). The significances of using biosensors are economical and can be produced in large number of samples and require less sample size and easy to operate even by non-skilled analyst (Hammond et al., 2016). In the detection of pesticides, biosensors pave the way to a more

efficient analysis, with greater precision, and a low cost (Zamora-sequeira et al., 2019). Nowadays, different types of biosensors are available, such as cell based biosensors, enzyme based biosensors, immunosensors, nucleic acid based biosensors, etc.

Enzyme Linked Immunosorbant Assay (ELISA), also used for detection pesticides in different kinds of food matrices especially fruit and vegetables (Malarkodi et al., 2017) and water samples (Xiang et al., 2019). In a last 4 year program, highly specific and sensitive antibodies against several groups of pesticides were studied. The study reported that direct and indirect competitive ELISA protocols were developed with the capability of reaching the detection limits ranging from 0.01 to 2.24 ppb (Chang et al., 2018). Commercial ELISA kits have been under development through technical transfer of the antibodies and the assay protocols to biotechnology companies since 2016 (Chang et al., 2018).

2.12. Validation

Validation is a thorough examination obtains realistic and unequivocal evidence that ensures either the procedure is effectively applicable for its purpose (Fajgelj and Ambrus, 2007). Validation proving that any approach, strategy, experimental procedure, laboratory staff, instrumentation, and room conditions selected for the method will function in a proper way under a fixed set of conditions (Gowik, 2009); as well as, checks if every measurement in routine analysis will obtain values close enough to the true value. Therefore, it verifies if the method is suitable to be used in quality control and research support.

The validation consists the determination of quality parameters namely; selectivity, accuracy (recovery percentages and precision), linearity, sensitivity (detection and quantification limits), and matrix effect (Magnusson, 2014, Galuszka et al., 2013). Aware of its importance, a number of international renowned organizations have offered guidance about method validation

namely; American Society for Testing and Material (ASTM), Environmental Protection Agency (EPA), European Commission referring Directorate for Health and Food Safety (DG SANTE and SANCO), European Cooperation for Accreditation (ECA), European Medicines Agency (EMA) (EMEA,2011), Food and Agricultural Organization (FAO), Food and Drug Administration (FDA), International Organization for Standardization (ISO), US Pharmacopoeia, World Health Organization (WHO) (Rambla-Alegre et al., 2012).

Consequently, many validation guidelines, with different scopes, have been issued to describe the validation parameters with their acceptance criteria which include.

Accuracy/trueness is the closeness between the concentrations provided by the analytical assay (calculated from the peak area through the calibration curve) and the true value which shows the systematic errors affect the result (Miller and Miller, 2018). Accuracy is usually determined in one of three ways. First, it can be assessed by analyzing sample of known concentration (reference materials) and comparing the measured value to the true value. The second approach is to compare test results from the new method with results from an existing alternate well-characterized procedure that is known to be accurate. The third approach based on the recovery of known amounts of analyte, is performed by spiking analyte in blank matrices.

Precision is also the closeness of agreement between the detector responses obtained by several individual measurements of a homogeneous sample, under stipulated conditions. In another word, precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent of deviations (EC No. SANTE/11813/2017). In chromatography science, the precision is expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Linearity also is the ability of the method to provide a signal directly proportional to the concentration of the analyte in the sample. Calibration standard curves for the linearity can be generated in two formats depending upon the methodology: standards in solvent and standards fortified into control matrix. The last option is more preferable, because the values would incorporate into calculation to correct the matrix effect (McMurry, 2014). The linearity should be evaluated across the working range of the analytical method and described by a linear regression plot of known concentration versus response signals using a minimum five different concentrations. The evaluation of the analytes for each calibration point should be carried out across at least three separate runs (Manav et al., 2018). Acceptability of linearity data is often judged by examining the correlation coefficient (r^2) and errors of intercept of the linear regression line.

Sensitivity is the lowest analyte concentration that can be measured with acceptable accuracy and precision (McMurry, 2014). In chromatographic analysis, it is calculated as the derivative of the peak area regarding the concentration, thus the slope of the calibration curve with the standard deviation of the intercept would inter the sensitivity values assessment. Finally, the values are expressed by the limit of detection **(**LOD) and limit of quantitation (LOQ) (Galuszka et al., 2013).

When a signal near the background noise is obtained, it must be decided if it corresponds to random responses of the blank or to the presence of the analyte. According to EC No. SANTE 11813/2017 guidelines, the fluctuation in baseline noise has a strong impact on LOD and LOQ values; hence, validation of LOD and LOQ in any condition is mandatory.

Limit of detection (LOD) is a value establishes the minimal concentration that provides a signal for 95% of the samples (i.e. a false negative rate of 5% is accepted) which can be reliably

differentiated from the background noise. Therefore, signals over that produced by the LOD are assigned to the analyte, whereas inferior values are attributed to the background noise. In the LOD assay, the presence of the analyte can be assessed, but not quantified with reliable accuracy and precision (EMEA, 2009). It is expressed as a concentration at a specified signal-to-noise ratio, usually 3:1. In the case of selectivity, the presence of false detects should be verified using non spiked samples. For each commodity group, a basic validation of a qualitative method should involve analysis of at least three samples spiked at the anticipated LOD.

LOD is calculated based on the standard deviation of the responses (SD) and the slope (y) of the calibration curve at levels approximating the LOD according to the formula: LOD =3.3(SD/y). The standard deviation (SD) of the response can be determined based on the standard errors of the matrix- matched standards on the regression line (Barganska et al., 2018).

The limit of quantitation (LOQ) in EC No. SANTE 11813/2017 criteria, is also defined as the lowest concentration of an analyte in a sample that can be quantified with acceptable precision under the stated operational conditions of the method. It should be remembered that the determination of LOQ is a compromise between the concentration and the required precision and accuracy, hence when the LOQ concentration level decreases, the precision increases. It has been recognized the 10:1 signal-to- noise ratio as typical LOQ. like in LOD, two additional options that can be used to determine LOQ, visual non-instrumental methods and calculating the LOQ (Luzardo et al., 2014, Barganska et al., 2018).

The calculation method is again based on the SD of the responses and the slope of the calibration curve (y) according to the formula: LOQ =10 (SD/y) (Barganska et al., 2018). Again, the SD of the responses can be determined based on the matrix-matched standards on the regression line. As in LOD, for LOQ an appropriate number of samples should be analyzed at the

limit to validate the level. One additional detail should also be considered; both the LOQ and the LOD can be affected by the chromatography. Hence, in HPLC and GC, sharper peaks result in a higher signal to-noise ratio, resulting in lower LOQ and LOD (EMEA, 2009). The LOQ should be less than the studied analyte found concentration the real sample (FDA, 2018).

About **matrix effects**, signal suppression/ enhancement that related to the commodity, is commonly called "matrix effect", may cause serious problems including inaccurate quantitation, low analyte detectability and increased method uncertainty (Uclés et.al., 2017). During extraction matrix constituents can be co-extracted and later co-eluted with analysed components and can consequently interfere with analyte identification and quantification. The co-extracted compounds, especially lipids, tend to adsorb by chromatographs especially in GC system such as injection port and column, resulting in poor chromatographic performance (Panseri et al., 2013). In LC and GC, matrix effects may impact the separation and detection steps in the analysis and leading to inaccurate quantitation and analyte detectability, reducing method ruggedness, and/or reporting of false results (Barganska et al., 2018). Hence, unavoidable presence of co-extracted matrix components in the final extract should be assessed at the validation stage.

For the assessment of the matrix effect, several designs of experiments have been proposed, for example, postextraction spiking experiments, and isotopic labelled internal standard (IS) spiking experiments; however post extraction spiking which is also called matrix-matched calibration standards is the most preferable to compensate of matrix effects (George et al., 2018), in which the standards are added to the extracts of blank matrix and used for calibration, which must be the same type as the real matrix sample (Peris-Vicente et al., 2013). According to EC No.SANTE 11813/2017 criteria, matrix effect percentages is assessed in the base of ME% standard values in which 100% indicates no effect, less than 100% indicates

ionization suppression and over 100% indicates ionization enhancement due to co-eluting sample compounds. From this definition, mostly in chromatography techniques, misunderstandings arise because the expression of "reduce matrix effect" does not mean reduced value of %ME, but also means the ME% ionization value becoming closer to 100%.

2.13. Prevalence of pesticides in meat

Pesticide residues include any derivatives of pesticides such as conversion products, metabolites, and reaction products considered being of toxicological significance, and growing concern due to possible adverse effects on humans (Panseri et al., 2013).

Different kinds of pesticides have been found animal tissues already, and many studies dealing with pesticides residues in animal tissues. In a study, high level of DMT (0.3- 1.13 mg/kg) found from sheep and goat meat samples collected near Sulaimaniyah (Abdurrahman, 2016). Researchers also found high DMT levels (0.4mg/kg) in beef samples collected in Baghdad (Khashan, 2016). CMT residual level (2.2 and 2.7 mg/kg) have been also found in beef samples in Faisalabad-Pakistan, where CMT applied in veterinary medicine and agriculture extensively (Muhammad et al., 2010). Moreover, cattle and sheep meat samples tested for CMT and DMT in Poland where rarely CMT and DMT are used in agriculture and livestock and found no residue for both (Niewiadowska et al., 2010). CMT and DMT were also found low level (< 0.05 and 0.013 mg/kg, respectively) in Rome (Italy) from 50 beef samples (Stefanelli et al., 2009). Moreover, researchers have found HCB in only 7% of lamb and beef in Jordan (Ahmad et al., 2010), and low residual level of HCBs was found in less than 10% of the analyzed cattle and sheep carcasses in Egypt (Sallam and Morshedy, 2008). Studies also found HCB < 0.05 mg/kg from cattle meat samples in Rome (Italy) (Stefanelli et al., 2009), while no HCB found in lamb samples collected in Almeria- Spain (Frenich et al., 2006).

Researchers also found high α-HCH in cattle (0.074 mg/kg) and sheep (0.039 mg/kg) meat samples collected in Andhra Pradesh-India (Kiranmayi et al., 2016). High level of α, β, and γ-HCH (0.0460, 0.0355 and 0.0626 mg/kg, respectively) were also found in cattle meat samples collected in Hyderabad, Telangana-India (Singh, 2017). The α-HCH was also found (<0.05 mg/kg) in beef samples collected in Rome (Italy) (Stefanelli et al., 2009).

Similarly, α, β, and γ, δ-HCH have been found in sheep meat samples (0.028, 0.035, 0.045, and 0.014 mg/kg, respectively) in Hyderabad, Telangana-India (Singh, 2017), and from mutton samples (0.18, 0.18, 0.6 and 0.42 mg/kg of residues of α, β, γ and δ-HCH, respectively from samples collected Coimbatore-India (Suganthy and Kuttalam, 2003). Other isomers such as γ-HCH (0.019 mg/kg), and δ-HCH (0.074 mg/kg) were also found in goat meat samples collected in Coimbatore-India (Suganthy et al., 2009). In another study, low level of α-HCH found in beef samples collected in Egypt which was 0.0089 and 0.004 mg/kg in samples had been imported from India and Brazil, respectively (Aboul-Enien et al., 2010).

Regarding fat tissues, high level of HCH mix isomers have been found in goat adipose tissues samples (0.573 mg/kg) collected in Punjab-India (Bedi et al., 2005), this supported by researchers who found high level of HCH (mixed isomers) residue in goat adipose tissue (0.084 – 0.18 mg/kg) in India (Singh et al., 2015).

Moreover, researchers have found high level of CPS (0.34 -0.35 mg/kg) in cattle meat samples collected in Andhra Pradesh-India (Muhammad et al., 2010). Similarly, for mutton samples, high residual level (0.46 mg/kg) in samples collected in Coimbatore-India (Suganthy et al., 2009); while, no CPS noticed in cattle and sheep meat samples collected in Hyderabad, Telangana-India (Singh, 2017). Low CPS residue (0.026.5 mg/kg) also found in cattle meat samples collected in Badrashen and Giza (Aboul-Enien et al., 2010), which was coincidence

with finding low CPS residue (0.016 mg/kg) in cattle meat samples collected in Egypt markets, which had been originally imported from Sudan (Aboul-Enien et al., 2010).

Yet, no enough study carried out to detect and/or quantify FTN residue in cattle, sheep and goats tissues; however, studies found FTN in fish (> 0.01µg/kg) (Akan et al., 2014), and whole milk (≥ 0.02mg/kg) (Anwar et al., 2011).

2.14. Minimizing pesticides residual levels in food

Pesticides are used indiscriminately and make the food unsafe for consumption. Besides, the presence of residues above the permissible levels is a major barrier in the approval of meats (Tilahun et al., 2016). Therefore, it is significant to evaluate, simple, cost effective strategies to minimize pesticide residue concentration in the food commodities. The strategies including food processing methods which are performed at home or industry such as washing, peeling, canning, and cooking, which are consider as proper methods to the reduce of pesticide residues in food and increase its quality (Keikotlhaile et al., 2010).

2.14.1. Heat treatment

To dissipate pesticides residues in food commodities, several heat treatment methods have been applied; namely, moist heat treatment such as braising, boiling, pressure cooking, stewing and dry heat treatment, such as broiling, barbequing, grilling, frying, roasting, baking, etc. (Yun-Sang et al., 2016). Studies represent that heat treatment cause to considerable reduction of pesticide residues in food commodities (Muthukumar et al., 2010, Witczak, 2009, Rajashekar et al.,2007); coincidently, they agreed with that the reduction level depends on thermal temperature, duration of heat treatment, methods of treatment, types of pesticides, and types of commodities. To confirm the safety of meats, the levels of pesticides should be determined

professionally before and after heat treatments which is important to develop some pragmatic procedures to increase the food safety along with the reduction in consumption of pesticide residues (Kiranmayi et al., 2016, Yun-Sang et al., 2016).

Reduction concentration of pesticide residues from heat treated meat and fat tissues could be through some physicochemical processes, i.e. decomposition, evaporation, co-distillation and thermal degradation, etc. which may vary with the chemical nature of the individual matrix and pesticide (Muresan et al., 2015). The most common heating processes used to reduce the pesticide load in raw meat and fat were boiling, broiling which used by consumes in the world (Goldwyn and Blonder, 2016), which have been focused in this study.

2.14.1.1. Boiling process

Boiling is a rapid heat treating of foods in a liquid at its boiling point (Vaclavik and Christan, 2008). Boiling is widely used for meat, by cooking meat in water at the boiling point of 100°C (Sobral et al., 2018).Certain boiling processes would reduce the levels of chemical contaminants in foods (Keikotlhaile et al., 2010, Kaushik et al., 2009). Boiling temperature and duration both play important role in the reduction of stored pesticide, beside the chemical and physical property of boiled compound (Muresan et al., 2015, Atkins et al., 2018). Researchers have boiled meat and obtained different results in terms of pesticides reduction levels because of using their different temperature and boiling period during the process.

In a study, boiling at 100^{o}C for 30 min was tested on α-HCH in beef samples and reduced 32.60 % in naturally contained α-HCH samples and 44.71 % in spiked samples. Isomers of β, γ, δ-HCH also reduced in natural contained samples by 15.2, 47.9, 12.3%, respectively, while in spiked samples were 27.5, 58.3, and 57.9%, respectively (Singh, 2017). The same boiling period and temperature were tested on mutton and noticed that α, β, γ, δ-HCH reduced in naturally

contained samples by 13.79, 27.76, 22.87, and 36.17%, respectively and in spiked samples were 50.67, 45.81, 48.27, and 38.0%, respectively. The α, β, γ, δ-HCH content in natural and spiked pork samples also tested by boiling temperature and reduced by 19.44,14.98,14.85 and 20.87% in natural and 15.81, 26.01, 26.7, and 25.25% in spiked samples, respectively (Singh, 2017).

The reduction in the levels of various OCPs after boiling of meat is also reported by Sallam and Morshedy (2008), who used 100^{o}C for 90 min and noticed overall reductions of 40.4%, 55.0%, 32.4%, 33.5%, 29.2%, 42.7% and 38.2% in DDTs, γ-HCH, dieldrin, aldrin, endrin, toxaphene and HCB contents, respectively. Boiling spiked meat at 100^{o}C for 30 min could also reduce OCPs by 31.6 % (dieldrin) and 33.2% (aldrin) (Krianmayi et al., 2016). Similarly, boiling for 30 min in low-density water impermeable polyethylene bags reduced the OCPs by 58.3% (α-Endosulfan) and 55.93% (β-Endosulfan) of pesticides in buffalo meat (Muthukumar et al., 2010).

Boiling at 100^{o}C for 30 min also tested on spiked cattle meat and mutton with CPS, and noticed the reduction level of 57.6 % and 58.5% for cattle meat and mutton samples, respectively (Singh, 2017). The same study reported that the result for spiked pork, fish and chicken after boiling was different because they noticed reduction level of 33.33%, 44.57% and 48.52% respectively, which were different from the reduction level in cattle and sheep meat samples.

2.14.1.2. Broiling process

Intense heating of foods in an oven at 170−288^{o}C is typical broiling process (Goldwyn and Blonder, 2016). Broiling differs from grilling and barbequing by the heat source in which is not just below the food. In the broiling processes, foods is heated intensively which hydrolysis the lipids at temperature raise above 100°C (Muresan et al., 2010). Broiling method used to enhance the flavor of meat in which browning occurring on the surface of the meat; this is accomplished

using dry heat. During broiling, thermo-degradation of fats is happened when the temperature is around 180°C for 30−60 min (Muresan et al., 2015). In broiling process, higher fat is remained in the tissue compared to boiling process (Utama et al., 2018), because the surface temperature well exceed 100°C, leading to evaporation of moisture in the food and dry or crisp the surface; hence, the a part of compounds are degrade, evaporate but not washed-out and the rest could be decomposed and remained in the matrix tissue (Muresan et al., 2015).

2.14.2. Other methods

Pressure cooking is another commonly used method in which providing heat and pressure together, produce high thermal steam and transferred to meat and affects far more quickly. Pressure cooking (121^{o}C, 30 min) has been tested on spiked beef with OCPs including aldrin and dieldrin, and reduced their concentration by 35.9- 34.6%, respectively (Kiranmayi et al., 2016). The method (130^{o}C for 1 hr) also tested on spiked fish with aldrin and dieldrin, and showed the reduction percentages of 91.4 and 91.3%, respectively (Muresan et al., 2015).

Frying is another commonly used heating treatment, in which the food fried for a short time. Two types of frying procedures is mostly used namely; pan frying on a thin layer of fats and oils, and deep frying, in which the food product is dipped into a fried oil (Boskou et al., 2006). In both types, the oil is heated to about 180–200°C for 2–10 min. Frying methods can destroy only the surface pesticides especially pan frying. However, the effect of deep frying is higher; it affects nutritional values and destroys antioxidants (Boskou and Elmadfa, 2011).

In a study, frying process was tested on spiked fish with α-HCH, β-HCH, γ-HCH, heptachlor, aldrin, heptachlor epoxide isomer B, pp'-DDE, endrin, pp'-DDT, pan-frying for 10 min caused significant losses in concentrations of most studied OCPs. The dissipation rate

ranged between 10-74% in different types of OCPs, but the most dissipated OCPs were β-HCH (Witczak, 2009). Another study tested pan-frying at 180^{o}C for 10 min on spiked pork with aldrin and dieldrin pesticides; reduction of 64.3% and 64.5% were noticed for aldrin and dieldrin, respectively (Muresan et al., 2015).

In Baking in which the heat surrounds foods which is also called indirect broiling, which is same as broiling process performed in lower temperature. It has been also used for reduction pesticides in foods samples. Baking process (110^{o}C, 60 min) was tested on spiked pork with aldrin and dieldrin and showed dissipation of 52.4 and 53.7 %, respectively (Muresan et al., 2015).

Grilling also refers to intense direct heat similar broiling cooking process with only one major difference. In grilling, the heat source is below (like with a barbecue grill), but in oven broiling, the heating source is above. Grilling at 260^{o}C has also commonly used because it retains aroma and flavor in the foods (Sun, 2016). In a study, grilling process tested on spiked fish with α-HCB, β-HCB, γ-chlordane, aldrin and found the redaction level of 100, 90, 11, 67%, respectively. They also revealed that the reduction in OCPs concentrations by ratio of 11% to 100% depending upon the type of pesticide and its sensitivity to heat (Fatin et al., 2016).

Chapter Three

MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

Table 3.1. Chemicals and compounds used in the study

No.	Chemicals	Exclusive	Manufactured by.	Origin
1	Cypermethrin (CMT)	94%	Sigma-Aldrich GmbH	Germany
2	Deltamethrin (DMT)	99%	Sigma-Aldrich GmbH	Germany
3	Hexachlorobenzene (HCB)	98%	Sigma-Aldrich GmbH	Germany
4	Hexachlorocyclohexane (α-HCH)	98%	Sigma-Aldrich GmbH	Germany
5	Chlorpyrifos (CPS)	96%	Sigma-Aldrich GmbH	Germany
6	Fenitrothion (FTN)	95.5%	Sigma-Aldrich GmbH	Germany
7	Blank samples (meat and fat)	FP	BARCC	Brazil
8	Hexane	99%	Merck Ltd.	Germany
9	Acetonitrile (MeCN)	99.5%	Merck Ltd.	Germany
10	Acetic acid (A.A)	99.9%	Merck Ltd.	Germany
11	Primary and Secondary Amine (PSA)	40 µm P.S	Merck Ltd.	Germany
12	Octadecylsilane (C18)	50 µm P.S	Merck Ltd.	Germany
13	Sodium Chloride (NaCl)	99.5%	Merck Ltd.	Germany
14	Anhydrous Magnesium Sulfate $MgSO_4$	99.5%	Merck Ltd.	Germany

3.1.2. Equipment

Table 3.2. The main instrument and essentials used in the study

No.	Instruments	Manufactured Co.	Origin
1	LC- 10AV series System(HPLC)	Shimadzu Corporation	Japan
2	SPD-10A Ultraviolet detector (UV)	Shimadzu Corporation	Japan
3	QP Gas Chromatograph (GC)	Shimadzu Corporation	Japan
4	QP Mass spectrometry Detector (MS)	Shimadzu Corporation	Japan
5	HPLC column C18 (50 × 4.6 mm I.D, 3 µm P.S)	Supelco Analytical Co.	UK
6	GC column DB-5 (30 m DB-5 × 0.25 mm I.D, 0.1 µm thickness)	Supelco Analytical Co.	UK
7	Stream nitrogen evaporator	LabX	USA
8	Oven	Memmert 93/42 EEC	Germany
9	Water bath	HAAKE	USA
10	Refrigerator	Profilo	Turkey
11	Freezer	Hitachi	Japan
12	Autoclave	KSG Sterilisatoren GmbH	Germany
13	Vortex mixer	REAX top, Heidolph,	Germany
14	Centrifuge	Sigma	UK
15	Electric meat grinder	SANSUI	China
16	Sensitive balance	Mettler Toledo	USA
17	Thermometer	Acculab	USA
18	Syringe filters (0.45 and 0.20 µm pore size)	Supelco Analytical Co.	UK
19	50 mL falcon tube	Fisher Scientific	USA
20	Poly propylene Centrifuge Tubes	Fisher Scientific	USA
21	Ice box	JY-CL	China
22	Micropipettes	Eppendorf ®	Germany
23	Polyethylene Zip Bag	Four star plastics	China

3.2. Sample collection

3.2.1. Real samples

Meat (n =150) and fat (n = 150) samples were collected from carcasses of local animals raised from Darbandikhan, Said Sadiq, Arbat, Bazian, Piramagrun districts, after slaughtering the animals at Sulaimani new slaughterhouse. The collection strategy was equalization of sampling from cattle (n = 50), sheep (n = 50) and goats (n = 50) carcasses.

All samples were taken from hind quarter of animals and packed in polythene zip lock bags in cold box with respective labels in order to avoid any contamination transferred to laboratory and kept at −18^{o}C. Each sample was divided into three equivalent portions to make 450 samples (150 × 3) for meat and 450 (150 × 3) for fat samples. The first portion of meat (n = 150) and fat (n = 150) samples was straightforwardly prepared for extraction and analysis by HPLC−UV and GC−MS. The second portion of meat (n = 150) and fat (n = 150) was transferred for boiling heat treatment; then, prepared for extraction and analysis. The third portion of meat (n = 150) and fat (n = 150) samples was also transferred for broiling heat treatment then, prepared for extraction and analysis by HPLC-UV and GC-MS.

All samples were collected from July to October form adult cattle, sheep and goats of 12 − 18 months old.

3.2.2. Blank samples

Blank meat (250 g) and fat (250 g) samples were acquired from cattle free from the studied pesticides. Aliquot meat and fat samples used for selectivity test, aliquot spiked for recovery studies, and the rest used to prepare matrix- matched calibration standards and sensitivity studies. The blank samples verified to confirm the existence or below limit of detection (BLD) of studied pesticides before the use for spiking studies.

3.3. Standards preparation

Individual stock solutions were prepared including of cypermethrin (CMT), deltamethrin (DMT), hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH), chlorpyrifos (CPS), and fenitrothion (FTN). Concentrations of 100 mg/L were prepared from standards with acetonitrile in a pyrex glass vials, and stored at −18°C in a dark amber bottle. Working standard solutions (WSS) were prepared at a concentration of 50 mg/L from the stock solution in acetonitrile. Solutions were prepared for internal multistandards, multistandards for spiking meat and fat samples for recovery, linearity, and sensitivity studies. All the solutions were protected against light with aluminium foil and stored at − 4°C.

Table 3.3. Standard preparations and spiking levels in meat and fat tissues

Working Standard Solution (WSS)	**Used Vol (µL) of single standard**	**Conc. of analytes (µg/ 20 µL)**	**Used Vol (µL) multistandard sol.**	**Total conc. of six analytes (µg/120 µL)**	**Matrices (meat and fat) weight (g)**	**Conc. of each analyte in 2g matrix (mg/kg)**
Preparation for meat samples						
1mg/L	20	0.02	120	0.12	2	0.01
2.5 mg/L	20	0.05	120	0.3	2	0.025
5 mg/L	20	0.1	120	0.6	2	0.050
10 mg/L	20	0.2	120	1.2	2	0.1
20 mg/L	20	0.4	120	2.4	2	0.2
50 mg/L	20	1	120	6	2	0.5
Preparation for fat samples						
1 mg/L	20	0.02	120	0.12	2	0.01
2 mg/L	20	0.04	120	0.24	2	0.020
5 mg/L	20	0.1	120	0.6	2	0.050
10 mg/L	20	0.2	120	1.2	2	0.1
20 mg/L	20	0.4	120	2.4	2	0.2
50 mg/L	20	1	120	6	2	0.5

3.4. Chromatography operating parameters

3.4.1. HPLC-UV System

The pesticides concentrations were detected by high performance liquid chromatography (HPLC) system. The eluted peaks were monitored by SPD-10A UV detector. To follow Al-Rimawi, (2014) method with some modification depending on and Kromidas recommendations in 2017, separation was performed on a reversed phase using C18 (50 × 4.6 mm I.D, 3 µm P.S) column.

The column temperature was controlled at 35°C and the mobile phase (Water (% 0.1 A.A) /MeCN 20:80, v/v)) as an isocratic elution, and pumped at a flow rate of 1mL/ min and the wave length of the UV detector was fixed at 260 nm. Each sample was run for 10 min.

3.4.2. GC-MS System

Gas chromatograph QP GC was equipped with a QP mass spectrophotometer detector (MS) to detect and quantify the pesticide residues. To follow Sartarelli et al. (2012) method with some modifications, separation was performed with capillary column of a 30m DB-5, with 0.25 mm I.D and 0.1 µm film thicknesses. The injector, interface, and ion source temperatures were kept at 250°C. Splitless injection (1.0 min) was also performed with using helium as a carrier gas with a flow rate of 0.75 mL/min. The oven temperature was set at 4°C /min from 120°C to 190°C. Next, the temperature was increased from 32°C / min to 270°C, and was held for 4 min. The mass spectrometer was operated with scan mode, put between *m/z* 45 and *m/z* 475 daltons, which can detect analytes in a solvent to limit of 1.0mg/kg. Lower concentrations can be detected in the SIM (single ion monitoring) for each compound.

3.5. Preparation real samples:

3.5.1. Extraction samples

Based on previous original QuEChERS technique for extraction (Anastassiades et al., 2003) and cleanup procedure (Lehotay et al., 2005b) were applied with little modification. Meat and fat samples were blended well prior to use. Meat samples (2g) were taken and transferred to a 50 mL falcon tube. For the first step of extraction, single − phase extraction was performed by adding 4 mL acetonitrile (MeCN) (containing 1% Glacial A.A) to the meat samples.Then,1.6 g anhydrous magnesium sulfate ($MgSO_4$), and 0.4 g sodium chloride (NaCl) were added and the mixture agitated in a vortex mixer for 1 min. The mixture was centrifuged at 3000 rpm for 3 min at room temperature to separate the phases which is called liquid-liquid partitioning (LLP). For the second step which is called dispersive solid-phase extraction (d-SPE), the cleanup was performed by taking the supernatants, corresponding to the organic solvent MeCN, and transferred to a tube containing 70 mg primary secondary amine (PSA), 70 mg octadecylsilane (C18), and 150 mg $MgSO_4$. The tube was shaken by hand for half a min, and centrifuged at 3000 rpm for 1 min. The supernatant was filtered by syringe filter (0.45µm pore size). The filtered supernatant was subjected to evaporation under a stream of nitrogen to remain 1 ml and stored at 4°C. Samples were transferred for analysis by HPLC-UV and GC-MS.

For fat samples, the extraction and cleanup procedures used were adapted with the original developed QuEChERS method for extraction (Mastovska and Lehotay, 2006), and cleanup (Lehotay et al., 2005a) procedure with little modifications.The samples were thawed at 4°C overnight and blended prior to use. Blended samples (2g) were transferred to a 50 mL Falcon tube. For the first step, double - phase extraction was performed by adding 5 mL hexane to the tube and agitated in a vortex mixer for 1 min; next, 10 mL MeCN (containing 1% (v/v) of A.A)

was added and mixed by vortex mixer for 1 min. Then, 1.6g anhydrous $MgSO_4$, and 0.5 g NaCl were added and the mixture was agitated again in a vortex mixer for 1 min. The mixture was centrifuged at 3000 rpm for 3 min to separate the phases (liquid-liquid partition). The upper phase, corresponding to the organic solvent hexane, was drawn off with the aid of a pipette and discarded. The next phase, corresponding to the organic solvent MeCN, was transferred to a tube containing 70 mg of the adsorbent PSA and 150 mg $MgSO_4$. The tube was shaken by hand for half a min and centrifuged at 3000 rpm for 1 min. The supernatant was filtered by syringe filters (0.45 and 0.20 µm pore size, successively).The filtered supernatant of each sample was subjected to evaporation under a stream of nitrogen to remain 1ml and stored at 4°C. Samples were transferred for analysis by HPLC-UV and GC-MS.

3.5.2. Quantification of pesticides residues in real sample

The concentration of the pesticides residues in mg/kg was calculated for meat and fat samples as follow (Singh, 2017)

$$\text{Pesticides residue (mg/kg)} = \frac{\text{A s}}{\text{A std}} \times \frac{\text{C std}}{\text{Ws}} \times \frac{\text{Vf}}{\text{Vs}} \times \text{I}_{\text{std}}$$

Table 3.4. Retention times and peak areas in meat and fat samples analysed by HPLC-UV

Pesticides		R.t (min)	AS spiked	AS MMC	AS Std
Meat samples					
1	Hexachlorobenzene	1.91	117171	125346	132980
2	α-Hexachlorocyclohexane	3.25	118087	127890	135893
3	Cypermethrin	4.42	105936	111593	118121
4	Chlorpyrifos	5.31	117147	129342	134662
5	Deltamethrin	6.5	119747	125093	133772
6	Fenitrothion	7.75	100206	109893	115331
Fat samples					
1	Hexachlorobenzene	1.91	136730	149728	165783
2	α-Hexachlorocyclohexane	3.25	119803	130901	141998
3	Cypermethrin	4.42	105766	118001	132330
4	Chlorpyrifos	5.31	103512	112990	124381
5	Deltamethrin	6.5	109958	120377	131183
6	Fenitrothion	7.75	91569	105344	114991

Table 3.5. Retention times and peak areas in meat and fat samples analysed by GC-MS

Pesticides		R.t (min)	AS spiked	AS MMC	AS Std
Meat samples					
1	Hexachlorobenzene	2.93	108294	113898	118909
2	α-Hexachlorocyclohexane	3.83	139257	146894	152243
3	Fenitrothion	5.46	184876	189410	195989
4	Chlorpyrifos	7.44	116927	124000	128801
5	Cypermethrin	8.52	105032	110798	116102
6	Deltamethrin	9.75	73940	80782	84401
Fat samples					
1	Hexachlorobenzene	2.93	104619	115785	125002
2	α-Hexachlorocyclohexane	3.83	144281	158980	169984
3	Fenitrothion	5.46	178831	182786	197001
4	Chlorpyrifos	7.44	116301	124879	137091
5	Cypermethrin	8.52	107274	116878	129900
6	Deltamethrin	9.75	79878	88080	99102

Values are referring concentration of 10 mg/L standards at spiking level of 0.1 mg/kg. R.t = retention time, AS = peak areas, MMC= Matrix-Matched Calibration standards.

According to EC No.SANTE 11813/2017, discrepancies between the concentrations of new and old standard solutions may normally differ by more than ±10%, which could be due to a number of factors such as, simply analyte degradation, analyte precipitation, solvent evaporation, differences in the purities between the old and new reference standards; hence the readings were accepted as above.

3.6. Validation study

All methods in this study validated according to internationally accepted criteria of EC No..SANTE/11813/2017 i.e., selectivity, accuracy (recovery percentages), precision, linearity, sensitivity (limit of detection and quantification), and matrix effects.

3.6.1. Method specificity and selectivity

Analytical single standards form stock solutions were injected to HPLC and GC system separately to identify the analytes retention time. To improve the analytical retention time validations, multistandard solutions injected into HPLC and GC system and maximum retention time tolerance range checked based on EC No.SANTE/11813/2017 criteria (± 0.2 min). This is performed because the detector response of individual pesticides in multipesticide calibration standards may be affected by one or more of the other pesticides in the same solution.

3.6.2. Recovery

Recovery values were obtained by spiking meat and fat samples with specific standard concentrations. The spiked samples analysed and the obtained concentrations pesticides after spiking were compared with the same concentrations of standard been spiked into samples. For meat and fat samples, multistandard solutions were spiked into samples in different concentrations separately (0.01, 0.025, 0.05, 0.1 and 0.01, 0.020, 0.05, 0.1 for meat and fat

samples, respectively). Samples homogenized for two minutes and left for 40 minutes to allow the pesticides to penetrate into the matrix. Then, spiked meat and fat samples were transferred for extraction by QuEChERS method.

The injection concentration of 0.01 to 0.1 mg/ kg was considered for fortification in meat and fat samples for recovery study, this is because; firstly, the amount of analyte added to the test portion is advised to be around one to five times the estimated amount of the analyte already present in the sample, which is recommended by EC No. SANTE/11817/2017. This technique performed to obtain the highest method reliability during screening. The analyses were performed in three successive days with the same instruments, same analyst and same atmosphere temperature. The data were collected and average values were calculated per day for each analyte to validate the results.

In recovery study, the spiked concentration values were found as this equation (Nilsen, 2010)

$$\text{Conc} = \frac{\text{As} - \text{I}}{\text{Y}}.$$

The recovery values were also found as this equation (Nilsen, 2010)

$$\text{Recovery \%} = \frac{\text{Found conc.post spiking}}{\text{Initial conc.}} \times 100$$

The results of recovery for each analyte were assessed with minimum and maximum recovery values which has been allowed by EC No. SANTE/11813/2017 criteria (70% − 120%). The ability of the method to screen the target analyte was expressed by Extraction Efficiency (EE %), which is also evaluated by the recovery percentages. Since it's impossible to extract more analyte concentration than the initial amount in the samples, the extraction efficiency was not validated to be greater than 100%, while validated for recoveries. Hence, any tiny found

concentration greater than initial, was accounted as an error in signals (signal enhancement) due to matrix co-extractive effect.

3.6.3. Precision

The method precision in this study was determined by intermediate precision (inter-day) assay. The intermediate precision was expressed by results of deviations of three successive days (n = 3) which referred nine analyses involving three analyses per each day. The precision values were also evaluated under EC No. SANTE/11813/2017 (≤ 20%).

3.6.4. Matrix-Matched Calibration assay (MMC)

Matrix-matching standards (standards added to blank extracts) were performed regarding EC No. SANTE/11813/2017 recommendations, to minimize matrix effects during analysis. Standard solutions prepared at a concentration of 50 mg/L of acetonitrile. For meat samples, matrix-matched standards were prepared by diluting solutions to prepare 1, 2.5, 5, 10, 20 and 50 mg/L. Similarly for fat samples, standard solutions were diluted to prepare 1, 2, 5, 10, 20 and 50 mg/L. Later, 120 µL of each prepared multistandard solution spiked into the extracted blank solutions of meat and fat samples to obtain concentrations of 0.010, 0.025, 0.050, 0.100, 0.200 and 0.500 mg/kg of spiked meat and 0.010, 0.020, 0.050, 0.100, 0.200 and 0.500 mg/kg of spiked fat extracts. Matrix-matched standard solutions of meat and fat samples transferred to HPLC-UV and GC-MS for analysis. The peak areas were obtained and the MMC curves were built for each analyte.

3.6.5. Linearity

The linearity can be tested using standard solutions or spiked blank samples. The latter option is more preferred because the slope and intercept values in the calibration curve of spiked standards would incorporate the calculation of sensitivity study (EC No. SANTE/ 11813/2017). Hence, the matrix-matched standards were used to evaluate linearity across the working range of the analytical method. To construct X and Y axis for the calibration curves, spiked concentrations plotted versus the recorded peak areas. All the calibration datasets of 0.010, 0.025, 0.050, 0.100, 0.200 and 0.500 mg/kg for meat and 0.010, 0.020, 0.050, 0.100, 0.200 and 0.500 mg/kg for fat samples were used to construct the calibration curves.

Later, the standard deviation of intercept (responses) (SDI) values and calibration curve slopes (y) proposed to validate the test sensitivity. The acceptability of the regression model was confirmed by the determination of correlation coefficients (r^2). The linearity of the model for significance level (0.05) was validated by calculating standard error of intercept (responses) (SEI) for each analyte and was used to assess method of sensitivity.

3.6.6. Sensitivity Assay

The concentration of analytes that produce a signal peak of 3 folds up the background noise of the chromatogram was set as the limit of detection (LOD) value (Al-Rimawi, 2014). Hence, LOD was calculated using the equation LOD = 3.3 × SD / b, where b is the slope of the calibration curve and SD is the standard deviation of the curve intercept (Sahu and Nelapati, 2018). The concentration of analytes that produce a signal peak of 10 folds up the background noise of the chromatogram, were set as the limit of quantification (LOQ) of the method (Al-Rimawi, 2014). Hence, the limit of quantification was calculated as LOQ = 10 × SD / b (Sahu

and Nelapati, 2018). LODs were kept in minimum for each analyte; hence, several spiking levels were tried till the acceptable LOD and LOQ obtained for each pesticide.

3.6.7. Matrix Effect

In order to evaluate the influence of matrix components on the detector's response, the matrix effect was assessed. Quantitation was performed by comparing peak areas of calibration standards involving matrix-matching (standards added to blank extracts) and non-matrix-matching (standards in solutions). The evaluation was performed by comparing peak areas of the matrix-matched solutions with the analytical multistandards signals at the same concentration. For this approach the analytical multistandard solutions with known concentration of 10 mg/L was prepared and analysed with HPLC-UV and GC-MS for meat and fat samples to obtain the standards peak area (standards signal) called AS_{standard}. Next, a blank sample extracts were spiked with multianalytes to prepare concentration 0.1 mg/kg (corresponding 10 mg/L for each analyte); thereafter, analysed with HPLC-UV and GC-MS for meat and fat samples. The giving peak area (matrix-matched solution signal) was expressed as $AS_{\text{MM sample}}$.

The data were put into the ME% equation and the ionization suppression /enhancement effect were calculated as follow: (Vu-Duc et al., 2019, Rutkowska et al., 2018).

$$\text{ME\%} = \frac{AS\ \text{post extrraction spiked matrix}(AS\ \text{MMsample}) - AS\ \text{standard}}{AS\ \text{standard}} \times 100$$

Matrix effect percentages assessed in the base of ME% standard values in which less than 100% indicates ionization suppression and over 100% indicates ionization enhancement due to co-eluting sample compounds (Pang, 2018). The "reduce matrix effect" does not mean reduced value of %ME, but also means the ME% ionization value becoming closer to 100%.

3.7. Thermal treatment study

3.7.1. Boiling process

Meat (n =150) and fat (n =150) samples were about 50g, and placed in low-density water-impermeable polyethylene bags separately. Next, all samples were cooked in boiling water at100°C for 30 min using a water bath and the temperature were monitored with thermometer during the test. After the boiling process, all the treated samples were transferred to -10^{o}C and prepared for extraction.

3.7.2. Broiling process

Similarly, meat (n = 150) and fat (n =150) samples were about 50g, and placed on a glass bowl and broiled in a preheated air oven at 176°C for 20 min, and being turned upside every 5 min during the test. The oven temperature was also monitored by the oven thermometer gauge during the experiment. After the broiling process, all the treated samples were transferred to -10^{o}C and prepared for extraction

3.8. Processing of results and statistical data analysis

The findings of this project were designed and analysed statistically by using computer programs of Excel (Analysis ToolPak, Regression), SPSS Software (One-way ANOVA, Post Hoc = Duncan, Student t-test). Multiple ranges were used to significantly compare means ($p \leq 0.05$) of pesticide residue concentrations between and within species, HPLC-UV and GC-MS data, boiling and broiling data, and between and within districts data.

Chapter Four

RESULTS AND DISCUSSION

4.1. Optimization of extraction and cleanup in meat and fat samples

Many food types have a fat composition of 2% – 20% including milk, fish, shellfish, liver, kidney, meat from poultry, pork, cattle, small animals, and eggs. In fatty food samples, occurring pesticides are fat and water soluble, so analytical methods should focus on non-polar and polar pesticides (Hercegová et al., 2007).

QuEChERS method can extract polar and non-polar pesticides in the same matrix, it depends what modification is performed in the procedure during extraction. In this study, same extraction method used for samples analysed by HPLC and GC, because, when samples extracted and cleanup by QuEChERS method, it can be injected to both LC and GC with providing best recovery (Mastovska and lehotay, 2006).

Since the concentrations of pesticides in any kind of food samples including meat, are quite low; hence, sample pretreatment for pesticide analysis can be considered a key step in the whole analysis process which include pesticide extraction method (Liu et.al., 2016).

For extraction pesticides in meat samples, at the beginning of the study original QuEChERS methods were applied for extraction (Anastasides et al., 2003) and for cleanup (Lehotay et al., 2005b). The method contained liquid-liquid partitioning (LLP), in which single-phase extraction and performed and for the second step (cleanup step), which is called dispersive solid-phase extraction (d-SPE). The obtained recovery values ranged between 60 to 74% for HPLC and GC analysis, with high RSD values (>20%) which was not acceptable. Hence, the QuEChERS method was modified. By reduction sample weight from 10g to 2g, 10mL MeCN to 4 mL, 4g MgSO4 to 1.6 g and 1g of NaCl to 0.4g in the extraction step, and in the cleanup step

increasing amount of each PSA and C18 from 50 mg to 70 mg, acceptable recoveries of 78.08 - 101.5% and 79.2 to 104.3% (Table 4.1) obtained in HPLC and GC analysis, respectively which were in the range of EC No. SANTE/11813/2017 guidelines (70 –120%) and acceptable precision (RSD) values obtained which ranged from 0.5 -15.7% and 0.32 to14.6% in HPLC and GC respectively, which were satisfactory according to EC No. SANTE/11813/2017 (≤ 20).

Similarly, for fat samples, the original QuEChERS method was considered for first step of extraction (Mastovska and lehotay, 2006), and original QuEChERS method used for second step of extraction which specified for high fat samples (Lehotay et al., 2005a).

The recovery values obtained for the pesticides varied from 65 to 78, with high RSD values (>20%) in HPLC and GC analysis which was not acceptable. Hence, the method was modified in the second test samples weight (2 gm) used, and remaining 5mL hexane, 10mL MeCN, and 0.5 g NaCl, same as original procedure with reduction 4 g $MgSO_4$ to 1.6 g, in the extraction step. In the cleanup step also PSA amount increased from 50 mg to 70 mg and remaining $MgSO_4$ (150 mg) same as original procedure. Single and double filtration steps also performed with the extracted solutions for meat and fat samples, respectively.

For fat samples, the obtained recovery and RSD rates were between 77.3 -106.2 % in HPLC and 81.5 to 98.6% in GC analysis (Table 4.1), which were in the range of EC No. SANTE/11813/2017 guidelines. The relative standard deviation (RSD) were also between 0.23 - 12.9 in HPLC and 0.3- 9.3% in GC analysis, which were in the range of EC No. SANTE/11813/2017 guidelines.

Modification of QuEChERS method has also been proposed by Sartarelli et al., (2012) for extraction of 3 PYRs, OPPs, and PPs, in meat and fat by GC-MS and also used by Oliveira et al, (2018), for extraction of 188 PYRs, OCs and OPs in meat and analysed by HPLC- MS. Other

kind of modification of QuEChERS method for extraction pesticides was also made by Paramasivam et al, (2011) for extraction 17 OCPs in meat and analyzing by GC-ECD.

Traditionally, pesticides have been extracted by liquid-liquid extraction (LLE) and dispersive- solid phase extraction (d-SPE); however, these extraction procedures are not recommended as they are time consuming, multistep procedures, and need large amounts of organic solvents. By contrast, the use of QuEChERS technique has recently become a very popular technique for the determination of pesticide residue in different foodstuffs, due this technique skips many complicated analytical steps used in the traditional methods, ease to modify, requires low solvent, incurs low cost of the analysis per sample and consume little time and work (Kim et al., 2019). Hence, it has been widely used and has been modified many times for determination of multiclass pesticide residues in different foodstuffs such as vegetables and fruits (Singh et al., 2018, Chawla et al., 2018), cereals (Koesukwiwat et al., 2009), honey (Bargańska et al., 2018), meat (Oliveira et al., 2018, Arioli et al., 2019), and fat (Zamariola et al., 2017).

In this study, for the first step of extraction (LLP) in meat samples, original QuEChERS method (Anastasiades et al., 2003) was used because, these method do not need buffer for the extraction. While, for LLP in fat samples, originial QuEChERS method was used (Mastovska and lehotay, 2006), because it involves hexane, which serves defatting and provides the best extraction process when combined with acetonitrile in the extraction procedure.

For the second step, (d-SPE) the original QuEChERS methods of Lohety et al, (2005a) and Lohety et al., (2005b) was used for fat and meat samples, respectively this is because it involves large mass of PSA, this is due to the fact that PSA, C18 and $MgSO_4$, in extraction of pesticides provide high level of cleanup (Okihashi et al., 2007).

Regarding the chemical components used in QuEChERS technique, the MeCN was selected for the extraction of pesticides in meat and fat samples because of its effectiveness in the extraction of polar and non-polar pesticides from the diverse range of matrices (Paramasivam et al., 2011), and miscible with water and it can penetrate water-based matrices for the extraction of the target analytes (Lee et al., 2017).

It has also been demonstrated that phase separation is more successfully achieved using MgSO4 and NaCl together rather than $MgSO_4$ alone because the addition of NaCl leads to the presence of fewer co-extractives. The salt combination (NaCl, $MgSO_4$) is commonly used as a drying salt because they effectively bind with organic acids, polar material and/or glucosides, removes residual water, and produce exothermic hydration reaction which elevates temperatures that required to efficiently extract of non-polar pesticides (Lehotay, 2011).

The cleanup step in meat samples extraction was improved by increasing PSA and C18 from 50 mg to 70 mg. PSA can effectively eliminate matrix components such as organic acids, certain polar pigments, and sugars from the meat and fat extracts. C18 can also thoroughly remove fatty acids which are considered the main co-extract of the non-polar pesticides in meat, among other components (Zamariola et al., 2017, Paramasivam et al., 2011).

For cleanup in meat and fat samples, the addition of filtration step(s) was/were another modification which prompts the cleanup and prepares the solution for injection (Paramasivam et al., 2011).

The difficulties of the method for the extraction pesticides are when minor or major modification performed with the extraction method, such as extraction volume, and last concentrate evaporation temperature (Li et al., 2014). Therefore, several volumes and amount of sorbents were tested to achieve acceptable recoveries.

Co-extractives in extract produce extra peaks, poor peak resolution and loss of detector sensitivity (Stefanelli et al., 2009). Therefore, in this study "cleanup step" was mostly considered to optimize and adapt the QuEChERS procedure with chromatography; firstly, to obtain acceptable recovery, RSD, maximize extraction efficiency, and peak resolution; secondly, because insufficient sample cleanup from the co-extractives in the extract causes deterioration chromatographic system and detectors which precludes reliable results (Koesukwiwat et al., 2009).

Under the optimized chromatographic conditions, a satisfying separation was achieved for HPLC and GC technique with symmetrical and high resolution peaks in the retention times of 1.91 to 7.75 min for HPLC (Appendices B1, B3) and 2.93 to 9.75 min for GC (Appendices B5, B7) for the six analytes. These due to the minimum co-extractives were presence in the meat and fat samples extracts.

4.2. Validation study

Detection capability must not be confused with that the non-detected compounds are not present in the matrices, it may present but in the level below the detection limit (BDL).This can only be proved by validation studies, which showing the specific method that is employed on specific matrices and detect the studied compounds as present at the levels of concern or quantifying the exact existence level (Zeigenbaum and Stone, 2009), this is totally recommended by EC No. SANTE/11813/2017.

In this study, the blank extracts eliminated a false positive in the extraction process, analytical standards also spiked blank removed false negatives, and spiked blanks assessed the extraction efficiency, matrix effect and calibration curves checked both, sensitivity and linearity

in the working range of concentrations in order to avoid quantitation mistakes caused by possible matrix co-extractives and instrumental fluctuations.

4.2.1. Recovery values

4.2.1.1. HPLC analysis

In HPLC analysis, the recovery values and RSDs were obtained using spiked samples at four different concentrations for meat and fat samples. Acceptable recoveries were obtained (78.08–101% and 77.3-106.2 % for meat and fat samples, respectively) which meets the EC No. SANTE/11813/2017 guidelines. Hence, the extraction procedure that employed in this study was efficient in recovering the amount of residues present in meat and fat samples. These results demonstrating that the matrices co-extracts of meat and fat samples have little affected the extracted analytes (Table 4.1).

Table 4.1. Recovery and relative standard deviations (RSD %) in different spiked concentrations of meat and fat samples.

Spiked level (mg/kg)	**Recovery ± (RDS %), n=3**					
	Meat samples					
	CMT	**DMT**	**HCB**	**α-HCH**	**CPS**	**FTN**
0.01	78.08±5.9	97±15.7	80.8±5.1	83.8±13	85.9±11	97.7±2.5
0.025	90.4±5.4	101± 5.7	98±9.3	83.7±11	79±11.9	96.6±3
0.05	88.1±8	101±5.8	94.6±7.5	88.5±5.9	88.8±10	97.6±2.1
0.10	97.7±1	99.6±1.7	98.7±0.5	97.1±1.1	97.9±1	99.2±0.7
Fat samples						
0.01	96.7±3.7	77.3±4.2	80.9±6.5	98.8±13	93.8±6.1	91.5±12
0.020	94.8±2.3	81.1±7	82.3±12.3	88.6±9	97.7±7.7	106.2±3.9
0.05	104.5±1.2	86.5±2.7	93.9±12.9	102±1.6	103.7±3	99.04±4.7
0.10	99.1±0.23	97.4±0.6	97.2±1.2	98.8±0.4	99.2±0.5	100.06±1

Moreover, the intermediate precision was expressed by the RSD of the results of nine analyses performed on three different days (n=3). In the HPLC analysis, the obtained inter-day RSD was 0.5-15.7% for meat and 0.23-12.9% for fat samples in the four different spiked concentrations, which meets the EC No. SANTE/11813/2017 guidelines (RSD ≤ 20%) (Table 4.1).

4.2.1.2. GC analysis

In GC-MS analysis, satisfactory recovery values and RSDs were also obtained for meat and fat samples in four different concentrations, which ranged from 79.2 –104.3% and 81.5 – 98.6 % for meat and fat samples, respectively and meets the criteria reported in EC No. SANTE/11813/ 2017. Hence, it can be stated that the extraction procedure that applied in this experiment was efficient in recovering the maximum amount of residues present in the samples.

The precision in GC–MS was also determined by intermediate precision (inter – day). The RSD of the results of nine analyses performed on three different days. The obtained RSD was 0.32 –14.6% for meat and 0.3 – 9.3% for fat samples, which meets EC No. SANTE/11813/2017 guidelines (RSD ≤ 20%) (Table 4.2).

According to Vogel et al., (1989), yields close to 100% are quantitative, yields above 90% are excellent, yields above 80% are very good, yields above 70% are good, yields above 50% are fair, and yields below 40% called poor recovery and the method is inapplicable; hence, it can be stated that overall recoveries obtained for meat and fat samples in HPLC and GC analysis in this study are good to quantitative and advisable to be applied.

Table 4.2. Recovery and relative standard deviations (RSD %) in different spiked meat and fat samples.

Spiked level (mg/kg)	**Recovery ± (RDS %), n=3**					
	Meat samples					
	CMT	DMT	HCB	α-HCH	CPS	FTN
0.01	79.2±7.4	85.8±1.7	85.8±13.09	82.7±3.8	88.3±13.3	97.6±6.3
0.025	82.7±7	88.9±1.8	86.1±10.2	104.3±4.6	91.4±8.6	96.2±3.1
0.05	86.8±4.1	93.5±1.3	88.6±14.6	98.7±5.1	92.1±9	96.3±4.8
0.10	97.3±0.9	98.1±0.3	98.07±1.5	97.5±3.1	98.6±1.5	99.6±0.6
Fat samples						
0.01	91.2±4.9	88.7±4.5	87±5.3	83.2±7.6	82.5±7.3	81.5±5
0.020	88.4±2.3	86.3±6.8	85.9±3.8	86.8±6.9	87.6±9.3	84.5±3.4
0.05	93.5±2.1	92.5±2.8	91.7±5	90 ±5.2	95.6 ±3.3	90.8±2.2
0.10	98.6±0.3	98.3±0.6	98.3±0.4	98.2 ±0.3	97.9±1.2	97.6±0.4

These results agreed with the results obtained by Oliveira et al. (2018), who obtained recoveries value of 70.2 – 108.5% with RSD 4 –20 % using modified QuEChERS method with HPLC–MS to quantify multiresidues of 188 PYR, OCPs and OPPs in beef meat, and it is also agreed with modified QuEChERS method conducted by Paramasivam et al. (2011), who obtained recoveries values between 70 –110 % and 84 – 99 % with RSD 4 – 9.29 and 2.97–8.42% in spiked and naturally contaminated sheep meat samples respectively, for detection of 17 OCs and 11 PYRs by GC–ECD. Results in this study also agreed with Zamariola et al. (2017) recovery values (75% to 93% with RSD <13%) using modified QuEChERS method in extract spiked fat with 10 PYRs and OCs pesticides analysed by GC–ECD.

However, the recovery values of extraction method of this study was better than recovery percentages (81-129 %) and RSD (0.4–27 %) for meat samples and recovery percentages (70 – 123%) with RDS (0.5 –25 %) for fat samples obtained using modified QuEChERS method from

spiked beef meat and fat with 3 PYRs, OPs, and PPs pesticide that analysed by GC–MS (Sartarelli et al., 2012).

Finally, accuracy results in this study was not better than accuracy obtained by Letta and Attah., (2013), who obtained recovery values of 82.9 – 99.2% and RDS of 1.4 –7.2% using other methods like modified SPE for extraction of 8 OCPs in meat samples in five concentration level and analysis by GC-MS.

It is worth to mention that, some of the tested compounds showed lower recovery than others. This might be due to some kind of interaction which could not be corrected with matrix-matching, or could be due to samples that have been decomposed through extraction and/or cleanup, or evaporation (Okihashi et al., 2007), or could be occasionally related to the active sites in the chromatography system because liners and columns have active sites which cause the system to give a lower response after running a few samples; therefore, in chromatography methods more than 5% variability recovery for the entire methods process is a usual consideration due to the positive bias on a chromatography system (Smith, 2013). Some recoveries percentages and RSD also presented high (>100%) in HPLC and GC data for meat and fat samples, this could be signal enhancement of co-extractives in the extract.

High RSD in some analytes in HPLC and GC might be due to the high amount of co-extractives that interfered in the chromatography system (Okihashi et al., 2007). The main analytical problem in chromatographic analyses of foods has been reported to be the complexity of the matrix together with interfering co-extractive substances which may deteriorate the chromatographic columns (Frenich et al., 2006). Therefore, the analysis of three groups of pesticides in meat and fat samples are recommended to be cleaned up thoroughly prior to the injection.

4.2.2. Linearity

Acceptability of linearity data is judged by examining the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot. A correlation coefficient of 0.999 is generally considered as evidence of acceptable fit of the data to the regression line EC No. SANTE/11813/2017.

In this study, the linearity was evaluated by constructing regression graphs and the acceptability of the regression model was confirmed by using all the calibration datasets. In the HPLC-UV analysis, the obtained correlation coefficients (r^2) for the six pesticides ranged between 0.9998 - 0.9999 for meat and fat samples. In GC-MS analysis, the coefficients (r^2) of the six studied pesticides were also ranged from 0.9997 to 0.9999 for meat and fat samples for the five different spiking levels, which considered as acceptable for the data to the regression line.

The residual plots presented that both used methods are sufficiently sensitive and little errors presented and randomly distributed around the concentration axis (Appendices A). According to (Kaonga et al., 2015), the response of the detector to any analysed pesticide by LC and GC was found to be dependent on the studied matrix properties; therefore, the achieved slope (y) values for the studied analytes in meat were close to each other, which was also true for fat matrices. This could be due to the fact that the six anlytes extracted from same composition matrices of meat and fat.

It was expected to obtain the lower slope values for the analytes in the fat matrix compared with meat due to differences in the composition of the matrices, while, the way of extraction and cleanup in QuEChERS might have minimised the co-extractives in both matrices which also minimised the slop differences (Appendices A). This can be supported by a study in which GC-MS was used for quantification of extracted PYRs, OPs, and PPs in meat and fat

samples by QuEChERS method and obtained almost about same slope values in meat and fat samples (Sartarelli et al., 2012).

4.2.3. Sensitivity assay, (Limit of detection (LOD) and Limit of quantification (LOQ))

Basic validations of qualitative and quantitative methods in this study were spiking of extracted samples by five different concentrations to obtain data for LOD and LOQ values. The LOD may be defined as the minimum concentration of contaminant (pesticide) in a food sample that can just be qualitatively detected, but not quantitatively determined, under a pre-established set of analysis conditions; whereas, the LOQ is the minimum concentration of a contaminant in a food sample that can be determined quantitatively with an acceptable accuracy and consistency by applying the complete analytical method. Hence, LOD could confirm the "below detection limit", presence and non-presence values, and LOQ could determine the ability of the analytical method to quantify the lowest concentration of the analyte in the given sample. These all due to the fact that the quantitative screen analytes in matrices needs a very sensitive method and reliable assessment technique and must meet the criteria of EC No. SANTE /11813/2017.

In this study, the HPLC analysis showed that the LOD values ranged from 0.003 to 0.013 and 0.003 to 0.016 mg/kg for meat and fat samples, respectively and the LOQ ranged from 0.011 to 0.039 and 0.010 to 0.048 mg/ kg for meat and fat samples, respectively. In GC-MS analyais, the LOD values also ranged from 0.004 to 0.014 and 0.0052 to 0.014 mg/kg in meat and fat samples, respectively. The LOQ also ranged from 0.012 to 0.043 and 0.015 to 0.044 mg/kg respectively.

The LOQ vales in all meat and fat samples in HPLC and GC analysis were below the respective found pesticide concentrations. Regarding SANTE/11813/2017 criteria, this refers that the method can detect the studied pesticides at an adequately low level which accentuates its

validity and provides typical sensitivity. Very low LOD in this study also confirmed the acceptable sensitivity of the method, because the aspect of low LOD is the key-factor for evaluating analytical methods, as the lower LOD values, the lower probability of false negative results

Table 4.3. HPLC-UV analysis presenting, linearity range, regression equation, coefficients (r^2), limits of detection (LOD) and quantification (LOQ) for meat and fat samples (mg/ kg).

Pesticides	Linearity range	Regression equation	Regression equation	r^2	r^2	LOD	LOD	LOQ	LOQ
		Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
CMT	0.01-0.5	y = 1067.8x + 2148.6	y = 1160.1x + 2090.4	0.9998	0.9998	0.013	0.016	0.039	0.048
DMT	0.01-0.5	y = 1219.2x + 1995.8	y = 1200.3x + 87.178	0.9999	0.9999	0.009	0.006	0.028	0.019
HCB	0.01-0.5	y = 1249.x - 785.5	y = 1495.2x + 178.01	0.9999	0.9999	0.006	0.004	0.018	0.013
α-HCH	0.01-0.5	y = 1262x - 145.5	y = 1299.3x + 829.9	0.9999	0.9999	0.005	0.003	0.017	0.010
CPS	0.01-0.5	y = 1258x + 2226.7	y = 1128.5x + 1365.4	0.9998	0.9998	0.012	0.014	0.039	0.044
FTN	0.01-0.5	y = 1105.x - 630.4	y = 1057.8x - 455.23	0.9999	0.9999	0.003	0.003	0.011	0.010

Table 4.4. GC-MS analysis presenting, linearity range, regression equation, coefficients (r^2), limits of detection (LOD) and quantification (LOQ) for meat and fat samples (mg/ kg).

Pesticides	Linearity range	Regression equation	Regression equation	r^2	r^2	LOD	LOD	LOQ	LOQ
		Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
CMT	0.01-0.5	y = 1195.5x + 502.26	y = 1266.3x - 385.31	0.9999	0.9999	0.014	0.0148	0.043	0.044
DMT	0.01-0.5	y = 820.48x + 835.28	y = 908.83x - 1953.9	0.9999	0.9998	0.009	0.0111	0.027	0.033
HCB	0.01-0.5	y = 1120.2x + 1060.5	y = 1139.7x + 1142	0.9999	0.9999	0.006	0.0059	0.019	0.017
α-HCH	0.01-0.5	y = 1442.3x + 846.02	y = 1572.2x + 414.92	0.9999	0.9999	0.006	0.0054	0.020	0.016
CPS	0.01-0.5	y = 1102 x + 2010.9	y = 1082.7x + 3710.9	0.9998	0.9997	0.014	0.0052	0.043	0.015
FTN	0.01-0.5	y = 1900.6x + 679.8	y = 1860.5x - 2091.8	0.9999	0.9999	0.004	0.0064	0.012	0.019

4.2.4. Matrix effect study

In any multiresidue pesticide extraction method, there could be a residual matrix effect which making it difficult to quantify some specific compounds in certain cases. Signal suppression/enhancement, commonly called "matrix effect", may cause serious problems including inaccurate quantitation, low analyte detectability and increased method uncertainty (Uclés et al., 2017). Determination of the matrix effect allows the assessment of the reliability of an existing HPLC and GC method (Matuszewski et al., 2003).

According to the HPLC readings in this study, matrix effects (ME %) ranged between − 4 to − 6.5% for meat and − 7.9 to − 10.9 % for fat samples, while in GC was − 3.35 to − 4.58% for meat and − 6.47 to − 11.2% for fat samples. In both methods, the ME% were in the range of low or mild signal suppression effect, because if the impact of the matrix was in the range from −20% to +20%, which is considered as a mild signal suppression or enhancement effect, and If ME% was higher than ±20%, it is considered as a medium effect, and ≥ ± 50, is considered as a strongly affected (Bargańska et al., 2018). Such distributions can depend not only on the matrix effect but also on the compound-matrix combination, and finally the detection technique (Pang, 2018, Bargańska et al., 2018, Rutkowska et al., 2018). Obtaining mild or low ME% in this study was attributed due to the matrix corrections performed through matrix-matched standards in meat and fat samples.

Table 4.5. Matrix effects in meat and fat samples detected by HPLC-UV, spiked level 0.1 mg/kg versus standard concentration of 10 mg/L

Pesticides / ME%	CMT	DMT	HCB	α-HCH	CPS	FTN
Meat samples						
Collected %	94.4±1.8	93.5±2.8	94.2±2.5	94.1±2.7	96.0±1.7	95.2±2.0
Loss %	- 5.6	- 6.5	- 5.8	- 5.9	- 4	- 4.8
Fat samples						
Collected %	89.1±2.6	91.7±2.9	90.3±1	92.1±3	90.8±2.8	91.6±1.8
Loss %	- 10.9	- 8.3	- 9.7	- 7.9	- 9.2	- 8.4

Table 4.6. Matrix effects in meat and fat samples detected by GC-MS, spiked level 0.1 mg/kg versus standard concentration of 10 mg/L.

Pesticides / ME%	CMT	DMT	HCB	α-HCH	CPS	FTN
Meat samples						
Collected %	95.4±3.4	95.7±5.0	95.7±2.6	96.4±2.8	96.2±4.5	96.6±1.8
Loss %	- 4.58	- 4.28	- 4.21	- 3.51	- 3.72	- 3.35
Fat samples						
Collected %	89.9±2.7	88.8±3.5	92.7±2.2	93.5±2.3	91.1 ±0.8	92.7±1.7
Loss %	- 10.1	- 11.2	- 7.3	- 6.47	- 8.90	- 7.3

The matrix effects of fat samples in this study were more than that found by Zamariola et al., (2017), who used QuEChERS with GC-MS and found ME% of -3% in PYRs (cis-permethrin), -5% in OCP (dieldrin), while it was less than matrix effect of trans-permethrin (+14%) and α-endosulfan (+21%).

ME% is not similar between analytes and matrices in both HPLC and GC methods, and same pesticides in different matrix always show different matrix effect (Pang et al., 2006). This difference may due to variation between different commodities to detector responses. Matrix effects is different between each pesticide and different between meat and fat matrices this is

because when using chromatography, the matrix effect depends on the co-elution of each individual pesticide with co-extracted matrix components, which also vary between different commodities (Kromidas, 2017).

In this study, the ME% in fat samples showed higher than in meat samples in both LC and GC method. This could be due to, analytical problems associated with co-extractive extraction, which are well known due to matrix interferences and causes signal suppression (Castillo et al., 2012).

When a food extract is injected, the matrix components tend to fill (block) active sites in the inlet and column, thus it reduces of susceptible analytes due to irreversible adsorption and/or degradation (Vitha, 2016). This phenomenon results in a higher transfer of these analytes to the HPLC and GC column and consequently higher or lower signals exists for solutions with matrix compared with matrix-free solutions.

Practically, no cleanup method completely removes all the matrix components from a crude extract. Therefore, occasionally the matrix components injected into chromatographic system may lead to false in results, low analyte detectability, inaccurate quantitation (Hercegová et al., 2006).

4.3. Real sample analysis

4.3.1. HPLC analysis

In the HPLC analysis, the kind of pesticide that found in the highest concentration was DMT in sheep fat samples (0.256 mg/kg)(Table 4.7) followed by HCB in cattle fat samples (0.247 mg/kg), then DMT in sheep meat samples (0.218 mg/kg) and HCB in cattle meat samples (0.213 mg/kg). High concentration of α-HCH also found in fat samples of cattle (0.204 mg/kg) and sheep (0.201 mg/kg).

Table 4.7. Residual levels of pesticides in cattle, sheep and goat tissues analysed by HPLC-UV

Pesticides	Cattle		Sheep		Goats	
	Meat	Fat	Meat	Fat	Meat	Fat
CMT	$0.067^{A,v} \pm 0.004$	$0.079^{AB,v} \pm 0.005$	$0.110^{C,w} \pm 0.002$	$0.176^{D,wx} \pm 0.006$	$0.079^{AB,wx} \pm 0.003$	$0.091^{B,w} \pm 0.002$
DMT	$0.060^{A,v} \pm 0.005$	$0.108^{B,w} \pm 0.008$	$0.218^{C,z} \pm 0.011$	$0.256^{D,y} \pm 0.010$	$0.104^{D,y} \pm 0.006$	$0.126^{B,x} \pm 0.005$
HCB	$0.213^{C,x} \pm 0.009$	$0.247^{D,y} \pm 0.009$	$0.136^{B,xy} \pm 0.005$	$0.194^{C,wx} \pm 0.006$	$0.094^{A,xy} \pm 0.004$	$0.121^{B,x} \pm 0.005$
α-HCH	$0.161^{B,w} \pm 0.009$	$0.204^{C,x} \pm 0.008$	$0.157^{B,y} \pm 0.007$	$0.201^{C,x} \pm 0.005$	$0.093^{A,xy} \pm 0.005$	$0.113^{A,x} \pm 0.005$
CPS	$0.135^{C,w} \pm 0.008$	$0.200^{E,x} \pm 0.007$	$0.113^{B,wx} \pm 0.004$	$0.169^{D,w} \pm 0.005$	$0.073^{A,w} \pm 0.005$	$0.108^{B,w} \pm 0.020$
FTN	$0.043^{B,v} \pm 0.002$	$0.080^{D,vw} \pm 0.003$	$0.061^{C,v} \pm 0.003$	$0.095^{E,v} \pm 0.005$	$0.024^{A,v} \pm 0.002$	$0.031^{AB,v} \pm 0.003$

^{A,B,C,D,E} Different superscript letters denote significant differences within row ($p < 0.05$). ^{v,w,x,y,z}: Different superscript letters denote significant differences within column ($p < 0.05$), Values refer mean of 50 samples ± Standard error of the mean (SEM).

Regarding pesticides, statistically there was no significant difference between pesticides residual levels of CMT, DMT and FTN in cattle meat samples ($P > 0.05$); this was also true for α-HCH and CPS. In sheep meat samples, no significant difference was also noticed between HCB and α-HCH. There was also no difference between CMT, and CPS residual levels in sheep

meat samples. In goat meat samples, no significant difference noticed between residual concentrations between HCB, α-HCH and between CPS and CMT.

Regarding fat samples no significant difference ($P > 0.05$) found between CMT and FTN and between α-HCH and CPS in cattle samples, while in sheep fat samples no difference were noticed between CMT, HCB, and CPS. There was also no difference between HCB and α-HCH in sheep fat samples which was true for goat samples. In goat fat samples, no difference also found between DMT, HCB, and α-HCH residual concentration. No difference also seen between residual concentrations of CMT and CPS in goat fat sample.

Regarding to species versus meat samples, significant difference were found between cattle, sheep and goat meat samples in terms of residual levels of the "six pesticides" except CMT between cattle and goat samples and α-HCH between cattle and sheep meat samples

Regarding species versus fat samples, there were also significant difference between residual levels of the six studied pesticides in cattle, sheep and goats samples, except DMT between cattle and goat fat samples, and α-HCH, between cattle and sheep samples.

However, CPS and FTN are two pesticides in the same group of OPs, but they presented in different levels. High CPS concentration was found in fat samples of cattle, sheep and goat which were 0.200, 0.169 and 0.108 mg/kg, respectively which were more than that found in meat samples of cattle, sheep and goat samples (0.135, 0.113 and 0.073 mg/kg, respectively); while, the lowest pesticides concentration found in this study was FTN in meat (0.043, 0.061, and 0.024 mg/kg) and fat (0.080, 0.095, and 0.031 mg/kg) samples of cattle, sheep and goats, respectively.

The level of CMT and DMT detected in cattle, sheep and goat meat tissues were high and exceeded their MRLs (0.05 and 0.03 mg/kg, respectively) set by EU R No. 396/2005. Except that, CMT residue in fat sample which was less than CMT MRL (0.2 mg/kg) set by EU R No.

2377/90, 2004. DMT concentration in fat samples also found in level exceeded the DMT MRL (0.05 mg/ kg) set by EU R No. 396/2005.

The levels of HCB and α-HCH in meat samples of cattle, sheep, and goat also exceeded the HCB and α-HCH MRLs (0.01 mg/ kg) set by EU R No. 978/2017, beside that HCB just in cattle fat samples was exceeded HCB MRL in fat (0.2 mg/kg) set by EU R No. 396/2005. Residual levels of α-HCH in cattle and sheep fat samples were also higher than MRL of α-HCH in fat (0.2 mg/kg) set by EU R No. 396/2005, while it was not in goat fat samples.

CPS residual levels in cattle, sheep and goat meat and fat samples were also high and exceeded than MRL (0.05 mg/kg) set by EU R No. 396/2005, By contrast, the residual level of FTN in cattle and goat samples were low and below MRLs (0.05 mg/ kg) set by EU R No. 396/2005, except in cattle fat samples. In sheep meat and fat samples, FTN residual levels also exceeded the FTN MRL.

Regarding the dominant pesticide in every species, in cattle species HCB was the highest pesticide concentrations in their fat and meat samples (0.213, and 0.247 mg/kg respectively), followed by α-HCH in meat and fat samples (0.161, 0.204 mg/kg respectively).While, in sheep and goat samples, DMT was highest pesticide concentrations, as level of 0.218, 0.256 mg /kg in sheep meat and fat samples respectively, and 0.104, 0.126 mg/kg in goat meat and fat samples respectively.

4.3.2. GC analysis

In the GC-MS analysis, DMT residual level in sheep fat samples presented the highest level (0.248 mg/kg). Followed by, HCB in cattle fat samples (0.236 mg/kg). This was also true for meat samples in which DMT showed the highest level of residual concentration (0.210 mg/kg), followed by HCB in cattle meat samples (0.204 mg/kg). Similar in HPLC analysis, FTN residual level showed the lowest concentration in goat meat (0.019 mg/kg). CPS as an OPP was expected to be found in low level, but it was found at high level in goat meat (0.071 mg/kg) and fat (0.093 mg/kg) samples.

Table 4.8. Residual levels of pesticides in cattle, sheep and goat tissues analysed by GC-MS

Pesticides	Cattle		Sheep		Goats	
	Meat	Fat	Meat	Fat	Meat	Fat
CMT	$0.060^{A,w} \pm 0.004$	$0.075^{B,v} \pm 0.005$	$0.102^{D,w} \pm 0.004$	$0.169^{E,wx} \pm 0.005$	$0.076^{B,w} \pm 0.003$	$0.087^{C,w} \pm 0.002$
DMT	$0.057^{A,w} \pm 0.005$	$0.104^{B,w} \pm 0.007$	$0.210^{D,z} \pm 0.010$	$0.248^{E,z} \pm 0.010$	$0.100^{B,x} \pm 0.005$	$0.122^{C,y} \pm 0.006$
HCB	$0.204^{D,z} \pm 0.008$	$0.236^{E,y} \pm 0.009$	$0.131^{B,x} \pm 0.005$	$0.185^{C,xy} \pm 0.006$	$0.091^{A,x} \pm 0.004$	$0.114^{B,xy} \pm 0.004$
α-HCH	$0.152^{C,y} \pm 0.009$	$0.194^{D,x} \pm 0.008$	$0.151^{C,y} \pm 0.006$	$0.191^{D,y} \pm 0.005$	$0.090^{A,x} \pm 0.005$	$0.109^{B,x} \pm 0.005$
CPS	$0.124^{C,x} \pm 0.007$	$0.192^{E,x} \pm 0.006$	$0.110^{BC,w} \pm 0.004$	$0.156^{D,w} \pm 0.004$	$0.071^{A,w} \pm 0.005$	$0.093^{B,w} \pm 0.004$
FTN	$0.040^{C,v} \pm 0.002$	$0.077^{E,v} \pm 0.003$	$0.059^{D,v} \pm 0.003$	$0.089^{F,v} \pm 0.004$	$0.019^{A,v} \pm 0.002$	$0.029^{B,v} \pm 0.003$

^{A,B,C,D,E} Different superscript letters denote significant differences within row ($p < 0.05$). ^{v,w,x,y,z}: Different superscript letters denote significant differences within column ($p < 0.05$), Values refer mean of 50 samples ± Standard error of the mean (SEM).

Regarding species versus pesticides concentrations, there was significant difference noticed between the six pesticides residual levels in meat between the three species samples ($P < 0.05$) except α-HCH between cattle and sheep meat samples which was also true for CPS. Regarding fat samples, there was significant difference between the six pesticides residual levels

between the three species samples except residual levels of α-HCH between cattle and sheep samples.

The level of DMT and CMT detected in cattle, sheep, and goat meat samples were high and exceeded the DMT and CMT MRLs (0.03 and 0.05 mg/kg, respectively) set by EU R No. 396/2005. DMT levels in fat samples in the three species also were exceeded the MRL (0.05 mg/kg) set by EU R No. 396/2005. However, CMT residual level in fat samples found in high concentration, there were still less than MRLs (0.2 mg/ kg) for fat tissue set by EU R No. 2377/90, 2004.

The levels of HCB that detected in cattle, sheep, and goat meat samples also exceeded the HCB MRL (0.01 mg/kg) set by EU R No. 978/2017. The residual level of α-HCH in cattle, sheep and goat meat sample was also exceeded α-HCH MRL (0.01 mg/kg) set by EU R No. 978/2017, while in fat samples; the found residual levels of α-HCH were less than its MRL (0.2 mg/kg) set by EU R No. 396/2005, this was also true for HCB, except cattle fat samples.

Regarding OPs, the CPS residual level in cattle, sheep, and goat meat and fat samples exceeded the CPS MRL (0.05 mg/kg) set by EU R No 396/2005. However, the FTN residual concentrations were less than the studied PYRs, OCPs and CPS; it was exceeded the FTN MRLs (0.05 mg/kg) set by EU R No. 396/2005 in sheep meat and fat samples and even in cattle fat samples.

Similar in HPLC analysis, the highest concentration of pesticide were DMT in sheep meat and fat samples (0.210, 0.248 mg/kg, respectively) followed by HCB in cattle meat and fat samples (0.204, 0.236 mg/kg, respectively).

Concerning pesticides concentrations in meat samples, there was no significant difference between CMT and DMT residual levels in cattle meat samples, the levels of rest pesticides

residuals were different in cattle samples. In sheep meat samples, no significant difference also noticed between the residual level of CMT and CPS, the rest pesticides residual levels were different significantly. In goat samples, no difference noticed between residual levels of DMT, HCB and α-HCH, and no difference also noticed between CMT and CPS in the same goat meat samples.

In fat samples, statistically no significant difference noticed between CMT and FTN and between α-HCH and CPS in cattle samples. While, in sheep samples, no difference was noticed between the residual levels of CMT and CPS and between HCB and α-HCH. Similarly, in goat samples, no difference noticed between residual levels of CMT and CPS, and between HCB and α-HCH, and between HCB and DMT.

Results of DMT in HPLC-UV and GC-MS, agreed with other studies that also found high level of DMT (0.3- 1.13 mg/kg) in sheep and goat meat samples respectively, that collected near Sulaimani (Abdurahman, 2016). The result also agreed with the study that found high DMT levels (0.4mg/kg) in beef in Baghdad (Khashan, 2016). Researchers also found high CMT residual level (2.2- 2.7 mg/kg), in beef samples in Faisalabad-Pakistan, where CMT applied extensively in veterinary medicine and agriculture (Muhammad et al., 2010). However, this result disagrees with researchers who tested cattle and sheep meat for CMT and DMT in Poland and found no residue for both (Niewiadowska et al., 2010), and they reported that the result could be due to the CMT and DMT are rarely used by their local farmers. CMT and DMT were also found at low level (< 0.05 and 0.013 mg/kg, respectively) in Rome (Italy) from 50 beef samples (Stefanelli et al., 2009), because CMT and DMT are not used as a routine pesticides in most of their live stocks.

Results of HCB residue in meat using HPLC and GC was disagree with that found by Ahmad et al., (2010), who detected low HCB residue in 7% of lamb and beef in Jordan. It also disagree with the study that found low residual level of HCB in less than 10% of the analyzed cattle and sheep carcasses in Egypt (Sallam and Morshedy, 2008), and the study that found HCB < 0.05 mg/kg from cattle meat samples in Rome (Italy) (Stefanelli et al., 2009). These researchers found low level of HCB in meat tissues think that the results could be due to where farmers are not use HCB as a routine pesticide in their fields because of its alternatives PYRs and OPPs.

Results of α-HCH residual level in this study agreed with a study that found high α-HCH in cattle meat (0.074 mg/kg) and sheep meat (0.039 mg/kg) samples collected in Andhra Pradesh-India (Kiranmayi et al., 2016). It is also agreed with study that found high level of α, β, and γ-HCH (0.0460, 0.0355 and 0.0626 mg/kg, respectively) in cattle meat samples, and in sheep meat samples (0.028, 0.035, 0.045, and 0.014 mg/kg, respectively) collected in Hyderabad, Telangana-India (Singh, 2017). The α-HCH have also been found (< 0.05 mg/kg) in beef samples collected in Rome (Italy) (Stefanelli et al., 2009), and in Benin City (Southern Nigeria) (Tongo and Ezemonye, 2015), and in mutton samples (0.18 mg/kg) collected in market in Coimbatore-India, where α-HCH is routinely used by farmers (Suganthy and Kuttalam, 2003). While, very low level of α-HCH (0.0089 and 0.004 mg/kg) quantified in beef samples which had been imported from India and Brazil respectively to Egypt (Aboul-Enien et al., 2010).

Regarding fat tissues, high level of HCH (mixed isomers) residue have been detected in goat adipose tissue (0.084 – 0.18 mg/kg), that collected in different districts in India (Singh et al., 2015). They also found α-HCH in 16% of same samples at concentrations of 0.231 ± 0.13 006 mg/kg in India.

Results of CPS in this study also agreed with results of other studies found high level of CPS (0.34 and 0.35 mg/kg in winter and summer, respectively) in cattle meat samples collected in Andhra Pradesh-India (Muhammad et al., 2010). Similarly, results of CPS for mutton samples agreed with residual level of CPS that found (0.46 mg/kg) in mutton samples collected in Coimbatore-India (Suganthy et al., 2009); while, disagreed with results of CPS (below detection limit) noticed in cattle and mutton samples collected in Hyderabad, Telangana-India (Singh, 2017). Low CPS residue has also found in cattle meat samples collected in Badrashen and Giza (0.026.5 mg/kg), and samples collected in Egypt markets (0.016 mg/kg), which had been originally imported from Sudan to Egypt (Aboul-Enien et al., 2010).

Yet, no enough study carried out to detect and/or quantify FTN residue in cattle, sheep and goats tissues; however, studies found FTN in other animal like in fish (> 0.01µg/kg) (Akan et al., 2014) and animal products such as whole milk (≥ 0.02 mg/kg) (Anwar et al., 2011).

Some pesticides residual levels found in above studies are almost about same as in our study, but some differed from residue levels found in this study. The reasons cannot be critically stated because it is related to several factors.

First, high concentration pesticides residues are always found in animals tissues that are on contaminated feeds and water by pesticides especially highly environmental resistance pesticides which are used in agriculture such as OCPs including HCB and HCH, and OPPs including CPS and FTN (Tongo and Ezemonye, 2015) and/or in animals that are treated with pesticides to control external parasite such as PYRs including CMT and DMT (Choudhary et al., 2018).

Second, inadequate information concerning the age of animals used for above studies, because older age could present higher residual level than young ages if they are on same contaminated feeds (Kumar et al., 2013). This could be due to the reduction function of liver and

kidneys, because liver and kidneys in older adults become less efficient in eliminating pesticides from the body (Risher et al., 2010). Coincidentally, the highly acidic (pH 2) gastric juices (young animals) reduce absorption the amount of some active pesticide from the gut.

Third, in adequate information about animal's body parts where the samples been taken from, because pesticides could be distributed in body parts differently. In a study, lower residual level of HCB found in pig (0.038 mg/kg) in dorsal fat samples than in perirenal fat tissue (0.079 mg/kg) (Bažulić et al., 2002).

Fourth, lack information about the period between animal treatment and slaughtering process could be another factor, because residual concentration and distribution in organ and body tissues depend period of time between drug consumption and slaughtering date of animals for instance, in a study CMT topically administrated (15 mg/kg) to sheep, and tested after 4, 7 and 14 days and found 0.66, 0.17, and 0.08 mg/kg in fat samples (Suárez and Eliis, 2002).

Fifth, in adequate information about the sample collection period and season is another reason to make difference with results in this study, because pesticides concentrations differ between the seasons in the same animal (Muhammad et al., 2010). In a study, atmospheric concentrations of OCPs have showed seasonal variation as the maximum and minimum concentration noticed in summer and winter, respectively (Yeo et al., 2004), which was agreed with Li et al., (2011) study found annual peak of OCPs in summer, this was also coincident with highest PYRs residue from meat tissues (Khashan, 2016) in summer compared to winter. Researchers have suggested that variation in levels of pesticide residues during different season may be due to frequent use a specific group of pesticide in a specific season, and environmental variation factors on some pesticides (Ashoub and Azam, 2016).

Sixth, luck information about animal weight could also be another reason, because higher residual level can be in animals with grater body fat and vice versa (Edwards, 2013). This is also depends on animals age, because fat tissues of older animals could have higher residue level than young aged animals in same weight if they are both raised in same field (Bill, 2016).

High level of PYRs (CMT and DMT) was found in cattle, sheep and goat samples. This could be attributed to extensive use of PYRs in livestock to control ticks, flies, fleas, lice, mites in animal fields. However, CMT and DMT found in higher level in sheep and goat samples than in cattle samples. This could be due to sheep and goats have exposed by DMT and CMT more than cattle. Dipping process which is mostly applied on sheep and goats to control ectoparasites could be a major reason to find high residue in sheep meat and fat samples, besides grazing outdoor on contaminated grass and crops with DMT and used for agriculture, as well as maternal transfer through the breast milk would be the minor reason (Darko and Acquaah, 2007). Similarly, finding high CMT in meat and fat samples especially in sheep and goat samples could be due to the extensive use of CMT especially alpha isomer to control aphids, fungus gnats, shore flies, thrips, cutworms, etc in greenhouses and open field cultivated area.

Finding, high HCB and α-HCH in cattle, sheep and goat tissues could be due to extensively use of HCB and α-HCH in agriculture to control white flies, aphids, mealy bugs, caterpillars, scale crawlers insect and most other insects. This is because HCB and α-HCH have wide spectrum of biological activities, high stability in the environment (Park et al., 2006). Compared to PYRs, and OPPs, the OCPs have the highest environmental resistance, low volatility, together with high lipophilic behavior which makes to be accumulated in environment for ages and deposit in water, soil, and agricultural products and contaminate animal feeds (Davis, 2014).

Generally, the residual levels of HCB and α-HCH, in cattle samples were higher than their level in sheep and goat samples; means the exposing degree in cattle could be higher than sheep, and goats. This result agreed with the results obtained by Singh (2017) who also found higher level of α-HCH in cattle meat compared to that of sheep meat. Feed represent a main source for contamination of pesticides through the feed-chain for bovine (Panseri et al., 2013), and farmers mostly use leftover parts of crops, fruit and vegetables such as cucumber, cabbage, tomatoes, and watermelon after harvesting as a source of feed for their animals, and these leftovers may contain large amounts of pesticides such as HCB and HCH. Hence, the high incidence of HCB and α-HCH in bovine have originated from the feed, due to massive using of HCB and α-HCH to treat of seasonal crops controlling the fungal disease and the mosquito and /or used as an antifouling agent. Lower OCPs in sheep and goats compared to cattle samples could be due to the fact that they are mostly grazing far from agricultural or rural area (Darko and Acquaah, 2007), and farmers mostly use leftover parts of crops, for their cattle but not for sheep and goats.

High incidence of CPS in cattle, sheep and goat samples also indicating the extensive exposure to CPS, which mostly used in horticulture viticulture, and forestry on a wide range of crops, in residential and nonresidential applications to control cutworms, corn root worms, cockroaches, grubs, flies, termites, fire ants, and lice (Davis, 2014).

However, FTN residual levels in sheep samples exceeded MRL, it was much less than CMT, DMT, HCB, α-HCH and CPS residual level in meat and fat samples. Lower residual levels of FTN could be due to the less use of FTN, due to high prices, its less biological spectrum activity and low stability in the environment (Deguine et al., 2017).

Overall, finding high levels of pesticides in animal tissues in this study could be justified by the reality of over use or over dose levels of pesticides by farmers (Al-Zahra and Najim,

2017). Butchers also slaughter the animals without knowledge on the withholding periods of veterinary drugs, and they may slaughter animals or harvest crops only few days after treatment (Khan et al., 2017). Moreover, after dipping the animals, farmers disposed their pesticide containers around the dipping area and contaminate the whole soil and grazing area; therefore, the contaminated materials such as contaminated empty containers or old application equipment are another source of pesticide residues and pasture contaminations (Tarla et al., 2014). This agreed with the results of a survey that found, 78% of the farmers disposed their pesticide containers around the spray area, and 6% used the containers for domestic use. The contents of the containers can be washed into the environment and water bodies thus contaminating the water quality (Turyahikayo, 2013).

Finally, the time of dipping animals in ectoparasiticides and seasonal crops that pesticide used for is in the summer causes to higher levels of residue in animal tissues during this time. The samples in this study were collected in mid of summer to about mid of autumn, which could justify the presence of higher pesticide levels in the animal tissue.

4.4. Comparisons of residual concentrations between meat and fat samples

The results in this study showed that residual levels of the six pesticides in cattle, sheep and goat fat tissues were higher than the residual levels in muscle tissues (Tables 4.7, 4.8). This could be due to the fact that the studied pesticides were lipophilic and have high tendency to be stored in fat tissues than in muscle tissues, and fat has been confirmed as target tissue able to store pesticides with lipophilic behavior; however, muscle tissues can also store lipophilic pesticides due to its fat content, as well as low lipophilic behavior of some pesticides can partly be stored in meat due to water content of meat; hence, the level of pesticides residues in fat tissue

depends on lipophilicity behavior of the pesticides, and higher lipophilic pesticide may store more than lower lipophilic behavior pesticides in fat and vice versa (Panseri et al., 2013).

Obtaining higher level of lipophilic pesticides in fat tissues than in muscle tissues of same animals agreed with other studies that found higher residual level of OCPs (Lindane, Aldrin, Dieldrin, Endosulfan, DDT and DDE) in fat than in lean beef in the same animal (Darko and Acquaah, 2007), and also agreed with the study that found less concentration of HCH in muscle, than that in adipose tissue of same animals (Singh, 2015), and agreed with a study that found higher levels of HCH in goat adipose tissue compared to their level in muscle tissues (Bedi et al., 2005).

In low fat or fatty samples, lipophilic and hydrophilic pesticides can occur, so analytical methods should cover a wide pesticide polarity range. However, in fatty food samples, dominant pesticides are fat soluble, so analytical methods should focus on non-polar pesticides (Hercegová et al., 2007).

The distribution of pesticide in meat and fat tissue also depends on lipophilic behavior of the pesticides isomers, for instance α-HCH, has less lipophilic behavior than the other OCPs and has a lower tendency to be stored in fat compared with lindane (γ-HCH) (Ware, 2000); hence, same pesticides but different isomers may be distributed in meat and fat tissue differently.

Moreover, distribution of pesticides residual levels in meat and fat could be different between animals as it depends on body fat versus meat ratio, body parts that sample where taken at, and route of ingestion and (Suárez and Ellis, 2002). The more fatty animals tend to store higher and faster fat soluble pesticides and vice versa (Edwards, 2013). About route of ingestion, animals may have high residue in milk, blood, meat, liver and kidney directly after sudden huge dermal exposure and/or oral such as in dipping (Banerjee et al., 2015), but dermal absorbed

pesticides will not exceed residues in organs such as liver than oral taken, this was also confirmed by testing animals through bile duct canulation (EFSA, 2011).

Animal physiological status also plays important role on the residue level in tissues for example, during weight loss, the accumulated and stored pesticides in fat are slowly released into the bloodstream, and may shows higher pesticides and metabolites in blood and meat tissues; therefore, adipose tissue considered as a continual source of internal exposure of lipophilic pesticides during losing weight (Chevrier et al., 2000).

4.5. Residue levels in animals tissues regarding districts

4.5.1. Cattle samples

4.5.1.1. HPLC analysis

Statistically, there were significant differences between pesticide residual levels ($P< 0.05$) among some districts in samples analysed by using HPLC, but no pesticide type presented non-significant difference among the “five districts”. Moreover, no pesticide residue presented the highest or the lowest level over the “five districts”

Regarding pesticides concentration between districts, the highest level of pesticide residue in this study noticed in cattle meat and fat samples collected in Bazian, which was OCPs including HCB (0.307 mg/kg) in meat and (0.344mg/kg) in fat; followed by, α-HCH (0.255 mg/kg) in meat and (0.290 mg/kg) in fat samples. Bazian also presented the highest level of CMT concentration in meat and fat samples (0.101 and 0.125 mg/kg, respectively). The second district that presented highest concentrations in some pesticides (DMT and FTN) was Piramagrun. DMT showed 0.103 and 0.169 mg/kg and FTN showed 0.059 and 0.099 mg/kg in meat and fat samples, respectively, which are the highest DMT and FTN concentration among the five districts.

Moreover, Darbandikhan, presented the lowest concentrations of PYRs including CMT (0.035 and 0.043 mg/kg), DMT (0.023 and 0.050 mg/kg) for meat and fat samples, respectively. This was also true for OPPs including CPS (0.067 and 0.145 mg/kg), and FTN (0.028 and 0.064 mg/kg) in meat and fat samples, respectively. While, the lowest OCPs including HCB (0.162 and 0.190 mg/kg), and α-HCH (0.077 and 0.143 mg/kg) residue found in meat and fat samples collected in carcasses of animals reared Arbat respectively.

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4.5.1.2. GC analysis

Statistically, no pesticide type presented similar value over “all districts” and no pesticide presented the highest or lowest residue among “all districts”.

Similar to HPLC analysis, Bazian presented the highest concentration of pesticides residues in CMT (0.092 and 0.119 mg/kg), HCB (0.285 and 0.327 mg/kg), α-HCH (0.239 and 0.266 mg/kg), and CPS (0.197 and 0.253 mg/kg) in meat and fat samples respectively. While, the highest DMT (0.105 and 0.178 mg/kg) and FTN (0.056 and 0.098 mg/kg) residue level was found in meat and fat samples had been collected in animal carcasses reared in Piramagrun, respectively.

The lowest pesticides residue in terms of CMT (0.030 and 0.032 mg/kg), DMT (0.021and 0.048mg/kg), CPS (0.065 and 0.139 mg/kg), and FTN (0.025 and 0.060 mg/kg) found in cattle meat and fat samples, respectively, from samples taken from carcasses of animals reared in Darbandikhan. The lowest residue of HCB (0.154 and 0.169 mg/kg) and α-HCH (0.071 and 0.135 mg/k) also found in Arbat cattle meat and fat samples, respectively.

Table 4.9. HPLC-UV analysis, found concentrations of pesticides in cattle meat and fat samples.

Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	$0.035^{a} \pm 0.003$	$0.043^{a} \pm 0.002$	$0.023^{a} \pm 0.003$	$0.050^{a} \pm 0.004$	$0.178^{a} \pm 0.006$	$0.226^{ab} \pm 0.006$	$0.164^{c} \pm 0.005$	$0.189^{b} \pm 0.006$	$0.067^{a} \pm 0.006$	$0.145^{a} \pm 0.012$	$0.028^{a} \pm 0.002$	$0.064^{a} \pm 0.004$
Said Sadiq	$0.072^{b} \pm 0.005$	$0.080^{bc} \pm 0.007$	$0.073^{c} \pm 0.005$	$0.163^{c} \pm 0.006$	$0.172^{a} \pm 0.006$	$0.215^{a} \pm 0.006$	$0.110^{b} \pm 0.006$	$0.176^{b} \pm 0.007$	$0.122^{b} \pm 0.003$	$0.186^{b} \pm 0.009$	$0.045^{b} \pm 0.003$	$0.076^{a} \pm 0.004$
Arbat	$0.037^{a} \pm 0.003$	$0.052^{a} \pm 0.009$	$0.041^{ab} \pm 0.006$	$0.066^{a} \pm 0.003$	$0.162^{a} \pm 0.007$	$0.190^{a} \pm 0.011$	$0.077^{a} \pm 0.015$	$0.143^{a} \pm 0.007$	$0.128^{b} \pm 0.003$	$0.212^{c} \pm 0.006$	$0.041^{b} \pm 0.003$	$0.065^{a} \pm 0.004$
Bazian	$0.101^{c} \pm 0.004$	$0.125^{d} \pm 0.004$	$0.057^{bc} \pm 0.010$	$0.092^{b} \pm 0.011$	$0.307^{c} \pm 0.010$	$0.344^{c} \pm 0.023$	$0.255^{e} \pm 0.006$	$0.290^{d} \pm 0.020$	$0.228^{c} \pm 0.003$	$0.264^{d} \pm 0.006$	$0.044^{b} \pm 0.002$	$0.095^{b} \pm 0.005$
Piramagrun	$0.092^{c} \pm 0.006$	$0.096^{c} \pm 0.006$	$0.103^{d} \pm 0.007$	$0.169^{c} \pm 0.009$	$0.246^{b} \pm 0.014$	$0.257^{b} \pm 0.009$	$0.200^{d} \pm 0.006$	$0.222^{c} \pm 0.009$	$0.128^{b} \pm 0.003$	$0.195^{bc} \pm 0.007$	$0.059^{c} \pm 0.007$	$0.099^{b} \pm 0.007$

Table 4.10. GC-MS analysis, found concentrations of pesticides in cattle meat and fat samples.

Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	$0.030^{a} \pm 0.003$	$0.032^{a} \pm 0.002$	$0.021^{a} \pm 0.002$	$0.048^{a} \pm 0.004$	$0.166^{ab} \pm 0.006$	$0.210^{b} \pm 0.010$	$0.155^{c} \pm 0.004$	$0.187^{bc} \pm 0.004$	$0.065^{a} \pm 0.006$	$0.139^{a} \pm 0.011$	$0.025^{a} \pm 0.002$	$0.060^{a} \pm 0.003$
Said Sadiq	$0.066^{b} \pm 0.004$	$0.076^{bc} \pm 0.007$	$0.070^{c} \pm 0.004$	$0.140^{d} \pm 0.005$	$0.183^{b} \pm 0.004$	$0.216^{b} \pm 0.005$	$0.102^{b} \pm 0.003$	$0.168^{b} \pm 0.006$	$0.115^{b} \pm 0.002$	$0.177^{b} \pm 0.008$	$0.041^{b} \pm 0.003$	$0.072^{a} \pm 0.005$
Arbat	$0.036^{a} \pm 0.003$	$0.059^{b} \pm 0.009$	$0.047^{b} \pm 0.006$	$0.070^{b} \pm 0.002$	$0.154^{a} \pm 0.007$	$0.169^{a} \pm 0.008$	$0.071^{a} \pm 0.005$	$0.135^{a} \pm 0.007$	$0.121^{b} \pm 0.003$	$0.205^{c} \pm 0.005$	$0.037^{b} \pm 0.003$	$0.062^{a} \pm 0.004$
Bazian	$0.092^{d} \pm 0.004$	$0.119^{d} \pm 0.004$	$0.043^{b} \pm 0.004$	$0.083^{c} \pm 0.003$	$0.285^{d} \pm 0.008$	$0.327^{d} \pm 0.012$	$0.239^{e} \pm 0.005$	$0.266^{d} \pm 0.019$	$0.197^{c} \pm 0.011$	$0.253^{d} \pm 0.006$	$0.039^{b} \pm 0.003$	$0.090^{b} \pm 0.005$
Piramagrun	$0.078^{c} \pm 0.006$	$0.090^{c} \pm 0.006$	$0.105^{d} \pm 0.004$	$0.178^{e} \pm 0.005$	$0.233^{c} \pm 0.012$	$0.259^{c} \pm 0.011$	$0.193^{d} \pm 0.004$	$0.214^{c} \pm 0.007$	$0.120^{b} \pm 0.002$	$0.187^{bc} \pm 0.007$	$0.056^{c} \pm 0.007$	$0.098^{b} \pm 0.007$

Values present mean of 10 samples (mg/kg ± SEM). ^{a,b,c,d,e}: Different superscript letters denote significant differences within column ($p < 0.05$). Red and green colors refer the highest and lowest concentration levels, respectively.

Overall, in HPLC and GC analysis, the highest residual concentration found in carcasses of animals raised in Bazian and Piramagrun, while the lowest residual values were mostly presented in samples of carcasses raised in Darbandikhan, and these results can be attributed to the feeding program. Basically, cattle are grazing outdoor during the day and fed concentrate fodder at night. While, the pastures where the cattle fed in are different in terms of crop and grass types between districts.

In Darbandikhan, cattle are grazing out on left over harvested wheat, barley grain, grasses and rye in summer. While, in spring cattle are mostly graze out on grasses and in winter consume left over of seasonal crops such as, cabbage, cauliflower and lettuce etc, as well as, consume hay and concentrate feed at night. In Said Sadiq and Arbat cattle are grazing out on left over harvested wheat, barley grain in summer, as well as, in seasonal crops such as tomato, cucumber, courgette, and melons in open cultivated fields which are already treated by HCB, HCH, α-CMT, CPS and FTN pesticides; while, grasses and mixture of concentrate feed are fed to cattle in spring and winter, respectively. Left overs of seasonal crops such as cabbage, cauliflower and lettuce etc, also used sometimes; but mostly the crops cultivated in open fields instead of green houses.

In Bazian and Piramagrun, cattle also graze out on left over in summer seasonal crops such as tomato, cucumber, green beans, zucchini, and melons in open cultivated field which are already treated with α-CMT and/or DMT. Beside, greenhouse byproducts including deteriorate products and crops left over which are highly treated with α-CMT, HCB, α-HCH, CPS.

Besides outdoor contaminated pastures, left over crops, and contaminated water, concentrate feed could be another source of pesticides, however feeds made by feed factories

expected to be less contaminated due to the high processing temperature (80^{o}C) during pellet making in the machine.

4.5.2. Sheep samples

4.5.2.1. HPLC analysis

Regarding the deference in pesticide concentration in sheep samples between districts, there were significant differences between pesticide residual levels among these districts in samples analysed by using HPLC. No significant difference presented between the five districts regarding CMT residue in sheep meat samples ($P < 0.05$).

Samples collected from carcasses of sheep raised in Piramagrun presented the highest concentrations residue including CMT (0.119 and 0.227 mg/kg), α-HCH (0.215 and 0.227 mg/kg), CPS (0.142 and 0.190 mg/kg), and FTN (0.089 and 0.142 mg/kg) in meat and fat samples, respectively. While in Bazian district which is near Piramagrun presented greatest concentration of HCB in meat (0.166 mg/kg) and fat (0.246 mg/kg) samples. The highest DMT residue (0.304 and 0.328 mg/kg) among the five districts was found in meat and fat samples collected in carcasses of animals reared in Said Sadiq.

Moreover, the lowest residual levels of CMT (0.104 and 0.154 mg/kg), DMT (0.135 and 0.177 mg/kg), HCB (0.102 and 0.154 mg/kg), and α-HCH (0.109 and 0.170 mg/kg) found in meat and fat samples, respectively from the carcasses of sheep reared in Darbandikhan, which also presented very low residual level of CPS in meats and fat samples. While, the lowest CPS residue among the five districts was found in Arbats' sheep meat samples (0.090 mg/kg).

4.5.2.2. GC analysis

Statistically, there was also no significant difference between districts regarding CMT residue in sheep meat samples ($P > 0.05$), while no pesticides showed the highest or lowest residue level over all the "five districts".

The highest concentration of CMT (0.116 and 0.217 mg/kg), α-HCH (0.207 and 0.211 mg/kg), CPS (0.138 and 0.182 mg/kg), and FTN (0.085 and 0.132 mg/kg) was found in meat and fat samples of sheep reared in Piramagrun. Moreover, the highest HCB residue among the districts was found in Bazian sheep meat (0.161mg/kg) and fat (0.234 mg/kg) samples. DMT was the only pesticide presented the highest concentration in sheep meat (0.293 mg/kg) and fat (0.335 mg/kg) samples collected in carcasses of sheep fostered in Said Sadiq.

In contrast, Darbandikhan presented the lowest residue of CMT (0.090 and 0.149 mg/kg), DMT (0.135 and 0.170 mg/kg), HCB (0.097 and 0.142 mg/kg), and α-HCH (0.105 and 0.162 mg/kg) in sheep meat and fat samples, respectively. Regarding the OPPs in sheep carcasses, the lowest CPS residue level was found in Arbat meat (0.088 mg/kg) and fat (0.133 mg/kg) samples, while the lowest FTN residue level was in meat (0.033 mg/kg) and fat (0.054 mg/kg) samples collected in sheep carcasses reared in Said Sadiq.

Overall, in samples analaysis by HPLC and GC Piramagrun presented high residue level in all studied pesticides except DMT and HCB. While, Darbandikhan presented the lowest residual level in all the studied pesticides except CPS and FTN.

Table 4.11. HPLC-UV analysis, found concentrations of pesticides in sheep meat and fat samples.

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Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	$0.104^{a} \pm 0.004$	$0.159^{a} \pm 0.009$	$0.135^{a} \pm 0.009$	$0.177^{a} \pm 0.010$	$0.102^{a} \pm 0.005$	$0.154^{a} \pm 0.007$	$0.109^{a} \pm 0.012$	$0.170^{a} \pm 0.009$	$0.100^{ab} \pm 0.005$	$0.145^{a} \pm 0.008$	$0.054^{b} \pm 0.002$	$0.084^{b} \pm 0.004$
Said Sadiq	$0.108^{a} \pm 0.004$	$0.155^{a} \pm 0.014$	$0.304^{c} \pm 0.013$	$0.328^{c} \pm 0.017$	$0.154^{bc} \pm 0.009$	$0.207^{c} \pm 0.016$	$0.124^{a} \pm 0.011$	$0.180^{a} \pm 0.005$	$0.114^{ab} \pm 0.006$	$0.182^{b} \pm 0.006$	$0.034^{a} \pm 0.003$	$0.058^{a} \pm 0.018$
Arbat	$0.107^{a} \pm 0.003$	$0.161^{a} \pm 0.008$	$0.213^{b} \pm 0.012$	$0.247^{b} \pm 0.009$	$0.116^{a} \pm 0.004$	$0.194^{bc} \pm 0.006$	$0.168^{b} \pm 0.006$	$0.210^{b} \pm 0.009$	$0.090^{a} \pm 0.007$	$0.145^{a} \pm 0.009$	$0.051^{b} \pm 0.004$	$0.096^{b} \pm 0.006$
Bazian	$0.111^{a} \pm 0.007$	$0.175^{a} \pm 0.008$	$0.150^{a} \pm 0.007$	$0.236^{b} \pm 0.011$	$0.166^{c} \pm 0.009$	$0.246^{d} \pm 0.010$	$0.170^{b} \pm 0.006$	$0.216^{b} \pm 0.009$	$0.116^{b} \pm 0.010$	$0.184^{b} \pm 0.007$	$0.079^{c} \pm 0.004$	$0.096^{b} \pm 0.006$
Piramagrun	$0.119^{a} \pm 0.008$	$0.227^{b} \pm 0.010$	$0.286^{c} \pm 0.018$	$0.292^{c} \pm 0.020$	$0.144^{b} \pm 0.009$	$0.169^{ab} \pm 0.009$	$0.215^{c} \pm 0.012$	$0.227^{b} \pm 0.012$	$0.142^{c} \pm 0.010$	$0.190^{b} \pm 0.014$	$0.089^{c} \pm 0.006$	$0.142^{c} \pm 0.006$

Table 4.12 GC-MS analysis, found concentrations of pesticides in sheep meat and fat samples.

Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	$0.090^{a} \pm 0.008$	$0.149^{a} \pm 0.009$	$0.135^{a} \pm 0.009$	$0.170^{a} \pm 0.004$	$0.097^{a} \pm 0.005$	$0.142^{a} \pm 0.005$	$0.105^{a} \pm 0.011$	$0.162^{a} \pm 0.009$	$0.101^{ab} \pm 0.004$	$0.139^{a} \pm 0.008$	$0.052^{b} \pm 0.002$	$0.090^{b} \pm 0.006$
Said Sadiq	$0.093^{a} \pm 0.016$	$0.151^{a} \pm 0.013$	$0.293^{c} \pm 0.012$	$0.335^{d} \pm 0.015$	$0.148^{bc} \pm 0.009$	$0.220^{c} \pm 0.013$	$0.120^{a} \pm 0.011$	$0.171^{a} \pm 0.005$	$0.110^{ab} \pm 0.006$	$0.178^{b} \pm 0.007$	$0.033^{a} \pm 0.003$	$0.054^{a} \pm 0.005$
Arbat	$0.103^{a} \pm 0.004$	$0.158^{a} \pm 0.006$	$0.205^{b} \pm 0.011$	$0.222^{b} \pm 0.008$	$0.112^{a} \pm 0.004$	$0.184^{b} \pm 0.006$	$0.162^{b} \pm 0.006$	$0.206^{b} \pm 0.007$	$0.088^{a} \pm 0.007$	$0.133^{a} \pm 0.009$	$0.049^{b} \pm 0.004$	$0.089^{b} \pm 0.006$
Bazian	$0.107^{a} \pm 0.006$	$0.167^{a} \pm 0.007$	$0.145^{a} \pm 0.007$	$0.215^{b} \pm 0.010$	$0.161^{c} \pm 0.009$	$0.234^{c} \pm 0.009$	$0.163^{b} \pm 0.006$	$0.208^{b} \pm 0.013$	$0.112^{b} \pm 0.010$	$0.150^{a} \pm 0.010$	$0.076^{c} \pm 0.004$	$0.079^{b} \pm 0.004$
Piramagrun	$0.116^{a} \pm 0.007$	$0.217^{b} \pm 0.009$	$0.275^{c} \pm 0.017$	$0.296^{c} \pm 0.015$	$0.138^{b} \pm 0.009$	$0.147^{a} \pm 0.005$	$0.207^{c} \pm 0.011$	$0.211^{b} \pm 0.007$	$0.138^{c} \pm 0.010$	$0.182^{b} \pm 0.007$	$0.085^{c} \pm 0.005$	$0.132^{c} \pm 0.005$

Values present mean of 10 samples (mg/kg ± SEM). ^{a,b,c,d,e}: Different superscript letters denote significant differences within column ($p < 0.05$). Red and green colors present the highest and lowest concentration levels, respectively.

4.5.3. Goat samples

4.5.3.1. HPLC analysis

Statistically, all the six pesticide residue level showed difference level among the five districts. At the same time, there were no significant differences between the residual levels of some pesticides among some of districts. While, no pesticide residue showed the highest or lowest level over the “five districts. Regarding the deference in pesticide concentration in goat samples between districts, there were differences between pesticide residual levels among these districts using HPLC analysis, Piramagrun presented the highest residue concentration of PYRs including CMT (0.100 and 0.102 mg/kg), and DMT (0.143 and 0.162 mg/kg) in meat and fat samples, respectively. This was true for OPPs including CPS (0.120 and 0.206 mg/kg), and FTN (0.049 and 0.065 mg/kg) in meat and fat samples, respectively. The highest OCPs including HCB in meat (0.139 mg/kg) and fat (0.173 mg/kg) and α-HCH in meat (0.126 mg/kg) and fat (0.142 mg/kg) were found in samples collected in carcasses of goats reared in Bazian.

By contrast, Arbat showed the lowest CMT residue in meat (0.065 mg/kg) and fat (0.085 mg/kg) samples in comparison with other districts, which was almost about equal residual levels to Darbandikhan (0.066 and 0.086 mg/kg in meat and fat samples, respectively). Beside to finding low CMT residual concentration in samples collected in carcasses of animals reared Dabandikhan, it also showed the lowest DMT in meat (0.038 mg/kg) and fat (0.065 mg/kg) samples, which was also true for HCB that presented the lowest residual level in meat (0.071mg/kg) and fat (0.095 mg/kg) samples and α-HCH in meat (0.039 mg/kg) and fat (0.063 mg/kg). Arbat goat meat and fat samples also presented the lowest residual level of OPPs including CPS in meat (0.039 mg/kg) and fat (0.057 mg/kg), and FTN in meat (0.009 mg/kg) and fat (0.010 mg/kg) samples.

4.5.3.2. GC analysis

Statistically, all the six pesticide residue level showed significant difference over the “five districts, but only few non-differences noticed between the residual levels among some districts. No pesticide residue showed the highest or lowest residual level among all the “five districts”.

Similarly, there were significant differences between pesticide residual levels among these districts in sheep samples using GC analysis, Piramagrun presented the highest residual concentration of CMT in goat meat (0.096 mg/kg) and fat (0.098 mg/kg) and DMT in meat (0.136 mg/kg) and fat (0.155 mg/kg) samples. Samples collected from carcasses of animals reared in Piramagrun also presented the highest CPS residue in meat (0.115 mg/kg) and fat (0.143 mg/kg) samples; this was also true the FTN in meat (0.042 mg/kg) and fat (0.062 mg/kg) samples. While, Bazian presented the highest residual concentration of OCs including HCB (0.133 and 0.164 mg/kg) and α-HCH (0.121 and 0.138 mg/kg) in meat and fat samples, respectively.

The lowest residual levels of CMT, DMT, HCB, and α-HCH were also found in Drbandikhan which were 0.063, 0.036, 0.067, and 0.037 mg/kg in meat and 0.082, 0.060, 0.090, and 0.059 mg/kg in fat samples, respectively. CMT also found in Arbat (0.063 and 0.083 in meat and fat samples, respectively) which was almost equal level as in Darbandikhan. Arbat also presented the lowest OPPs residue including CPS and FTN as 0.037 and 0.009 mg/kg in meat samples and 0.054 and 0.010 mg/kg in fat samples, respectively.

Table 4.13.HPLC-UV analysis, found concentrations of pesticides in goat meat and fat samples.

Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	0.066^{a} ±0.004	0.086^{ab} ± 0.004	0.038^{a}± 0.002	0.065^{a} ± 0.003	0.071^{a} ±0.003	0.095^{a} ± 0.004	0.039^{a} ±0.002	0.063^{a} ± 0.003	0.042^{a} ±0.002	0.067^{a} ± 0.004	0.020^{b} ±0.003	0.029^{b} ± 0.003
Said Sadiq	0.073^{a} ±0.004	0.093^{a} ± 0.004	0.139^{c}± 0.002	0.145^{c} ± 0.004	0.081^{a} ± 0.005	0.118^{b} ± 0.009	0.081^{b} ±0.004	0.103^{b} ± 0.008	0.075^{b} ±0.006	0.093^{b} ± 0.005	0.010^{a} ±0.004	0.022^{b} ± 0.005
Arbat	0.065^{a} ± 0.002	0.085^{a} ± 0.003	0.092^{b}± 0.008	0.116^{b} ± 0.006	0.074^{a} ± 0.004	0.099^{a} ± 0.004	0.105^{c} ±0.003	0.129^{c} ± 0.011	0.039^{a} ±0.002	0.057^{a} ± 0.003	0.009^{a} ±0.003	0.010^{a} ± 0.001
Bazian	0.089^{bc} ± 0.004	0.091^{a} ± 0.005	0.108^{b}± 0.006	0.144^{cd} ± 0.007	0.139^{c} ± 0.004	0.173^{c} ± 0.002	0.126^{cd} ±0.01	0.142^{c} ± 0.004	0.093^{c} ±0.006	0.115^{c} ± 0.012	0.023^{b} ±0.003	0.037^{c} ± 0.002
Piramagrun	0.100^{c} ± 0.004	0.102^{b} ± 0.004	0.143^{c}± 0.001	0.162^{d} ± 0.006	0.105^{b} ± 0.004	0.119^{b} ± 0.007	0.114^{c} ±0.004	0.137^{c} ± 0.006	0.120^{d} ±0.005	0.206^{d} ± 0.010	0.049^{c} ±0.003	0.065^{d} ± 0.003

Table 4.14. GC-MS analysis, found concentrations of pesticides in goat meat and fat samples.

Pesticides / Locality	CMT		DMT		HCB		α-HCH		CPS		FTN	
	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat	Meat	Fat
Darbandikhan	0.063^{a} ± 0.003	0.082^{a} ± 0.003	0.036^{a} ± 0.002	0.060^{a} ± 0.003	0.067^{a} ± 0.003	0.090^{a} ± 0.004	0.037^{a} ± 0.002	0.059^{a} ± 0.003	0.044^{a} ± 0.005	0.064^{a} ± 0.004	0.019^{b} ± 0.003	0.035^{c} ± 0.002
Said Sadiq	0.071^{a} ± 0.004	0.085^{a} ± 0.004	0.103^{c} ± 0.007	0.148^{c} ± 0.007	0.104^{b} ± 0.003	0.112^{b} ± 0.006	0.078^{b} ± 0.004	0.081^{b} ± 0.006	0.086^{b} ± 0.005	0.111^{b} ± 0.012	0.008^{a} ± 0.001	0.019^{b} ± 0.003
Arbat	0.063^{a} ± 0.004	0.083^{a} ± 0.003	0.091^{b} ± 0.005	0.112^{b} ± 0.005	0.076^{a}± 0.003	0.094^{a} ± 0.003	0.103^{c} ± 0.004	0.131^{c} ± 0.005	0.037^{a} ± 0.003	0.054^{a} ± 0.003	0.009^{a} ± 0.001	0.010^{a} ± 0.001
Bazian	0.087^{b} ± 0.005	0.089^{ab} ± 0.003	0.133^{d} ± 0.002	0.136^{c} ± 0.009	0.133^{c} ± 0.004	0.164^{c} ± 0.002	0.121^{c} ± 0.011	0.138^{c} ± 0.002	0.074^{b} ± 0.006	0.090^{b} ± 0.005	0.018^{b} ± 0.004	0.020^{b} ± 0.005
Piramagrun	0.096^{b} ± 0.004	0.098^{b} ± 0.004	0.136^{d} ± 0.001	0.155^{c} ± 0.006	0.077^{a} ± 0.005	0.110^{b} ± 0.008	0.109^{c} ± 0.004	0.134^{c} ± 0.005	0.115^{c} ± 0.005	0.143^{c} ± 0.009	0.042^{c} ± 0.005	0.062^{d} ± 0.003

Values present mean of 10 samples (mg/kg ± SEM). ^{a,b,c,d,e}: Different superscript letters denote significant differences within column ($p < 0.05$). Red and green colors present the highest and lowest concentration levels, respectively.

Overall, in HPLC and GC analysis, the data showed that the highest residue levels of PYRs and OPPs found in goat tissue samples collected in carcasses of animals reared in Piramagrun, while the highest OCPs concentrations found in samples collected in carcasses of animals reared in Bazian. Hence, Bazian and Piramarun the two districts which are relatively close to each other, both showed higher pesticide residue in the three species than other districts. By contrast, Darbandikhan district showed the lowest pesticide residue in goat tissue samples regarding PYRs and OCPs. While, the lowest OPPs found in samples collected in carcasses of animals that reared in Arbat, this was also true for CMT.

In the all five districts, no regular feeding program for sheep and goats is applied. Sheep and goats are mostly grazing outdoor during the day and fed concentrate fodder at night. The pastures where the sheep and goats fed in are different in terms of crop types between districts, and grazing area in sheep and goats are different from that of cattle, because owners have a massive herd of sheep and goats are mostly take them to lands, mountains, meadow and far from agricultural area to graze, whereas the opposite in cattle as the owners mostly have small herd of cattle and let them to graze close around, rural, and agricultural area and feed mostly on left over of planted crops.

In Darbandikhan, the pastures for sheep and goats are mostly left over of harvested wheat barley grain, rye, oat and some seasonal crops in open fields, such as cucumber, tomato, melons and beans are occasionally used for animals, while greenhouses byproducts hardly ever used for animals.

In Said Sadiq and Arbat, sheep and goats mostly graze on grasslands, left over and harvested wheat, barley grain and less on rye and oat and almost no greenhouse byproducts are used for small ruminants, except some seasonal crop left over planted in open fields.

In Bazian and Piramagrun, sheep and goats usually graze out on grass in spring and on left over of wheat and barley grain in June to about the end of August. Deteriorated vegetables in open planted field and greenhouses byproducts are frequently fed to animals frequently such as, tomato, cucumber, eggplants, etc which are already treated with α-CMT, HCB, α-HCH, and CPS by tetra dose higher than open cultivated cops; as well as, contaminated grass around the cultivated areas are a main source of all animals feed in spring.

Generally, Bazian and Piramagrun presented the highest pesticides residue in cattle samples in this study. This indicated that farmers use these pesticides to treat open planted crops and greenhouses plants extensively and have caused to contaminate crops, grasslands, pastures and even might have contaminated water/environment. By contrast, Darbandikhan cattle samples presented the lowest PYRS, and OPPs and low OCPs residues. T his refers to less use of these studied pesticides by farmers in Darbandikhan; as well as, farmers plant their crops in open field lands instead of greenhouses, which uses much less pesticides.

In Bazian, sheep samples showed the highest concentration of OCPs especially HCB, this was true for goat samples which presented the highest HCB and α-HCH; while Piramagrun presented the highest PYRs (CMT, DMT) and OPPs (CPS, FTN) in meat and fat samples of goat samples, this is also true for sheep samples except DMT. Overall, Bazian and Piramagrun presented high concentration of all pesticides in sheep and goat tissue samples.

The main reason to find high residue of studied pesticides in these two districts is due to that sheep and goats usually sometimes graze on left over of wheat and barley grain, harvested deteriorate vegetables in open cultivated field and greenhouses byproducts which are already treated with α-CMT, HCB, α-HCH, CPS, and FTN by much higher dose than open cultivated cops.

While, samples collected in Darbandikhan presented lower pesticide residual concentration (CMT, DMT, HCB, and α-HCH) in sheep and goat samples. This indicated that the less used pesticides by farmers to treat animals and crops, as well as no greenhouses by-products used for animals.

Finding high PYRs in all the three animal species in the five districts indicated that the residue could mostly come from feeds because the use of DMT and CMT for cattle is not very common in veterinary medicine.

In this study, no pesticide presented the highest concentration over the five districts means farmers are not stick with a specific pesticide, but also they use pesticides randomly depends on firstly; prices, secondly; market availability, and thirdly; broad spectrum activity. Hence, the residual level of studied pesticides was significantly different among districts except CMT in sheep meat samples of all the five districts.

4.6. Heat treatment impacts

4.6.1. HPLC analysis

Boiling and broiling process could destroy pesticides residues differently. Boiling at 100°C for 30 min reduced the concentrations of the six pesticides significantly ($p < 0.05$) in cattle, sheep, and goat muscle and fat tissues except FTN in goat meat and fat samples ($p > 0.05$).

In meat samples, the highest reduction percentages noticed in PYRs (Table 4.17), including CMT and DMT ranged from 37.31 – 41.77% and 38.46 – 40 %, respectively. Followed by OCPs including α-HCH which showed the dissipation level of 36.65 – 37.63% which was higher than the reduction level in HCB (33.82 – 35.11%). The least reduction level in boiling process noticed in FTN which was 23.26 – 26.23%; followed by CPS which showed the second most reduced pesticide percentages (27.4 – 28.32%).

Similarly, the highest reduction level in fat samples was observed in PYRs including CMT and DMT that presented 34.07 – 41.48% and 32.54 – 39.81%, respectively, followed by the OCPs including α-HCH which presented reduction level of 33.91 –39% and HCB reduced by 30.58 – 34.54%. OPPs in fat samples again presented the lowest reduction level including FTN (22.5 – 27.37%); followed by CPS (25.93 – 28.99%).

On the other hand, broiling at 176°C for 20 min reduced the studied pesticides concentration in questionable level, and the effect of broiling was less than boiling significantly ($p < 0.05$), except in FTN in goat meat and fat samples and CPS in goat meat samples.

In broiling process for meat samples, the most reduced pesticides was PYRs including CMT and DMT which were ranged between 15.43 – 17.72% and 15.38 – 16.67%, respectively. Followed by, α-HCH which reduced by 11.83 – 13.98%, and then HCB reduced by 11.03 –12.77 %. Regarding OPPs, FTN presented the lowest reduction level (6.56 – 8.33%), followed by CPS which reduced by 9.33 – 11.11%.

Table 4.15. Residual levels of pesticides in meat samples analysed by HPLC after boiling and broiling processes

Pesticide	Species	Raw meat	Boiling (100°C, 30 min)	Broiling (176°C, 20 min)
CMT	Cattle	$0.067^{b} \pm 0.004$	$0.042^{a} \pm 0.003$	$0.056^{b} \pm 0.004$
	Sheep	$0.110^{c} \pm 0.002$	$0.066^{a} \pm 0.002$	$0.093^{b} \pm 0.002$
	Goat	$0.079^{cb} \pm 0.003$	$0.046^{a} \pm 0.002$	$0.065^{b} \pm 0.002$
DMT	Cattle	$0.060^{b} \pm 0.005$	$0.036^{a} \pm 0.003$	$0.050^{b} \pm 0.004$
	Sheep	$0.218^{c} \pm 0.011$	$0.133^{a} \pm 0.007$	$0.184^{b} \pm 0.009$
	Goat	$0.104^{c} \pm 0.006$	$0.064^{a} \pm 0.004$	$0.088^{b} \pm 0.005$
HCB	Cattle	$0.213^{c} \pm 0.009$	$0.140^{a} \pm 0.006$	$0.188^{b} \pm 0.007$
	Sheep	$0.136^{c} \pm 0.005$	$0.090^{a} \pm 0.003$	$0.121^{b} \pm 0.004$
	Goat	$0.094^{c} \pm 0.004$	$0.061^{a} \pm 0.003$	$0.082^{b} \pm 0.004$
α-HCH	Cattle	$0.161^{b} \pm 0.009$	$0.102^{a} \pm 0.006$	$0.141^{b} \pm 0.008$
	Sheep	$0.157^{c} \pm 0.007$	$0.098^{a} \pm 0.004$	$0.1385^{b} \pm 0.006$
	Goat	$0.093^{c} \pm 0.005$	$0.058^{a} \pm 0.003$	$0.080^{b} \pm 0.005$
CPS	Cattle	$0.135^{b} \pm 0.008$	$0.097^{a} \pm 0.006$	$0.120^{b} \pm 0.007$
	Sheep	$0.113^{c} \pm 0.004$	$0.081^{a} \pm 0.003$	$0.101^{b} \pm 0.004$
	Goat	$0.073^{b} \pm 0.005$	$0.053^{a} \pm 0.003$	$0.066^{ab} \pm 0.005$
FTN	Cattle	$0.043^{b} \pm 0.002$	$0.033^{a} \pm 0.002$	$0.040^{b} \pm 0.002$
	Sheep	$0.061^{b} \pm 0.003$	$0.045^{a} \pm 0.003$	$0.057^{b} \pm 0.003$
	Goat	$0.024^{a} \pm 0.002$	$0.018^{a} \pm 0.002$	$0.022^{a} \pm 0.002$

Raw and heat treated values, represent mean concentration (mg/kg ± SEM), n =50.

$^{a, b, c}$: Different superscript letters denote significant differences within a row ($p < 0.05$).

In fat samples, the most dissipated pesticides were also CMT and DMT that reduced by 16.32 –17.58% and 15.74 – 17.46 %, respectively (Table 4.17). Followed by α-HCH and HCB that dissipated by 13 –14.71% and 12.89 –14.05%, respectively. Among the six pesticides, FTN

presented the lowest reduction level (8.42 –10%) in fat samples, and CPS presented the second lowest reduction level (9 – 11.83%).

Table 4.16. Residual levels of pesticides in fat samples analysed by HPLC after boiling and broiling processes

Pesticide	Species	Raw fat	Boiling (100 °C, 30 min)	Broiling (176 °C, 20 min)
CMT	Cattle	$0.079^{b} \pm 0.005$	$0.049^{a} \pm 0.003$	$0.066^{b} \pm 0.004$
	Sheep	$0.176^{c} \pm 0.006$	$0.103^{a} \pm 0.003$	$0.146^{b} \pm 0.005$
	Goat	$0.091^{c} \pm 0.002$	$0.060^{a} \pm 0.001$	$0.075^{b} \pm 0.001$
DMT	Cattle	$0.108^{b} \pm 0.008$	$0.065^{a} \pm 0.005$	$0.091^{b} \pm 0.006$
	Sheep	$0.256^{c} \pm 0.010$	$0.156^{a} \pm 0.006$	$0.213^{b} \pm 0.008$
	Goat	$0.126^{c} \pm 0.005$	$0.085^{a} \pm 0.004$	$0.104^{b} \pm 0.005$
HCB	Cattle	$0.247^{c} \pm 0.009$	$0.162^{a} \pm 0.006$	$0.214^{b} \pm 0.008$
	Sheep	$0.194^{c} \pm 0.006$	$0.127^{a} \pm 0.004$	$0.169^{b} \pm 0.005$
	Goat	$0.121^{c} \pm 0.005$	$0.084^{a} \pm 0.003$	$0.104^{b} \pm 0.005$
α-HCH	Cattle	$0.204^{c} \pm 0.009$	$0.130^{a} \pm 0.006$	$0.174^{b} \pm 0.008$
	Sheep	$0.200^{c} \pm 0.005$	$0.122^{a} \pm 0.003$	$0.174^{b} \pm 0.004$
	Goat	$0.113^{c} \pm 0.005$	$0.076^{a} \pm 0.004$	$0.0997^{b} \pm 0.005$
CPS	Cattle	$0.200^{c} \pm 0.007$	$0.145^{a} \pm 0.005$	$0.182^{b} \pm 0.006$
	Sheep	$0.169^{c} \pm 0.005$	$0.120^{a} \pm 0.004$	$0.149^{b} \pm 0.003$
	Goat	$0.108^{b} \pm 0.020$	$0.080^{a} \pm 0.015$	$0.097^{b} \pm 0.018$
FTN	Cattle	$0.080^{c} \pm 0.003$	$0.062^{a} \pm 0.002$	$0.072^{b} \pm 0.003$
	Sheep	$0.095^{b} \pm 0.005$	$0.069^{a} \pm 0.003$	$0.087^{b} \pm 0.004$
	Goat	$0.031^{a} \pm 0.003$	$0.024^{a} \pm 0.002$	$0.028^{a} \pm 0.002$

Raw and heat treated values, represent mean concentration (mg/ kg ± SEM), n =50.

$^{a, b, c}$: Different superscript letters denote significant differences within a row ($p < 0.05$).

Table 4.17. Reduction percentages (R %) of meat and fat samples analysed by HPLC in boiling and broiling processes

Pesticide	Species	Boiling (100°C, 30 min)		Broiling (176°C, 20 min)	
		Meat (R%)	Fat (R%)	Meat (R%)	Fat (R%)
CMT	Cattle	-37.31	-37.97	-15.43	-16.32
	Sheep	-40.00	-41.48	-15.45	-17.05
	Goat	-41.77	-34.07	-17.72	-17.58
DMT	Cattle	-40.00	-39.81	-16.67	-15.74
	Sheep	-38.99	-39.06	-15.60	-16.80
	Goat	-38.46	-32.54	-15.38	-17.46
HCB	Cattle	-34.27	-34.41	-11.74	-13.77
	Sheep	-33.82	-34.54	-11.03	-12.89
	Goat	-35.11	-30.58	-12.77	-14.05
α-HCH	Cattle	-36.65	-36.27	-12.42	-14.71
	Sheep	-37.58	-39.00	-11.83	-13.00
	Goat	-37.63	-33.91	-13.98	-13.27
CPS	Cattle	-28.15	-27.50	-11.11	-9.00
	Sheep	-28.32	-28.99	-10.62	-11.83
	Goat	-27.40	-25.93	-9.33	-10.19
FTN	Cattle	-23.26	-22.50	-6.98	-10.00
	Sheep	-26.23	-27.37	-6.56	-8.42
	Goat	-25.00	-22.58	-8.33	-9.68

Boiling and broiling values for meat and fat samples represent mean of reduction concentration (n =50).

4.6.2. GC analysis

GC-MS analysis confirmed the HPLC data, in which boiling process could reduce pesticides significantly ($P < 0.05$), and the effect of boiling was significantly higher than broiling in reduction of all pesticide except FTN in goat meat and fat, and cattle meat samples and CPS in goat meat samples.

GC-MS analysis confirmed the HPLC data, in which boiling process could reduce pesticides significantly ($P < 0.05$), and the effect of boiling was higher than broiling in reduction of all pesticide except FTN in beef, goat meat and fat samples and CPS in goat meat samples.

The most reduced pesticides were PYRs group in meat samples including CMT (36.67 – 42.16%) and DMT (35 – 36.84%). OCPs presented slightly less reduction percentages compared to PYRs, because the highest reduction level that was noticed in α-HCH was 34.87 – 35.56 % (Table 4.20). However, HCB was in same group of pesticide with α-HCH, it showed lower reduction level (31.86 –34.36%) than in α-HCH. OPPs including FNT and CPS presented the lowest and second lowest reduction levels which were 21.05 – 25.42% and 23.94 – 29.84%, respectively.

Table 4.18. Residual levels of pesticides in meat samples analysed by GC after boiling and broiling processes.

Pesticide	Species	Raw meat	Boiling (100°C, 30 min)	Broiling (176°C, 20 min)
CMT	Cattle	$0.060^{b} \pm 0.004$	$0.038^{a} \pm 0.003$	$0.051^{b} \pm 0.003$
	Sheep	$0.102^{c} \pm 0.004$	$0.059^{a} \pm 0.002$	$0.086^{b} \pm 0.003$
	Goat	$0.076^{c} \pm 0.003$	$0.048^{a} \pm 0.003$	$0.0636^{b} \pm 0.002$
DMT	Cattle	$0.057^{b} \pm 0.005$	$0.036^{a} \pm 0.003$	$0.048^{b} \pm 0.004$
	Sheep	$0.210^{c} \pm 0.011$	$0.134^{a} \pm 0.007$	$0.1762^{b} \pm 0.009$
	Goat	$0.100^{c} \pm 0.005$	$0.065^{a} \pm 0.004$	$0.086^{b} \pm 0.005$
HCB	Cattle	$0.204^{c} \pm 0.008$	$0.139^{a} \pm 0.005$	$0.177^{b} \pm 0.007$
	Sheep	$0.131^{c} \pm 0.005$	$0.088^{a} \pm 0.003$	$0.114^{b} \pm 0.004$
	Goat	$0.091^{c} \pm 0.004$	$0.059^{a} \pm 0.002$	$0.0811^{b} \pm 0.003$
α-HCH	Cattle	$0.152^{b} \pm 0.009$	$0.099^{a} \pm 0.006$	$0.135^{b} \pm 0.008$
	Sheep	$0.151^{c} \pm 0.006$	$0.098^{a} \pm 0.004$	$0.130^{b} \pm 0.006$
	Goat	$0.090^{c} \pm 0.005$	$0.058^{a} \pm 0.003$	$0.080^{b} \pm 0.004$
CPS	Cattle	$0.124^{c} \pm 0.007$	$0.087^{a} \pm 0.005$	$0.110^{b} \pm 0.006$
	Sheep	$0.110^{c} \pm 0.004$	$0.082^{a} \pm 0.003$	$0.099^{b} \pm 0.004$
	Goat	$0.071^{b} \pm 0.005$	$0.054^{a} \pm 0.003$	$0.064^{ab} \pm 0.004$
FTN	Cattle	$0.040^{b} \pm 0.002$	$0.031^{a} \pm 0.002$	$0.036^{ab} \pm 0.002$
	Sheep	$0.059^{b} \pm 0.003$	$0.044^{a} \pm 0.002$	$0.054^{b} \pm 0.003$
	Goat	$0.019^{a} \pm 0.002$	$0.015^{a} \pm 0.002$	$0.017^{a} \pm 0.001$

Raw and heat treated values, represent mean concentration (mg/ kg ± SEM), n =50.

$^{a, b, c}$: Different superscript letters denote significant differences within a row ($p < 0.05$).

Similarly, in fat samples the most reduced pesticide percentage noticed in PYRs group including CMT and DMT which were 35.63 – 39.05% and 34.43 – 36.54%, respectively (Table 4.20). While, the OCPs presented lower reduction percentages than in PYRs because the reduction percentages in OCPs were 33.94 – 37.11 % and 33.33 – 36.44% in α-HCH and HCB,

respectively. OPPs including FTN and CPS presented the lowest (21.09 – 24.14%) and second lowest (26.28 – 29.69%) reduction percentages, respectively.

Broiling process also reduced pesticide concentration in meat samples, and the most reduced group was PYRs including CMT (15 – 16.47%) and DMT (14 – 16.10 %). Followed by, OCPs including α-HCH and HCB which were reduced by11.11 –13.91% and 10.89 –13.24%, respectively. The lowest reduction percentage in broiling process found in FTN (8.47 – 10.53 %), followed by CPS that presented the reduction level of 9.86 –11.29% (Table 4. 20).

In fat samples, PYRs group was the most reduced pesticides including CMT (14.33 - 18.34%) and DMT (13.93 – 17. 31%), followed by OCPs including α-HCH (12.04 – 13.74%) and HCB (11.40 –12.41%). The lowest reduction level was also noticed in OPPs including FTN (8.99 – 10.39%), followed by CPS (10.26 – 11.46%) (Table 4.20).

Table 4.19. Residual levels of pesticides in fat samples analysed by GC after boiling and broiling processes

Pesticide	Species	Raw fat	Boiling (100°C, 30 min)	Broiling (175°C, 20 min)
CMT	Cattle	$0.075^{b} \pm 0.005$	$0.046^{a} \pm 0.003$	$0.0642^{b} \pm 0.004$
	Sheep	$0.169^{c} \pm 0.005$	$0.103^{a} \pm 0.003$	$0.138^{b} \pm 0.004$
	Goat	$0.087^{c} \pm 0.002$	$0.056^{a} \pm 0.002$	$0.073^{b} \pm 0.001$
DMT	Cattle	$0.104^{c} \pm 0.007$	$0.066^{a} \pm 0.004$	$0.086^{b} \pm 0.006$
	Sheep	$0.248^{c} \pm 0.010$	$0.158^{a} \pm 0.006$	$0.207^{b} \pm 0.008$
	Goat	$0.122^{c} \pm 0.006$	$0.080^{a} \pm 0.004$	$0.105^{b} \pm 0.005$
HCB	Cattle	$0.236^{c} \pm 0.009$	$0.150^{a} \pm 0.006$	$0.2067^{b} \pm 0.008$
	Sheep	$0.185^{c} \pm 0.006$	$0.120^{a} \pm 0.004$	$0.1627^{b} \pm 0.006$
	Goat	$0.114^{c} \pm 0.004$	$0.076^{a} \pm 0.003$	$0.101^{b} \pm 0.004$
α-HCH	Cattle	$0.194^{c} \pm 0.008$	$0.122^{a} \pm 0.005$	$0.168^{b} \pm 0.007$
	Sheep	$0.191^{c} \pm 0.005$	$0.125^{a} \pm 0.003$	$0.168^{b} \pm 0.004$
	Goat	$0.109^{c} \pm 0.005$	$0.072^{a} \pm 0.003$	$0.0940^{b} \pm 0.008$
CPS	Cattle	$0.192^{c} \pm 0.006$	$0.135^{a} \pm 0.004$	$0.170^{b} \pm 0.006$
	Sheep	$0.156^{c} \pm 0.004$	$0.115^{a} \pm 0.003$	$0.140^{b} \pm 0.004$
	Goat	$0.093^{bc} \pm 0.004$	$0.068^{a} \pm 0.006$	$0.083^{b} \pm 0.008$
FTN	Cattle	$0.077^{c} \pm 0.003$	$0.059^{a} \pm 0.002$	$0.069^{b} \pm 0.003$
	Sheep	$0.089^{b} \pm 0.004$	$0.0702^{a} \pm 0.003$	$0.081^{b} \pm 0.004$
	Goat	$0.029^{a} \pm 0.003$	$0.022^{a} \pm 0.002$	$0.026^{a} \pm 0.003$

Raw and heat treated values, represent mean concentration (mg/ kg ± SEM), n=50.

$^{a, b, c}$: Different superscript letters denote significant differences within a row ($p < 0.05$).

Table 4.20. Reduction percentages (R %) of meat and fat samples analysed by GC in boiling and broiling processes

Pesticide	Species	Boiling (100^{o}C, 30 min)		Broiling (176 °C, 20 min)	
		Meat (R%)	Fat (R%)	Meat (R%)	Fat (R %)
CMT	Cattle	-36.67	-38.67	-15.00	-14.33
	Sheep	-42.16	-39.05	-15.69	-18.34
	Goat	-36.84	-35.63	-16.47	-16.09
DMT	Cattle	-36.84	-36.54	-15.79	-17.31
	Sheep	-36.19	-36.29	-16.10	-16.53
	Goat	-35.00	-34.43	-14.00	-13.93
HCB	Cattle	-31.86	-36.44	-13.24	-12.41
	Sheep	-32.82	-35.14	-12.98	-12.05
	Goat	-34.36	-33.33	-10.89	-11.40
α-HCH	Cattle	-34.87	-37.11	-11.18	-13.40
	Sheep	-35.10	-34.55	-13.91	-12.04
	Goat	-35.56	-33.94	-11.11	-13.74
CPS	Cattle	-29.84	-29.69	-11.29	-11.46
	Sheep	-25.45	-26.28	-10.00	-10.26
	Goat	-23.94	-26.88	-9.86	-10.75
FTN	Cattle	-22.50	-23.38	-10.0	-10.39
	Sheep	-25.42	-21.09	-8.47	-8.99
	Goat	-21.05	-24.14	-10.53	-10.34

Boiling and broiling values for meat and fat samples represent mean of reduction concentration (n=50).

In general, the HPLC-UV and GC-MS results presented that in boiling process, the residue of the six pesticides in the meat and fat samples reduced significantly ($p < 0.05$), except FTN in goat meat and fat samples and CPS in goat fat samples.

In boiling process, the HPLC-UV and GC-MS data showed that, the highest reduction percentages found in PYRs including CMT and ranged from 36.64 – 42.16% and DMT from 35 – 40% in meat samples. While, the least reduction percentages in HPLC and GC presented in OPPs residuals which was in FTN and ranged from 21.05 – 26. 23 and 21.09- 27.37 in meat and fat samples, respectively.

Similarly, in HPLC and GC analysis, the broiling process reduced PYRs more than OCPs and OPPs residues. The most reduced pesticide level in broiling presented in CMT in meat (15 – 17.72%), and fat samples (14.33 –18.34%). Moreover, the most reduced level presented in FTN which ranged from 6.56 –10.53% in meat and 8.42–10.39% in fat samples.

These results agree with some studies that spiked cattle meat with dieldrin and aldrin and boiled at 100^{o}C for 30 min, and noticed the reduction level of 31.6% and 33.2%, respectively (Krianmayi et al., 2016), and also agreed with the study that tested boiling at 100^{o}C for 30 min on sheep meat samples and noticed that naturally contained α, β, γ, δ-HCH samples reduced by 13.79, 27.76, 22.87, and 36.17%, respectively (Singh, 2017).

Furthermore, higher reduction (38.2%) level of OCPs (HCB) was also recorded in spiked cattle meat samples after using boiling at 100^{o}C for longer period (90 min) (Sallam and Morshedy, 2008). In natural contaminated meat samples, boiling at 100^{o}C for 30 min reduced α, β, γ, δ-HCH by 13.79, 27.76, 22.87, and 36.17%, in sheep meat samples, while in spiked samples reduced by 50.67, 45.81, 48.27, and 38.0%, respectively, and boiling at 100^{o}C for 30 min has also been tested on naturally contained α-HCH beef and reduced 32.60%, while in spiked samples reduced by 44.71% (Singh, 2017). Different reduction levels of other HCH isomers such as β, γ, δ also found in naturally contained pesticides and spiked beef samples which reduced by 15.2, 47.9, 12.3% and 27.5, 58.3, 57.9% for naturally and spiked contained

samples, respectively (Singh, 2017), due to variation in bonding of xenobiotic to some compounds of medium.

These results referring that the effect of heating treatments on reducing the level of pesticide depends on the kind of studied pesticide, which differ in their chemical and physical properties which make the pesticides have different heat susceptibility (Muthukumar et al., 2010), because dissipation level being influenced by the properties of individual pesticide, such as, different partition coefficients between the lipid and aqueous phase driven by differences in solubility, degree of volatilization, hydrolysis, which governed by different boiling points and different stability of pesticides s at high temperatures (Muresan et al., 2015).

In this study, the reduction level of PYRs and OCPs were almost close from each other, but not from OPPs reduction level; however, there is no strong correlation between a single physiochemical property of PYRs and OCPs. Since various parameters involving molecular weight, volatility (vapor pressure), hydrolysis rate and water solubility and lipophilic behavior impacts reduction rate (Hammond, 2014), no critical statement can be reported about the degree of each parameter grant to the amount loss of pesticides during boiling or broiling.

Moreover, results of heat treatment in this study could be different from studies that tested the impact of boiling and broiling on meat tissues, because they have used different critical limits in the parameters such as higher temperature, different heating period, and cooking process or using spiked samples rather than naturally contained samples, as the level of reduction of pesticide in natural contaminated meat samples are less than that of spiked samples (Singh, 2017). Hence, the reduction level of heat treated spiked samples may not correspond the realistic effect of heat treatment on dissipation pesticide level.

Regarding, the differences in the effect of heat treatments on the reduction levels of pesticides residues between the three species (cattle sheep and goat), which almost very close to each other (Table 4.17 and 4.20). This agreed with the study carried out by Singh (2017), who found no significant difference between the reduction levels observed between ruminants even differences in the reduction levels observed between ruminants versus pig and fish meat. The differences in reduction levels of pesticide residues through processing in the species is due to the variable kind of lipid as well as to the variation in the level of lipid content in their samples (Muresan et al., 2010).

Heat treatments can reduce pesticides in animal tissues, due to fat rendering induced by thermal temperature such as, co-distillation, thermal degradation and/or evaporation of fat, while the chemical nature of specific pesticides dictates which of those will prevail (Bajwa and Sandhu 2014, Đorđević and Đurović-Pejčev, 2016); and the effects of heat depends on the method of heating, heating period, and specific food item (Muresan et al., 2015, Kiranmayi et al., 2016, Letta and Attah, 2013).

The results of this study indicate that the effect of boiling is significantly higher than broiling in reduction of level of the studies pesticides this is agreed with Đorđević and Đurović-Pejčev, (2016) who found that moist heat treatment such as boiling and pressure cooking is significantly higher than dry cooking such as grilling and broiling. This could be due to the fact that in wet cooking (boiling) the boiled and steamed water droplets incorporate into the tissue and cells and wash out from the matrix (Perelló et al., 2010) and low fat content remained in boiled matrices due to its fat loss to the water (Utama et al., 2018); besides the provoke co-distillation with thermal degradation and evaporation occur due to the thermal effects and eliminate some pesticides (Perelló et al., 2010).Therefore, boiling procedures can extensively

release or remove fat from the product and tend to reduce the concentrations of the pesticides in the cooked food as confirmed previously (Utama et al., 2018, Utama et al., 2016, Muresan et al., 2015, Rawn et al., 2013).

By contrast, in dry heating such as, baking, roasting, grilling, broiling and barbecuing, the surface temperature may well exceed 100°C, leading melting lipids and evaporation of moisture in the matrices and dry or crisp (Muresan et al., 2015, and Perelló et al., 2010). Hence, a part of pesticide could be hydrolysis and co-distillated and the rest decomposed due to thermal degradation and remained in the matrix, which may vary depends on the chemical nature of the individual pesticides (Muresan et al., 2010).

Another cause in the reduction of the pesticides content is chemical transformations that are taking place during heat treatment has some roles in the reduction level (Muresan et al., 2010). Hence, the method ability for detection and quantification plays a vital role to obtain a precise result during analysis by reading the total compound, its metabolites, isomers and non-totally chemical transformed compounds.

However, less effects of broiling process on dissipation level of pesticide in meat were recorded compared to boiling process, broiling also converts fatty acids to carcinogenic substances such as advanced glycation end products (AGEs) (Nguyen and Katta, 2015), which have been linked to an increased risk of several diseases of heart and kidneys and skin aging (Prasad and Tiwari, 2017). At the same time, broiling is still preferred by consumers to obtain the maximum flavor with little sacrificing nutritional values (Goldwyn and Blonder, 2016). On the other hands, boiling at high temperatures destroys pesticides properly and improves food quality; but it dissolves and washes away water-soluble vitamins and almost of minerals (Yun-Sang et al., 2016 and Choi et al., 2016).

4.7. Comparisons between HPLC and GC resultants, performances and validations

4.7.1. Real sample concentrations

Methods based on LC and GC has been used in the analysis of a broad range of pesticides for several decades. Therefore, it is necessary to compare performances in terms of accuracy (recovery and deviations), linearity, sensitivity (limit of detection and quantification), and matrix effect of both technique to prove the suitability of methods specifically developed in this study.

Table 4.21. Concentration of the pesticides in the raw meat of cattle, sheep and goat samples analysed by HPLC-UV and GC-MS.

Pesticide	Meat samples					
	Cattle (mg/kg)		Sheep (mg/kg)		Goat (mg/kg)	
	HPLC	GC	HPLC	GC	HPLC	GC
CMT	0.067 ± 0.004	0.060 ± 0.004	0.110 ± 0.002	0.102 ± 0.004	0.079 ± 0.003	0.076 ± 0.003
DMT	0.060 ± 0.005	0.057 ± 0.005	0.218 ± 0.011	0.210 ± 0.011	0.104 ± 0.006	0.100 ± 0.005
HCB	0.213 ± 0.009	0.204 ± 0.008	0.136 ± 0.005	0.131 ± 0.005	0.094 ± 0.004	0.091 ± 0.004
α-HCH	0.161 ± 0.009	0.152 ± 0.009	0.157 ± 0.007	0.151 ± 0.006	0.093 ± 0.005	0.090 ± 0.005
CPS	0.135 ± 0.008	0.124 ± 0.007	0.113 ± 0.004	0.110 ± 0.004	0.073 ± 0.005	0.071 ± 0.005
FTN	0.043 ± 0.002	0.040 ± 0.002	0.061 ± 0.003	0.059 ± 0.003	0.024 ± 0.002	0.019 ± 0.002

Values represent mean of 50 samples ± SEM. No statistical difference was observed between HPLC-UV and GC-MS concentration in each species.

Table 4.22. Concentration of the pesticides in the raw fat of cattle, sheep and goat samples analysed by HPLC-UV and GC-MS.

Fat samples						
Pesticide	**Cattle (mg/kg)**		**Sheep (mg/kg)**		**Goat (mg/kg)**	
	HPLC	**GC**	**HPLC**	**GC**	**HPLC**	**GC**
CMT	0.079 ± 0.005	0.075 ± 0.005	0.176 ± 0.006	0.169 ± 0.005	0.091 ± 0.002	0.087 ± 0.002
DMT	0.108 ± 0.008	0.104 ± 0.007	0.256 ± 0.010	0.248 ± 0.010	0.126 ± 0.005	0.122 ± 0.006
HCB	0.247 ± 0.009	0.236 ± 0.009	0.194 ± 0.006	0.185 ± 0.006	0.121 ± 0.005	0.114 ± 0.004
α-HCH	0.204 ± 0.009	0.194 ± 0.008	0.201 ± 0.005	0.191 ± 0.005	0.113 ± 0.005	0.109 ± 0.005
CPS	0.200 ± 0.007	0.192 ± 0.006	0.169 ± 0.005	0.156 ± 0.004	0.108 ± 0.005	0.093± 0.004
FTN	0.080 ± 0.003	0.077 ± 0.003	0.095 ± 0.005	0.089 ± 0.004	0.031 ± 0.003	0.029 ± 0.003

Values represent mean of 50 samples ± SEM. No statistical difference was observed between HPLC-UV and GC-MS results in terms of residual concentrations in each species.

Both HPLC and GC method have been used for detection and quantification of same group of pesticides; however, they have some specifications for instance, GC-MS is useful for monitoring highly hydrophobic, volatile and small to medium molecular weight pesticides that are not ionized in the (electrospray ionization) ESI source of LC-UV, while, using LC-UV is allowing for analysis of larger molecular weight, polar and planer compounds in samples (He and Aga, 2019, Bargańska et al., 2018).

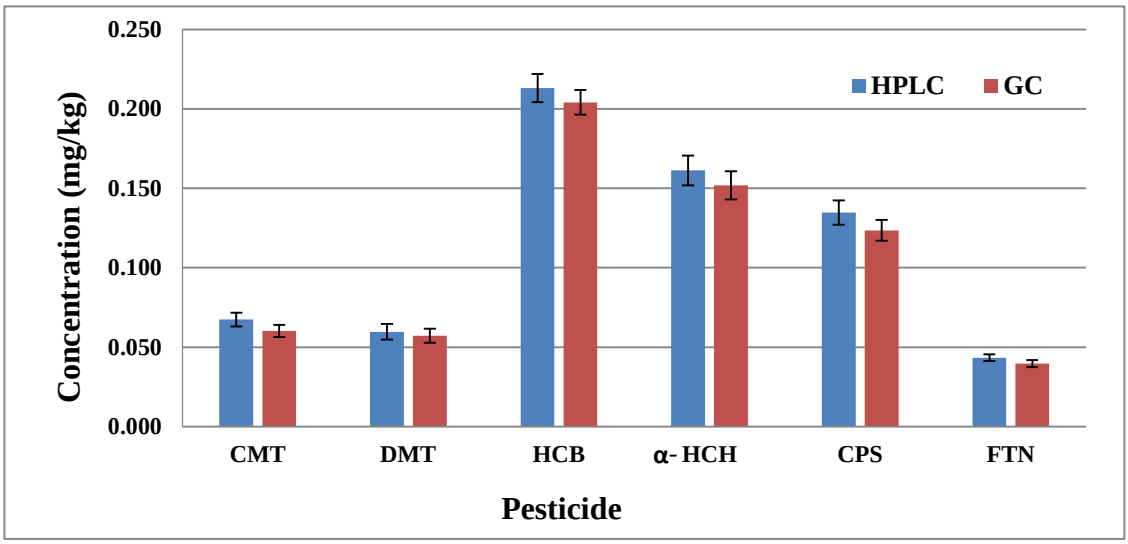


Figure 4.1. Concentration of the pesticides in the raw meat of cattle using HPLC-UV and GC-MS. Columns represent mean of 50 samples and error bars represent standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

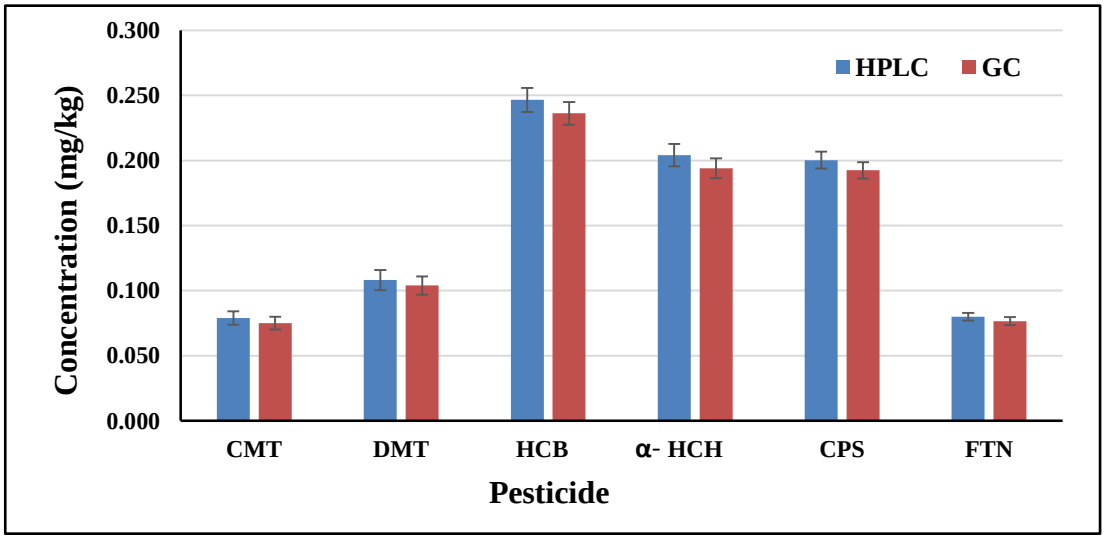


Figure 4.2. Concentration of the pesticides in the raw fat of cattle using HPLC-UV and gas GC-MS. Columns represent mean of 50 samples and error bars represent standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

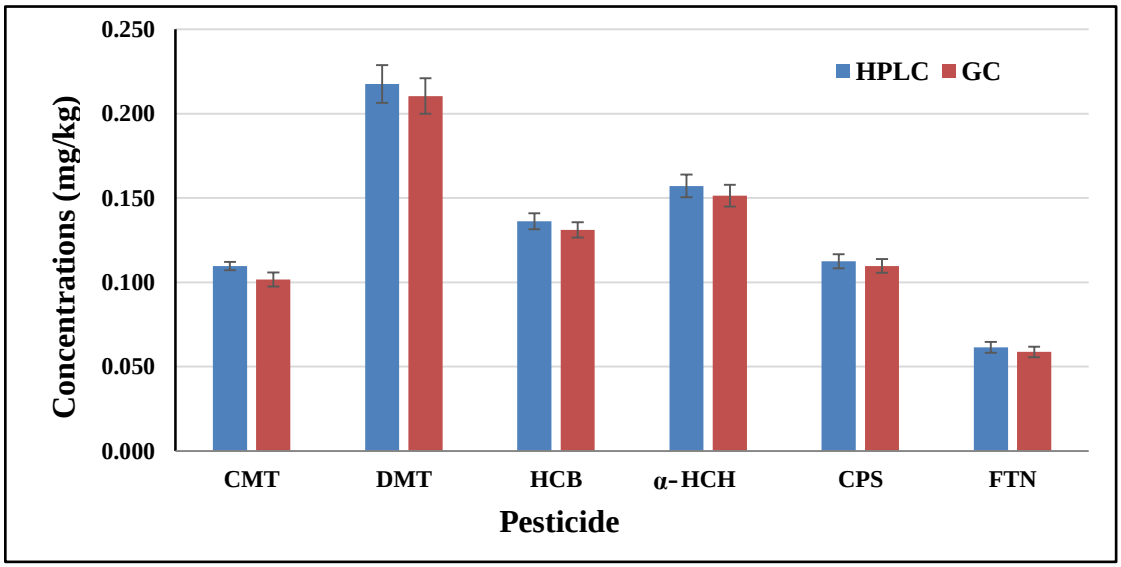


Figure 4.3. Concentration of the pesticides in the raw meat of sheep using HPLC-UV and GC-MS. Columns represent mean of 50 samples and error bars represent standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

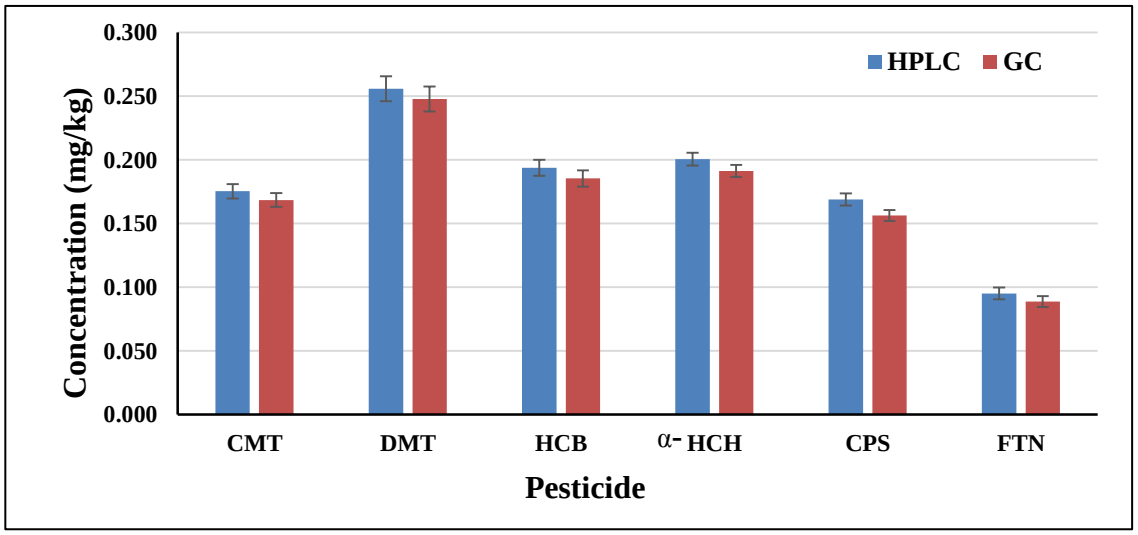


Figure 4.4. Concentration of the pesticides in the raw fat of sheep using HPLC-UV and GC-MS. Columns represent mean of 50 samples and error bars represent standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

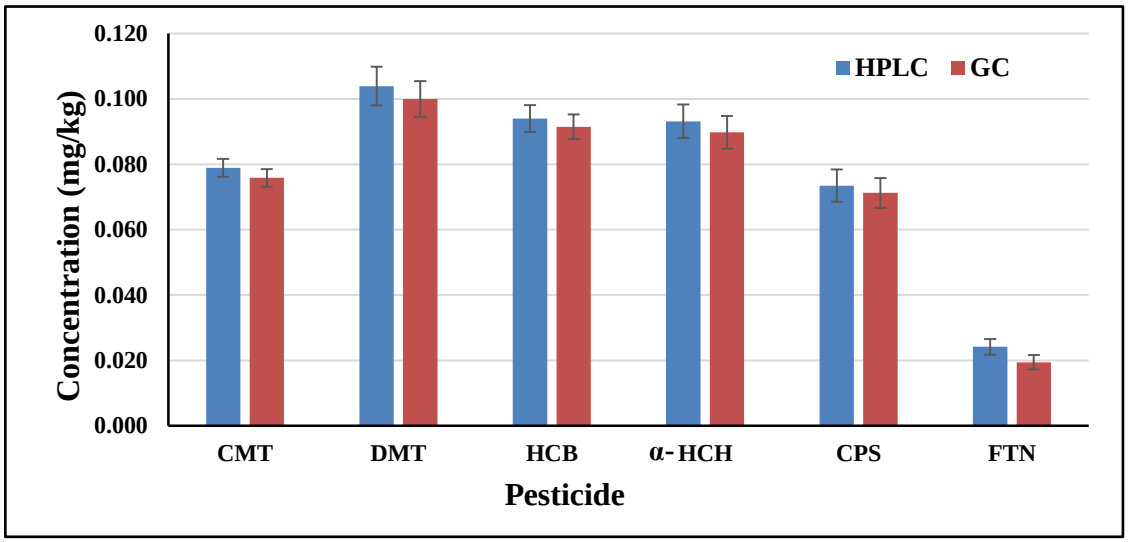


Figure 4.5. Concentration of the pesticides in the raw meat of goat using HPLC-UV and GC-MS. Columns represent mean of 50 samples and error bars represent standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

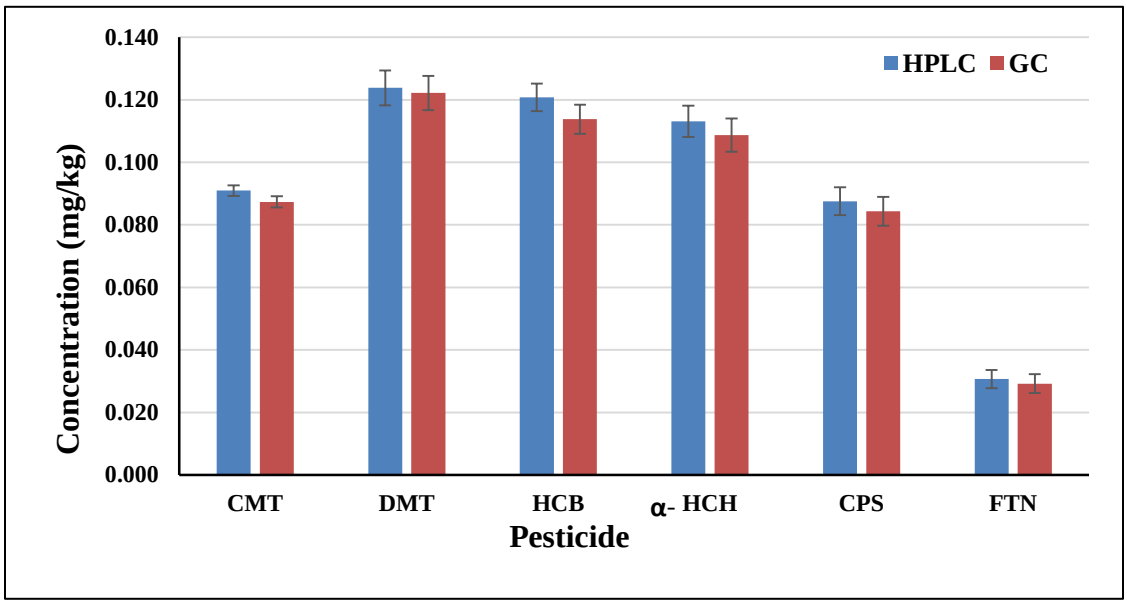


Figure 4.6. Concentration of the pesticides in the raw fat of goat using HPLC-UV and GC-MS. The columns represent the mean of 50 samples and the error bars represent the standard error of the mean (SEM). No statistical difference was observed between the groups using student t-test.

In this study, same sample weight used for preparation samples and injection into HPLC-UV and GC-MS, similar procedure of extraction was also used, in order to obtain almost close and acceptable recovery value, which agreed with studies that used both HPLC and GC for detection and quantification of multi pesticide residues in food products (Bargańska et al., 2018, Pang et al., 2006) and agreed with who used different kinds of detectors (Therdteppitak and Yammeng, 2003). However, HPLC-UV results presented little higher concentrations in all studied pesticides from cattle, sheep and goats meat and fat samples; statistically, there was no significant difference between HPLC and GC readings ($p > 0.05$) in terms of pesticides concentrations among meat samples, this was also true for fat tissues samples and these were also true for the three species. This could be due to the fact that HPLC is fit for wide range of pesticides molecular weights and allow the detection of compounds with wide polarities without concerning their chemical derivatization, as well as the use of high UV absorbance detector (wavelength 260 nm) in this study. This agreed with a study who noticed no significant differences ($p > 0.05$) between the results from LC and GC analyses for detection broad range of OCPs and OPPs in water (He and Aga, 2019), and also agreed with study determined six PYRs, OPPs, and PYZPs (Pyrazolopyrimidines) in honey bee samples using HPLC and GC and found no significant difference in pesticides concentration (Bargańska et al., 2018).

In LC and GC analysis, when a matrix is analysed to detect concentration of any analyte, same exact concentration cannot be obtained in reading for both methods (Bargańska et al., 2018). This could be due to the matrix effect, detector responses, columns; protocols used for HPLC and GC, and analytes physiochemical properties (Uclés et al., 2017).

4.7.2. Validation values differences

4.7.2.1. Accuracy and sensitivity (recovery values, linearity, LOD and LOQ)

The recovery in HPLC and GC seems to be similar in the analysis of meat samples. However, in GC analysis the recoveries for fat samples (81.5 to 98.6 %) are not plenty as in HPLC analysis (77.3 to 106.2 %) which means better recovery (Table 4.1), while still considered as similar of HPLC results because the difference between HPLC and GC recovery values are little and are both in acceptable range, this is also true for precision values in between GC (0.3 to 9.3 %) and HPLC (0.2 to 12.9 %) analysis.

Similarly, for meat samples, recoveries for GC (79.2 to 104.3%) and HPLC (78.08 to 101%) with precision of 0.32 to14.6% for GC and 0.5 to 15.7 % for HPLC, which are almost close together and in acceptable range.

This agreed with a study used similar extraction method (QuEChES) for pesticides multiresidues (PYRs, OPPs, and PYZP), and quantified by LC and GC and obtained almost similar recovery (75- 116%) for both GC and HPLC detection method (Bargańska et al., 2018).

Regarding recovery data it can be stated that GC accuracy is higher than HPLC in this study, while still cannot be recommend the GC performances over HPLC, because sensitivity of HPLC in terms of LOD and LOQ was obviously better than GC for six calibration points in the sensitivity assays, besides that for recoveries only four calibration points have incorporated to constriction the calibration curves.

Some compounds in HPLC analysis showed different recovery and precision to GC analysis, because the HPLC series mixture contains polar compounds and their recoveries might be affected by change of pH (Kromidas, 2017). While, different RDS in GC or LC analysis

might be caused by the different responses to co-extracts that interfered in GC or HPLC chromatograms.

In the linearity study, the results showed that the correlation coefficients (r^2) in HPLC method almost similar that of GC for the studied pesticides with few exceptions for instance, linearity in terms of correlation coefficients (r^2) for CMT (0.9999) in GC might be better than in HPLC (0.9998) for meat and fat samples. Whereas, in fat samples analysed by HPLC linearity of DMT (0.9999), and CPS (0.9998), seems to be better than linearity of DMT (0.9998) and CPS (0.9997) in fat samples analysed by GC. Better linearity in HPLC for DMT and CPS could be due to the fact that they are well transferred LC-UV and vice versa for CMT. Overall, same extraction method and spiking level for both HPLC and GC could provide almost similar and accepted linearity.

Moreover, all the LOD and LOQ values in HPLC for meat and fat samples were equal or less than in GC except LOD of CMT and CPS values in fat samples (Tables 4.3, 4.4). This confirm the higher sensitivity of HPLC-UV in quantification of four studied pesticides, because the lower LOD and LOQ, the more sensitive during analysis (Stefanelli et al., 2009). This agreed with study that compared LOD and LOQ between LC and GC and found little higher sensitivity in LC for twelve OPs, TZNs (Triazine), CARs, CADs (Chloroacetamide) pesticides compounds (He and Aga, 2019). The tiny difference between LOD in HPLC and GC is attributed to the same injection volume and the same dwell time employed (He and Aga, 2019).

The results demonstrate that the HPLC-UV method is an excellent choice for multiresidue analysis of studies pesticides in meat and fat samples if the pesticides extracted with QuEChERS method. In contrast to GC-based techniques, HPLC is not fit to volatile or low molecular weight compounds and allows for the detection of compounds with wide polarities. Coincidently, GC-

MS (using capillary column) is a better tool than LC (with any detector) for detection impurities or unknown compounds (Vitha, 2016). According to EC No.SANTE/11813/ 2017, GC is also highly preferred for detection of pesticides in animal tissues when the technique in conjunction with the MS/MS detector because it enables the determination of a wide spectrum of analytes without derivatization.

However, the selection of the most suitable method for pesticide analysis is not straightforward because the chemical and physical properties of pesticides from various classes differ considerably.

It is important to prioritize the target analytes based on the pesticide use in the study area and based on the research goals. For instance, if a study aims to determine the efficiencies of waste water treatment plants, then LC is the method of choice because most of these target chemicals will be polar and planar which are be ionized in ESI. On the other hand, if the aim is to determine the fate of legacy pesticides in high fat samples then GC-MS/MS may be a better choice because the dominant of analytes will be lipophilic.

For cases of analysis wide range of pesticides in different polarity and molecular weight, UV may use (variable wave length detector not single) because of absorbs wide range wavelength light. For complex matrices or fatty matrices and high fat matrices, GC-MS/MS can also mostly be used if the target compounds are highly lipophilic compound, unless any method else used for the quantification may need to be confirmed by another chromatographic technique.

4.7.2.2. Matrix effect study

In this study, the matrix co-extractives affected readings in LC and GC differently but not questionably.

Table 4.23. Matrix effects in meat and fat samples detected by HPLC-UV, and GC-MS in samples spiked level 0.1 mg/kg versus standard concentration 10 mg/L.

Pesticides / ME%	CMT	DMT	HCB	α-HCH	CPS	FTN
Meat samples						
HPLC-UV	- 5.6	- 6.5	- 5.8	- 5.9	- 4	- 4.8
GC-MS	- 4.58	- 4.28	- 4.21	- 3.51	- 3.72	- 3.35
Fat samples						
HPLC-UV	-10.9	-8.3	-9.7	-7.9	-9.2	-8.4
GC-MS	- 10.1	- 11.2	- 7.3	- 6.47	- 8.90	- 7.3

This agreed with study used QuChERS and GC and LC QqQ MS/MS (Triple quadrupole tandem mass spectrometry) for quantification of multiresidues and found no matrix effect in 10% of analyzed samples and found moderate effect in the rest of samples (Uclés et al., 2017). In this study, the ME% in meat samples showed lower than in fat samples, probably due to the difference matrices lipids percentages for meat, and fat samples caused higher matrix co-extractives in fat extracts.

According to Uclés et al., (2017), the matrix effects have a different character when using liquid chromatography and gas chromatography, while the difference does not depends only on method signal-responses, but also depends on the co-elution of each individual pesticide with co-extracted matrix components, which vary between different commodities to detector responses (Kromidas, 2017).

Chapter Five

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

1. It was concluded from the results of this study that the proposed method is well suited to accurate screening of CMT, DMT, HCB, α-HCH, CPS, and FTN in meat and fat tissues.

2. The analytical preparation QuEChERS method could successfully extract of six pesticides among three different pesticides groups in meat and fat tissue due to its optimzation with HPLC-UV and/or GC-MS technique for detection and quantification and enable to provide validations within the acceptable ranges according to international EC standards.

3. Method validation, demonstrated that the proposed method allows the quantification of studied residues in meat above the level of 0.01mg/ kg. This finding suggests that using one-step extraction procedure in LLP and not less than 70 mg of adsorbents in d-SPE for can cover the extraction procedure of PYRs, OCPs, and OPPs in muscle tissue; this is also true for fat samples with two-steps of extraction in LLP step with using hexane.

4. All of the six studied pesticides were found in cattle, sheep and goats samples as residues and their levels of contamination were mostly exceeding than MRLs specified by EC.

5. Regarding the effect of districts, Bazian and Piramagrun presented the highest pesticides residue in cattle, sheep and goat samples in this study. By contrast, Darbandikhan cattle, sheep and goat samples presented the low residue compared to other districts samples.

6. Regarding the effect of boiling (100^{o}C for 30 min), it destroyed pesticides significantly ($p < 0.05$), except in FTN in goat meat and fat samples, while the reduction level in broiling process (176^{o}C for 20 min) was questionable means in several of samples the reduction level was much less than in boiling and the heating effect was not significant. The highest reduction

percentages also found in PYRs including CMT, and DMT in meat and fat samples, while, the least reduction percentages presented in OPPs residuals which was in FTN and CPS in meat and fat samples.

7. The analytical performance HPLC and GC methods for the analysis of studied pesticides in meat and fat samples were compared. The results from the GC-MS and method for analytes that were detected in HPLC-UV were not significantly different, indicating that one of existing HPLC-UV and GC-MS methods can be used easily for small scale pesticide types in a specific and matrix. While, for wide range pesticide groups and analytes, a single method of chromatography cannot be considered, especially when the analytes properties vary such as volatility, lipophilicity, molecular mass, etc.

5.2. Recommendations

1. To critically confirm the studied methods, a plenty range of pesticides groups and compounds should be tested because using this method for only six pesticides in three groups may not confirm for the total group.

2. More research might be also considered to find out and quantify pesticide and other toxic chemicals in animal tissue like internal edible organs or animal product such as milk.

3. A study could also be performed for a larger group of PYRs, OCPs and OPPs to find out more about the effect of heating on various pesticides groups and the role of each pesticide parameter in the dissipation rate.

Using other heat treatments methods such as boiling under pressure, microwave could be tested to find out the best method to dissipate pesticides completely.

5. Using new rapid method for determination of pesticide in food including use of biosensor techniques.

REFERENCES

ABDOU, R. H. & SAYED, N. 2019. Antioxidant and Anti-Inflammatory Effects of Nano-Selenium against Cypermethrin-Induced Liver Toxicity. *Cell Biology*. 8: 53-65.

ABDULRAHMAN, P. 2016. *Determination of a Pyrethroids Insecticides Deltamethrin Residues in Sheep's and Goat's Meat in Sulaimaniya Province.* Master thesis of Veterinary Medicine. College of Veterinary Medicine/ University of Baghdad, Iraq.

ABOUL-ENEIN, A. M., ABOU ELELLA, F. & ABDULLAH, E. 2010. Monitoring of some organochlorines and organophosphorus residues in imported and locally raised chicken and bovine muscles in Egypt. *Journal of Applied Science Research.*6: 600-608.

ADAMOVICS, J. A. 2017. *Chromatographic analysis of pharmaceuticals.*New Jersey: Routledge Publishing.

AFFUL, S., ANIM, A. & SERFOR-ARMAH, Y. 2010. Spectrum of organochlorine pesticide residues in fish samples from the Densu Basin. *Research Journal Environmental Earth Science.* 2: 133-138.

AHMAD, L., KHAN, A., KHAN, M. Z., HUSSAIN, I., MAHMOOD, F., SLEEMI, M. K., LODHI, L. A. & ABDULLAH, I. 2012. Toxico-pathological effects of cypermethrin upon male reproductive system in rabbits. *Pesticide biochemistry and physiology.* 103: 194-201.

AHMAD, R., NIDA'M, S. & ESTAITIEH, H. 2010. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. *Chemosphere.*78: 667-671.

AIDOO, A. K., ARTHUR, S., BOLFREY–ARKU, G. & MOCHIAH, M. B. 2019. Pesticides Abuse and Health Implications in Ghana: A Review. *International Journal of Environment, Agriculture and Biotechnology.* 4: 874-883.

AKAN, J., ABDULRAHMAN, F. & CHELLUBE, Z. 2014. Organochlorine and organophosphorus pesticide residues in fish samples from Lake Chad, Baga, North Eastern Nigeria. *International Journal of Innovation, Management and Technology*. 5: 87-92.

AKRE, C. 2016. The Use of Pyrethroids, Carbamates, Organophosphates, and Other Pesticides in Veterinary Medicine . *In:* Desiderio, D.M.& Loo, J.A. *Chemical Analysis of Non-antimicrobial Veterinary Drug Residues in Food.* pp 383-426. New Jersey: John Wiley and Sons.

AKRE, C. J. & MACNEIL, J. D. 2006. Determination of eight synthetic pyrethroids in bovine fat by gas chromatography with electron capture detection. *Journal of AOAC International.* 89: 1425-1431.

AKTAR, W., SENGUPTA, D. & CHOWDHURY, A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology.* 2: 1-12.

AL-RIMAWI, F. 2014. A HPLC-UV method for determination of three pesticides in water. *International Journal of Advances in Chemistry.* 2: 1-8.

AL-ZAHRA, A. A. & NAJIM, H. 2017. Determination of insecticide Deltamethrin residues in local and imported raw milk samples collected from different animal's species and the effect of processing heat treatment on its content in milk. *The Iraqi Journal of Veterinary Medicine.* 41: 49-54.

ANASTASSIADES, M., LEHOTAY, S. J., ŠTAJNBAHER, D. & SCHENCK, F. J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC international.* 86: 412-431.

ANJU, A., RAVI S, P. & BECHAN, S. 2010. Water pollution with special reference to pesticide contamination in India. *Journal of Water Resource and Protection.* 2: 432-448.

ANWAR, T., AHMAD, I. & TAHIR, S. 2011. Determination of pesticide residues in fruits of Nawabshah district, Sindh, Pakistan. *Pakistan Journnal of Botany.* 43: 1133 -1139.

ARIOLI, F., CERIANI, F., NOBILE, M., VIGANO, R., BESOZZI, M., PANSERI, S. & CHIESA, L. M. 2019. Presence of organic halogenated compounds, organophosphorus insecticides and polycyclic aromatic hydrocarbons in meat of different game animal species from an Italian subalpine area. *Food Additives & Contaminants: Part A.* 36: 1-9.

ARSENOPOULOS, K., TRIANTAFILLOU, E. & PAPADOPOULOS, E. 2017. Reduced stress and fatigue indicators (cortisol and creatinine kinase) in dairy cattle due to fly repellency using deltamethrin (butox®, msd). *Bulgarian Journal of Veterinary Medicine.* 20: 123-129.

ASHOUB, M. & AZAM, A. E. 2016. Seasonal variations on the levels of some pesticide residues in dairy farms. *Benha Veterinary Medical Journal.* 30: 312-322.

ATKINS, P. W., DE PAULA, J. & KEELER, J. 2018. *Atkins' physical chemistry.* New York: Oxford university press.

BAJWA, U. & SANDHU, K. S. 2014. Effect of handling and processing on pesticide residues in food-a review. *Journal of Food Science and Technology.* 51: 201-220.

BANDEIRA, N. M., RIBEIRO, L. C., RIZZETTI, T. M., MARTINS, M. L., ADAIME, M. B., ZANELLA, R. & PRESTES, O. D. 2017. Evaluation of QuEChERS sample preparation for determination of avermectins residues in ovine muscle by HPLC-FD and UHPLC-MS/MS. *Journal of the Brazilian Chemical Society.* 28: 878-886.

BANERJEE, S., ROY, S. S., MANNAR, B. R., PANDEY, M., SHUKLA, U., MADHAVAN, M. & DHANESSCHAIYAKUPTA, U. 2015. Pesticide residues in animal feed and effects on animals and its products with special reference to endosulfan. *International Journal of Research in Ayurveda and Pharmacy.* 6: 371-374.

BARANOWSKA, I., BARCHAŃSKA, H. & PACAK, E. 2006. Procedures of trophic chain samples preparation for determination of triazines by HPLC and metals by ICP-AES methods. *Environmental Pollution.*143: 206-211.

BARGAŃSKA, Ż., KONIECZKA, P. & NAMIEŚNIK, J. 2018. Comparison of Two Methods for the Determination of Selected Pesticides in Honey and Honeybee Samples. *Molecules.* 23: 1-13.

BAŽULIĆ, D., SAPUNAR-POSTRUŽNIK, J., KUBALA DRINČIČ, H. & ET AL. 2002. Determination of hexachlorobenzene (HCB) in the perirenal and dorsal fatty tissues of pigs. *Acta Veterinaria Hungarica.*50 : 111-115.

BEDI, J., GILL, J. S., AULAKH, R., JOIA, B. & SHARMA, J. 2005. Contamination levels of DDT and HCH residues in different goat tissues. *The Indian Journal of Animal Sciences.*74: 11-13.

BELTIZ, D., GROSCH, W. & SCHIEBERLE, P. 2004. *Food Chemistry.* London: Springer.

BILL, R. L. 2016. *Clinical Pharmacology and Therapeutics for Veterinary Technicians.* Indiana: Elsevier Health Sciences.

BORDONI, L., NASUTI, C., FEDELI, D., GALEAZZI, R., LAUDADIO, E., MASSACCESI, L., LÓPEZ-RODAS, G. & GABBIANELLI, R. 2019. Early impairment of epigenetic pattern in neurodegeneration: Additional mechanisms behind pyrethroid toxicity. *Experimental Gerontology* .124: 110629.

BOSKOU, D. & ELMADFA, I. 2011. *Frying of Food: Oxidation, Nutrient and non Nutrient antioxidants,biologically active compounds, and high temperatures.* New Yok: CRC press.

BOSKOU, G., SALTA, F. N., CHIOU, A., TROULLIDOU, E. & ANDRIKOPOULOS, N. K. 2006. Content of trans, trans-2, 4-decadienal in deep-fried and pan-fried potatoes. *European Journal of Lipid Science and Technology.*108: 109-115.

BRADLEY, A. E., SHOENFELT, J. L. & DURDA, J. L. 2016. Carcinogenicity and mode of action evaluation for alpha-hexachlorocyclohexane: implications for human health risk assessment. *Regulatory Toxicology and Pharmacology.*76:152-173.

CARVALHO, F. P. 2017. Pesticides, environment, and food safety. *Food and Energy Security.* 6: 48-60.

CASTILLO, M., CARBONELL, E., GONZÁLEZ, C. & MIRALLES-MARCO, A. 2012. Pesticide residue analysis in animal origin food: procedure proposal and evaluation for lipophilic pesticides. *In:* Soundararajan, R.P. *Pesticides-Recent Trends in Pesticide Residue Assay*. pp 63-90. London: IntechOpen.

CASTILLO, M., GONZÁLEZ, C. & MIRALLES, A. 2011. An evaluation method for determination of non-polar pesticide residues in animal fat samples by using dispersive solid-phase extraction clean-up and GC-MS. *Analytical and Bioanalytical Chemistry.* 400: 1315-1328.

CHANG, S.-C., KAO, C.-H., SHEN-TU, H., CHOU, T.-M. & LO, S.-C. 2018. *Potential Application of Immunoassay for the Detection of Pesticide Residues in Agriculture. Conference*. Conference: Proceedings of the 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management of Key Crops. pp 122-133. Agricultural Laboratory Publishing

CHANGSHENG, L., HELIU, D., ZHICHENG, F., XIANYI, Y., LIN, C. & HUI, G. 2019. Portal vein gas and pneumatosis intestinalis: A case of intestinal necrosis caused by acute organophosphorus pesticide poisoning?. *Toxicology and Industrial Health.* 35: 482-485.

CHATZIMITAKOS, T. G., ANDERSON, J. L. & STALIKAS, C. D. 2018. Matrix solid-phase dispersion based on magnetic ionic liquids: An alternative sample preparation approach for the extraction of pesticides from vegetables. *Journal of Chromatography A.*1581: 168-172.

CHAWLA, P., KAUSHIK, R., SWARAJ, V. S. & KUMAR, N. 2018. Organophosphorus pesticides residues in food and their colorimetric detection. *Environmental Nanotechnology, Monitoring & Management.*10: 292-307.

CHEVRIER, J., DEWAILLY, E., AYOTTE, P., MAURIEGE, P., DESPRES, J. & TREMBLAY, A. 2000. Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *International Journal of Obesity.* 24: 1272-1278.

CHOI, Y.S., HWANG, K. E., JEONG, T.J., KIM, Y.B., JEON, K.H., KIM, E.M., SUNG, J.M., KIM, H.W. & KIM, C.J. 2016. Comparative study on the effects of boiling, steaming, grilling, microwaving and superheated steaming on quality characteristics of marinated chicken steak. *Korean Journal for Food Science of Animal Resources.* 36: 1-7.

CHOUDHARY, S., YAMINI, N. R., YADAV, S. K. & ANDAMIT SHARMA, M. K. 2018. A review: Pesticide residue: Cause of many animal health problems. *Journal of Entomology and Zoology Studies.*6: 330-333.

CHRUSTEK, A., HOŁYŃSKA-IWAN, I., DZIEMBOWSKA, I., BOGUSIEWICZ, J., WRÓBLEWSKI, M., CWYNAR, A. & OLSZEWSKA-SŁONINA, D. 2018. Current research on the safety of pyrethroids used as insecticides. *Medicina.* 54: 2-15.

CODEX ALIMENTARIUS. 2015. *Codex Alimentarius Commission: procedural manual, Joint FAO/WHO Food Standards Programme.* 24th ed. Rome: Food & Agriculture Organisations of United Nation and Word Health Organisation.

COSTA, L. G. 2018. Organophosphorus compounds at 80: some old and new issues. *Toxicological Sciences.*162: 24-35.

DAMALAS, C. & KOUTROUBAS, S. 2016. Farmers' exposure to pesticides: toxicity types and ways of prevention. *Toxics.* 4: 2-10.

DARKO, G. & ACQUAAH, S. O. 2007. Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science & Technology.* 4: 521-524.

DAUGAARD-PETERSEN, T., LANGEBÆK, R., RIGÉT, F. F., DYCK, M., LETCHER, R. J., HYLDSTRUP, L., JENSEN, J.E. B., DIETZ, R. & SONNE, C. 2018. Persistent organic pollutants and penile bone mineral density in East Greenland and Canadian polar bears (*Ursus maritimus*) during 1996–2015. *Environment International.* 114: 212-218.

DAVIS, F. R. 2014. *Banned: a history of pesticides and the science of toxicology,* London: Yale University Press.

DEGUINE, J., CAROLINE, G., PHILIPPE, L., RATNADASS, J. & ALAIN, A. 2017. *Agroecological Crop Protection.* Dordrecht: Springer Nature.

DHANANJAYAN, V. & RAVICHANDRAN, B. 2018. Occupational health risk of farmers exposed to pesticides in agricultural activities. *Current Opinion in Environmental Science & Health.* 4: 31-37.

DIMITROVA, R. T., STOYKOVA, I. I., YANKOVSKA-STEFANOVA, T. T., YANEVA, S. A. & STOYANCHEV, T. T. 2018. Development of analytical method for determination of organochlorine pesticides residues in meat by GC-ECD. *Revue Méd. Vét.*169: 77-86.

DING, R., CAO, Z., WANG, Y., GAO, X., LUO, H., ZHANG, C., MA, S., MA, X., JIN, H. & LU, C. 2017. The implication of p66shc in oxidative stress induced by deltamethrin. *Chemico-biological Interactions.* 278: 162-169.

DONG, K., DU, Y., RINKEVICH, F., NOMURA, Y., XU, P., WANG, L., SILVER, K. & ZHOROV, B. S. 2014. Molecular biology of insect sodium channels and pyrethroid resistance. *Insect Biochemistry and Molecular Biology.* 50: 1-17.

ĐORĐEVIĆ, T. & ĐUROVIĆ-PEJČEV, R. 2016. Food processing as a means for pesticide residue dissipation. *Pesticidi i Fitomedicina.*31: 89-105.

EC (European Commission). 2019a. *Maximum Residue Levels*. [Online] Available from .< https://ec.europa.eu/food/plant/pesticides /max_residue_levels_en.> Accesed 25th August 2019.

EC (European Commission). 2019b. *Detection methods. Eurropean Commisson*. [Online] Available from.< https://ec.europa.eu/food /plant/gmo/post_authorisation/detection methods_en.> Accessed 5th February 2020.

EC (European Comission). 2017. *Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed*. [Online] Availablefrom.<

https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.>. Accessed [23] February 2017.

ECPA (European Crop Protection Agency). 2019. *Maximum residue levels of pesticides.* Belgium: European Crop Protection Agency.

EDWARDS, C. 2013. *Environmental pollution by pesticides.* New York: Springer Science & Business Media.

EFSA (European Food Safety Authority). 2011. Scientific opinion on the science behind the revision of the guidance document on dermal absorption. *EFSA Journal.* 9: 1-73.

ELHALWAGY, M. E., DARWISH, N. S. & ZAHER, E. M. 2008. Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide. *Pesticide Biochemistry and Physiology.* 91: 81-89.

EMEA (European Medicines Agency). 2009. *Guidelines for the validation of analytical methods used in residue depletion studies.* Doc. Ref. EMEA/CVMP/VICH/463202/2009-CONSULTATION. pp 5-19. London: European Medicines Agency.

EMEA (European Medicines Agency). 2011. *Guideline on bioanalytical method validation. Committee for Medicinal Products for Human Use.* Doc Ref. CHMP/EWP/192217/2009. pp 1–22. London: European Medicines Agency.

ENSLEY, S. M. 2018. *Pyrethrin and Pyrethroids. In:* Gupta, R.C. *Veterinary toxicology.*3rd ed. pp 515-520. Kentucky: Academic Press.

ETO, M. 2018. *Organophosphorus pesticides.* Boca Raton/ Florida: CRC press.

FAJGELJ, A. & AMBRUS, A. 2007. *Principles and practices of method validation.* Cambridge: Royal Society of Chemistry.

FAO (Food and Agricultural Organization). 2017. *FAO STAT*. [Online] Available from.< http://www.fao.org/faostat/en/#home .> Accessed 20th November 2019.

FAO and WHO (Food and Agricultural Organization and World Health Organization). 2019. *WHO and FAO Codex Alimentarius International Food Standards.* Rome: [Online] Available at.< http://www.fao.org/fao-who-codexalimentarius/en/.> Accessed 15th November 2019

FAO/WHO (Food and Agricultural Organization and World Health Organization joint meeting). 2016. *Pesticide residue in food, Joint FAO/WHO Meeting on Pesticide Residues*. Rome:

[Online] Available from.< https://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/.> Accessed 7th October 2019.

FATIN, S., KAMEL, E., GAAFAR-REHAB, A. & SHAHEEN, A. 2016. Effect of Grilling on Pesticides Residues in O. niloticus Muscles. *Benha Veterinary Medical Journal* .30: 348-357.

FDA (Food and Drug Administration). 2018. *Guidance for industry: bioanalytical method validation*. [Online] Available from.< http://www. fda. gov/cder/Guidance/4252fnl. pdf>. Accessed 5th December 2019.

FERNÁNDEZ-GONZÁLEZ, R., YEBRA-PIMENTEL, I., MARTÍNEZ-CARBALLO, E., REGUEIRO, J. & SIMAL-GÁNDARA, J. 2013. Inputs of polychlorinated biphenyl residues in animal feeds. *Food Chemistry.*140: 296-304.

FRENICH, A. G., VIDAL, J. M., SICILIA, A. C., RODRÍGUEZ, M. G. & BOLANOS, P. P. 2006. Multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by gas chromatography–triple quadrupole mass spectrometry. *Analytica Chimica Acta.* 558: 42-52.

GALLO, M., FORMATO, A., IANNIELLO, D., ANDOLFI, A., CONTE, E., CIARAVOLO, M., VARCHETTA, V. & NAVIGLIO, D. 2017. Supercritical fluid extraction of pyrethrins from pyrethrum flowers (Chrysanthemum cinerariifolium) compared to traditional maceration and cyclic pressurization extraction. *The Journal of Supercritical Fluids*. 119: 104-112.

GAŁUSZKA, A., MIGASZEWSKI, Z. & NAMIEŚNIK, J. 2013. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. *TrAC Trends in Analytical Chemistry.* 50: 78-84.

GAMMON, D. W., LIU, Z., CHANDRASEKARAN, A., EL-NAGGAR, S. F., KURYSHEV, Y. A. & JACKSON, S. 2019. Pyrethroid neurotoxicity studies with bifenthrin indicate a mixed Type I/II mode of action. *Pest Management Science.* 75:1190-1197.

GARG, T. 2016. Environmental and occupational exposures and prostate cancer. *In:* Mydlo, J.H & Godec, C.J. *Prostate Cancer.*2nd ed. pp 191-198. New York: Academic Press

GEORGE, R., HAYWOOD, A., KHAN, S., RADOVANOVIC, M., SIMMONDS, J. & NORRIS, R. 2018. Enhancement and suppression of ionization in drug analysis using

HPLC-MS/MS in support of therapeutic drug monitoring: a review of current knowledge of its minimization and assessment. *Therapeutic Drug Monitoring.* 40: 1-8.

GHAVIDEL, F., SHAHTAHERI, S. J., JAZANI, R. K., TORABBEIGI, M., FROUSHANI, A. R. & KHADEM, M. 2014. Optimization of solid phase microextraction procedure followed by gas chromatography with electron capture detector for pesticides butachlor and chlorpyrifos. *American Journal of Analytical Chemistry.*5: 535-546.

GIDDINGS, J. C. 2017. *Dynamics of chromatography: principles and theory.* New York: CRC Press.

GOGOI, P., DAS, S., DAS, S. & KHAN, M. Z. A. 2016. Effect of organophosphorus insecticide, malathion on the division of meristems of Allium cepa L. *International Journal of Pure and Applied Bioscience.* 4: 114-122.

GOLDWYN, M. & BLONDER, G. 2016. *Meathead: The science of great barbecue and grilling.* New York: Houghton Mifflin Harcourt.

GÓMEZ-RÍOS, G. A., GIONFRIDDO, E., POOLE, J. & PAWLISZYN, J. 2017. Ultrafast screening and quantitation of pesticides in food and environmental matrices by solid-phase microextraction–transmission mode (SPME-TM) and direct analysis in real time (DART). *Analytical Chemistry.* 89: 7240 -7248.

GONZÁLEZ-CURBELO, M., SOCAS-RODRÍGUEZ, B., HERRERA-HERRERA, A., GONZÁLEZ-SÁLAMO, J., HERNÁNDEZ-BORGES, J. & RODRÍGUEZ-DELGADO, M. 2015. Evolution and applications of the QuEChERS method. *TrAC Trends in Analytical Chemistry.*71: 169-185.

GOWIK, P. 2009. The validation of methods for regulatory purposes in the control of residues. *Journal of Chromatography A.* 1216: 8051-8058.

GRANDJEAN, P. & LANDRIGAN, P. J. 2014. Neurobehavioural effects of developmental toxicity. *The Lancet Neurology.*13: 330-338.

GREER, J. B., MAGNUSON, J. T., HESTER, K., GIROUX, M., POPE, C., ANDERSON, T., LIU, J., DANG, V., DENSLOW, N. D. & SCHLENK, D. 2019. Effects of Chlorpyrifos on Cholinesterase and Serine Lipase Activities and Lipid Metabolism in Brains of Rainbow Trout (Oncorhynchus mykiss). *Toxicological Sciences.*172: 146-154.

GRIMALT, S. & DEHOUCK, P. 2016. Review of analytical methods for the determination of pesticide residues in grapes. *Journal of Chromatography A.* 1433: 1-23.

GYENWALI, D., VAIDYA, A., TIWARI, S., KHATIWADA, P., LAMSAL, D. R. & GIRI, S. 2017. Pesticide poisoning in Chitwan, Nepal: a descriptive epidemiological study. *BMC Public Health.* 17: 1-8.

HABOTTA, O., ABOUELMAGD, M., HALAWA, A. & UNO, S. 2018. Hepatic Metabolomics Profiling of Cyprinus Carpio After Acute Cypermethrin Toxicity. *Journal of Veterinary Healthcare.*1: 1-16.

HAMEY, P. Y. 2003. *The Complete Book of Pesticide Management, Science, Regulation, Stewardship, and Communication.* Oxford: Oxford University Press.

HAMMOND, D. 2014. *Heat treatment for insect control: developments and applications.*Oxford: Woodhead Publishing.

HAMMOND, J. L., FORMISANO, N., ESTRELA, P., CARRARA, S. & TKAC, J. 2016. Electrochemical biosensors and nanobiosensors. *Essays in Biochemistry.* 60: 69-80.

HARSHIT, D., CHARMY, K. & NRUPESH, P. 2017. Organophosphorus pesticides determination by novel HPLC and spectrophotometric method. *Food Chemistry.* 230: 448-453.

HE, P. & AGA, D. S. 2019. Comparison of GC-MS/MS and LC-MS/MS for the analysis of hormones and pesticides in surface waters: advantages and pitfalls. *Analytical Methods.* 11: 1436-1448.

HEDGES, L., BROWN, S., VARDY, A., DOYLE, E., YOON, M., OSIMITZ, T. G. & LAKE, B. G. 2019. Metabolism of deltamethrin and cis-and trans-permethrin by rat and human liver microsomes, liver cytosol and plasma preparations. *XenoBiotica.* 49: 388-396.

HERCEGOVÁ, A., DÖMÖTÖROVÁ, M. & MATISOVA, E. 2007. Sample preparation methods in the analysis of pesticide residues in baby food with subsequent chromatographic determination. *Journal of Chromatography A.*1153: 54-73.

HERCEGOVÁ, A., DÖMÖTÖROVÁ, M., KRUŽLICOVÁ, D. & MATISOVÁ, E. 2006. Comparison of sample preparation methods combined with fast gas chromatography–mass spectrometry for ultratrace analysis of pesticide residues in baby food. *Journal of Separation Science.* 29: 1102-1109.

HU, J. X., LI, Y. F., LI, J., PAN, C., HE, Z., DONG, H. Y. & XU, L. C. 2013. Toxic effects of cypermethrin on the male reproductive system: with emphasis on the androgen receptor. *Journal of Applied Toxicology.* 33: 576-585.

HÜBSCHMANN, H.-J. 2015. *Handbook of GC-MS: fundamentals and applications.* Pune: John Wiley & Sons.

IMMIG, J & NTN (National Toxics Network). 2010. *A list of Australia's most dangerous pesticides. World Wildlife Fund and National Toxics Network, Brisbane.* Bangalow: National Toxics Network.

IOERGER, B. P. & SMITH, J. S. 1993. Multiresidue method for the extraction and detection of organophosphate pesticides and their primary and secondary metabolites from beef tissue using HPLC. *Journal of Agricultural and Food Chemistry.* 41: 303-307.

JAYARAJ, R., MEGHA, P. & SREEDEV, P. 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology.* 9: 90-100.

JONES, R. R., BARONE-ADESI, F., KOUTROS, S., LERRO, C. C., BLAIR, A., LUBIN, J., HELTSHE, S. L., HOPPIN, J. A., ALAVANJA, M. C. & FREEMAN, L. E. B. 2015. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. *Occupational and Environmental Medicine.* 72: 496-503.

KAFKAS, E., DAGLIOGLU, N., YARPUZ-BOZDOGAN, N. & ZARIFIKHOSROSHAHI, M. 2019. *Pesticide analysis techniques, limitations, and applications*. Sarıçam/Adana: IGI Global.

KALACHOVA, K., PULKRABOVA, J., CAJKA, T., DRABOVA, L., STUPAK, M. & HAJSLOVA, J. 2013. Gas chromatography–triple quadrupole tandem mass spectrometry: a powerful tool for the (ultra) trace analysis of multiclass environmental contaminants in fish and fish feed. *Analytical and Bioanalytical Chemistry.* 405: 7803-7815.

KAN, C. & MEIJER, G. 2007. The risk of contamination of food with toxic substances present in animal feed. *Animal Feed Science and Technology.*133: 84-108.

KAONGA, C. C., TAKEDA, K. & SAKUGAWA, H. 2015. Diuron, Irgarol 1051 and Fenitrothion contamination for a river passing through an agricultural and urban area in Higashi Hiroshima City, Japan. *Science of the Total Environment.* 518: 450-458.

KARABASANAVAR, N. S., SINGH, S. P. & SINGH, M. K. 2012. Monitoring for chlorpyrifos residues in animal feed and fodder of Tarai region of Uttarakhand, India. *Toxicological & Environmental Chemistry.* 94: 275-280.

KARAGEORGOU, E. G. & SAMANIDOU, V. F. 2011. Development and validation according to European Union Decision 2002/657/EC of an HPLC-DAD method for milk multi-residue analysis of penicillins and amphenicols based on dispersive extraction by QuEChERS in MSPD format. *Journal of Separation Science.* 34: 1893-1901.

KAUSHIK, G., SATYA, S. & NAIK, S. 2009. Food processing a tool to pesticide residue dissipation–A review. *Food Research International.* 42: 26-40.

KAUSHIK, P. & KAUSHIK, G. 2007. An assessment of structure and toxicity correlation in organochlorine pesticides. *Journal of Hazardous Materials.*143: 102-111.

KAVVALAKIS, M. P. & TSATSAKIS, A. M. 2012. The atlas of dialkylphosphates; assessment of cumulative human organophosphorus pesticides' exposure. *Forensic Science International.* 218: 111-122.

KAZEMI, M., TAHMASBI, A. M., VALIZADEH, R., NASERIAN, A. A. & SONI, A. 2012. Organophosphate pesticides: A general review. *Agricalture Science Research.* 2: 512-522.

KEIKOTLHAILE, B. M., SPANOGHE, P. & STEURBAUT, W. 2010. Effects of food processing on pesticide residues in fruits and vegetables: a meta-analysis approach. *Food and Chemical Toxicology.* 48: 1-6.

KHALATBARY, A. R., GHAFFARI, E. & MOHAMMADNEGAD, B. 2015. Protective role of oleuropein against acute deltamethrin-induced neurotoxicity in rat brain. *Iranian Biomedical Journal.* 19: 247-253.

KHAN, M. S. & RAHMAN, M. S. 2017. *Introduction of Pesticides. In:* Khan, M. S. & Rahman, M. S. *Pesticide Residue in Foods.*pp 1-7. Steinhausen: Springer International Publishing.

KHAN, S., PRIYAMVADA, S., KHAN, S. A., KHAN, W. & YUSUFI, A. 2017. Studies on hexachlorobenzene (HCB) induced toxicity and oxidative damage in the kidney and other rat tissues. *International Journal of Drug Metabolism and Toxicology.* 1: 001-009.

KHASHAN, H. T. 2016. Determination of a pyrethroid insecticide deltamethrin residues in cattle meat in Baghdad Province/Al-Rusafa. *International Journal of Advance Reseach of Biological Sciences.* 3: 193-199.

KIM, K.-H., KABIR, E. & JAHAN, S. A. 2017. Exposure to pesticides and the associated human health effects. *Science of the Total Environment.* 575: 525-535.

KIM, L., LEE, D., CHO, H.K. & CHOI, S.D. 2019. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends in Environmental Analytical Chemistry.* ND: 1-54.

KINKELA, D. 2016. *Banned: A History of Pesticides and the Science of Toxicology.* Oxford: Oxford University Press.

KIRANMAYI, C. B., KRISHNAIAH, N., KUMAR, M. M., KUMAR, M. S., SUBHASHINI, N. & RAO, T. M. 2016. Multiresidue analysis of pesticides in beef and mutton samples and study on effect of cooking on residual levels of aldrin and dieldrin. *International Journal of Enviromental Technology.* 5: 195-203.

KOESUKWIWAT, U., LEHOTAY, S. J., MASTOVSKA, K., DORWEILER, K. J. & LEEPIPATPIBOON, N. 2009. Extension of the QuEChERS method for pesticide residues in cereals to flaxseeds, peanuts, and doughs. *Journal of Agricultural and Food Chemistry.* 58: 5950-5958.

KONGMEE, M., THANISPONG, K., SATHANTRIPHOP, S., SUKKANON, C., BANGS, M. J. & CHAREONVIRIYAPHAP, T. 2019. Enhanced mortality in deltamethrin-resistant Aedes aegypti in Thailand using a piperonyl butoxide synergist. *Acta Tropica.*189: 76-83.

KROMIDAS, S. 2017. *The HPLC-MS Handbook for Practitioners.* Boston: John Wiley & Sons.

KUMAR, J. M., KUMAR, S. D., KUBENDRAN, D. & KALAICHELVAN, P. 2013. Hexachlorobenzene-sources, remediation and future prospects. *International Journal of Current Research and Review.* 5:1-12.

KUMAR, P., SINGH, S., AHMAD, A. & RAO, V. 2008. Simultaneous detection of endosulfan and chlorpyrifos residues in buffalo meat using high performance liquid chromatography. *Analytical Chemistry: An Indian Journal.* 7: 103-109.

KUMAR, S., THOMAS, A. & PILLAI, M. 2011. Deltamethrin: Promising mosquito control agent against adult stage of Aedes aegypti L. *Asian Pacific Journal of Tropical Medicine.* 4: 430-435.

KUSS, S., AMIN, H. M. & COMPTON, R. G. 2018. Electrochemical detection of pathogenic bacteria—recent strategies, advances and challenges. *Chemistry–An Asian Journal.* 13: 2758-2769.

LAINSBURY, A. 2018. *The UK Pesticide Guide 2018.* London: BCPC.

LAMBERTH, C., JEANMART, S., LUKSCH, T. & PLANT, A. 2013. Current challenges and trends in the discovery of agrochemicals. *Science.* 341: 742-746.

LAQUITAINE, L., DURIMEL, A., DE ALENCASTRO, L. F., JEAN-MARIUS, C., GROS, O. & GASPARD, S. 2016. Biodegradability of HCH in agricultural soils from Guadeloupe (French West Indies): identification of the lin genes involved in the HCH degradation pathway. *Environmental Science and Pollution Research.* 23: 120-127.

LARI, S. Z., KHAN, N. A., GANDHI, K. N., MESHRAM, T. S. & THACKER, N. P. 2014. Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. *Journal of Environmental Health Science and Engineering*. 12: 2-8.

LARSEN, K. E., LIFSCHITZ, A. L., LANUSSE, C. E. & VIRKEL, G. L. 2019. In vitro and in vivo effects of chlorpyrifos and cypermethrin on blood cholinesterases in sheep. *Journal of Veterinary Pharmacology and Therapeutics.* 42: 548-555.

LASKOWSKI, D. A. 2002. *Physical and chemical properties of pyrethroids*. *In:* Pim, D. *Reviews of environmental contamination and toxicology*. pp 49-170. New York: Springer.

LEDOUX, M. 2011. Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. *Journal of Chromatography A.* 1218: 1021-1036.

LEE, J., KIM, L., SHIN, Y., LEE, J., LEE, J., KIM, E., MOON, J.-K. & KIM, J.-H. 2017. Rapid and simultaneous analysis of 360 pesticides in brown rice, spinach, orange, and potato using microbore GC-MS/MS. *Journal of Agricultural and Food Chemistry.* 65: 3387-3395.

LEHOTAY, S. J. 2011. QuEChERS sample preparation approach for mass spectrometric analysis of pesticide residues in foods. *Methods inMolecularBiology.*747: 65-91.

LEHOTAY, S. J., KOK, A. D., HIEMSTRA, M. & BODEGRAVEN, P. V. 2005a. 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.* 88: 595-614.

LEHOTAY, S. J., MAŠTOVSKÁ, K. & YUN, S. J. 2005b. Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *Journal of AOAC International.* 88: 630-638.

LETTA, B. D. & ATTAH, L. E. 2013. Residue levels of organochlorine pesticides in cattle meat and organs slaughtered in selected towns in West Shoa Zone, Ethiopia. *Journal of Environmental Science and Health, Part B.* 48: 23-32.

LI, Q., WANG, X., WANG, R., SUI, H., LI, W. & LI, L. 2011. Seasonal trends and potential sources of ambient air OCPs in urban and suburban areas in Dalian, China. *Journal of Environmental Monitoring.* 13: 1816-1822.

LI, R., HE, L., ZHOU, T., JI, X., QIAN, M., ZHOU, Y. & WANG, Q. 2014. Simultaneous determination of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol in duck muscle by modified QuEChERS coupled to gas chromatography tandem mass spectrometry (GC-MS/MS). *Analytical and Bioanalytical Chemistry.* 406: 2899-2907.

LIMA, H. R. S., DA SILVA, J. S., DE OLIVEIRA FARIAS, E. A., TEIXEIRA, P. R. S., EIRAS, C. & NUNES, L. C. C. 2018. Electrochemical sensors and biosensors for the analysis of antineoplastic drugs. *Biosensors and Bioelectronics.*108: 27-37.

LUCIANI-GIACOBBE, L. C., GUZMAN, M. L., MANZO, R. H. & OLIVERA, M. E. 2018. Validation of a simple isocratic HPLC-UV method for rifampicin and isoniazid quantification in human plasma. *Journal of Applied Pharmaceutical Science.* 8: 093-099.

LUSHCHAK, V. I., MATVIISHYN, T. M., HUSAK, V. V., STOREY, J. M. & STOREY, K. B. 2018. Pesticide toxicity: a mechanistic approach. *Experimental and Clinical Sciences Journal.*17: 1101-1136.

LIU, X.Q., LI, Y.F., MENG, W.T., LI, D. X., SUN, H., TONG, L. & SUN, G.X. 2016. A multi-residue method for simultaneous determination of 74 pesticides in Chinese material medica using modified QuEChERS sample preparation procedure and gas chromatography tandem mass spectrometry. *Journal of Chromatography B.*1015: 1-12.

LUZARDO, O. P., RUIZ-SUAREZ, N., VALERON, P. F., CAMACHO, M., ZUMBADO, M., HENRÍQUEZ-HERNÁNDEZ, L. A. & BOADA, L. D. 2014. Methodology for the identification of 117 pesticides commonly involved in the poisoning of wildlife using GC–MS-MS and LC–MS-MS. *Journal of Analytical Toxicology.* 38: 155-163.

LYDY, M., BELDEN, J., WHEELOCK, C., HAMMOCK, B. & DENTON, D. 2004. Challenges in regulating pesticide mixtures. *Ecology and Society.* 9: 1-15.

MACBEAN, C. 2015. *The Pesticide Manual.* London: British Crop Protection Council.

MAGNUSSON, B. 2014. *The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics.*Torino: Eurachem Publishing.

MAJDINASAB, M., MITSUBAYASHI, K. & MARTY, J. L. 2019. Optical and electrochemical sensors and biosensors for the detection of quinolones. *Trends in Biotechnology.* 37: 898-915.

MALARKODI, C., RAJESHKUMAR, S. & ANNADURAI, G. 2017. Detection of environmentally hazardous pesticide in fruit and vegetable samples using gold nanoparticles. *Food Control.* 80: 11-18.

MANAV, Ö. G., OLGUN, E. Ö. & ERMIŞ, E. 2018. Determination of organochlorinated pesticide and polychlorinated biphenyl congeners residues in chicken eggs by gas chromatography. *Food and Health.* 4: 264-273.

MASTOVSKA, K. & LEHOTAY, S. J. 2006. Rapid sample preparation method for LC− MS/MS or GC− MS analysis of acrylamide in various food matrices. *Journal of Agricultural and Food Chemistry.* 54: 7001-7008.

MATTHEWS, G. 2015. *Pesticides: health, safety and the environment.* Oxford: John Wiley & Sons.

MATTHEWS, G. 2019. Pesticides: Residues in Crops and Their Application. *Outlooks on Pest Management.* 30: 85-87.

MATTHEWS, G. 2018. *A History of Pesticides.*Oxford: CABI.

MATUSZEWSKI, B., CONSTANZER, M. & CHAVEZ-ENG, C. 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC− MS/MS. *Analytical Chemistry.*75: 3019-3030.

MCMURRY, J. E. 2014. *Organic Chemistry with Biological Applications*Boston/ Massachusetts: Cengage Learning.

MCNAIR, H. M., MILLER, J. M. & SNOW, N. H. 2019. *Basic gas chromatography.* Hoboken/New Jersey: John Wiley & Sons.

MEDINA-BUELVAS, D., ESTRADA-MUÑIZ, E., FLORES-VALADEZ, M. & VEGA, L. 2019. Genotoxic and immunotoxic effects of the organophosphate metabolite diethyldithiophosphate (DEDTP) in Vivo. *Toxicology and Applied Pharmacology.* 366: 96-103.

MELIGY, A., AL-TAHER, A., ISMAIL, M., AL-NAEEM, A., EL-BAHR, S. & EL-GHAREEB, W. 2019. Pesticides and Toxic Metals Residues in Muscle and Liver Tissues Of Sheep, Cattle and Dromedary Camel In Saudi Arabia. *Slovenian Veterinary Research.* 56: 157–166.

MIE, A., RUDÉN, C. & GRANDJEAN, P. 2018. Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. *Environmental Health.* 17: 1-5.

MILLER, J. & MILLER, J. C. 2018. *Statistics and chemometrics for analytical chemistry.* Philadelphia: Coronet Books Inc.

MOIROUX, N., DJÈNONTIN, A., ZOGO, B., BOURAIMA, A., SIDICK, I., PIGEON, O. & PENNETIER, C. 2018. Small-scale field testing of alpha-cypermethrin water-dispersible granules in comparison with the recommended wettable powder formulation for indoor residual spraying against malaria vectors in Benin. *Parasites & Vectors.*11: 1-8.

MOSSA, A.T. H., MOHAFRASH, S. M. & CHANDRASEKARAN, N. 2018. Safety of natural insecticides: toxic effects on experimental animals. *BioMed Research International.* 2018: 1-17.

MOSTAFALOU, S. & ABDOLLAHI, M. 2017. Pesticides: an update of human exposure and toxicity. *Archives of Toxicology.* 91: 549-599.

MUHAMMAD, F., AKHTAR, M., RAHMAN, Z., FAROOQ, H., KHALIQ, T. & ANWAR, M. 2010. Multi-residue determination of pesticides in the meat of cattle in Faisalabad-Pakistan. *Egyptian Academic Jurnal of Biol.ogical Sciencves.*2: 19-28.

MURESAN, C., COVACI, A., SOCACI, S., SUHAROSCHI, R., TOFANA, M., MUSTE, S. & POP, A. 2015. Influence of Meat Processing on the Content of Organochlorine Pesticides. *Journal of Food Processing & Technology.* 6: 1-6.

MUREȘAN, C., TOFANĂ, M. & LASLO, C. 2010. Effect of several technological and kitchen treatments on hexachlorocyclohexan residues in pork meat. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture.* 67: 328-333.

MUTHUKUMAR, M., REDDY, K. S., REDDY, C. N., REDDY, K. K., REDDY, A. G., REDDY, D. J. & KONDAIAH, N. 2010. Detection of cyclodiene pesticide residues in buffalo meat and effect of cooking on residual level of endosulfan. *Journal of Food Science and Technology.* 47: 325-329.

NADA, A. & SALEHA, Y. 2016. Genotoxic effect of an organophosphorus pesticide "ethephon" on somatic and germ cells of male mice. *Biosciences Biotechnology Research Asia.*5: 1-8.

NARDELLI, V., CASAMASSIMA, F., GESUALDO, G., LI, D., MARCHESIELLO, W. M., NARDIELLO, D. & QUINTO, M. 2018. Sensitive Screening Method for Determination of Pyrethroids in Chicken Eggs and Various Meat Samples by Gas Chromatography and Electron Capture Detection. *Journal of Agricultural and Food Chemistry.* 66: 10267-10273.

NASUTI, C., GABBIANELLI, R., FALCIONI, M. L., DI STEFANO, A., SOZIO, P. & CANTALAMESSA, F. 2007. Dopaminergic system modulation, behavioral changes, and oxidative stress after neonatal administration of pyrethroids. *Toxicology.* 229: 194-205.

NAYYAR, N., SANGWAN, N., KOHLI, P., VERMA, H., KUMAR, R., NEGI, V., OLDACH, P., MAHATO, N. K., GUPTA, V. & LAL, R. 2014. Hexachlorocyclohexane: persistence, toxicity and decontamination. *Reviews on Environmental Health.* 29: 49-52.

NCCP (National Cancer Control Policy). 2015. *Position statement - Pesticide and cancer*. Cancer Council Australia.[Online] Available from .< https://wiki.cancer.org.au/policy /Position_statement_-_Pesticides_and_cancer.> Acessed 12th August 2019.

NGUYEN, H. P. & KATTA, R. 2015. Sugar sag: glycation and the role of diet in aging skin. Skin Therapy Letter. 20: 1-5.

NIEWIADOWSKA, A., KILJANEK, T., SEMENIUK, S. & ZMUDZKI, J. 2010. Determination of pyrethroid residues in meat by gas chromatography with electron capture detection. *Bulletin of the Veterinary Institute in Pulawy.* 54: 595-599.

NILSEN, C. H. 2010. *Chromatography of metabolites in plasma and urine following oral administration of anthocyanin rich capsules.*Master theisis in Science and Technology. University of Stavanger, Norway.

OKIHASHI, M., KITAGAWA, Y., OBANA, H., TANAKA, Y., YAMAGISHI, Y., SUGITATE, K., SAITO, K., KUBOTA, M., KANAI, M. & UEDA, T. 2007. Rapid multiresidue method for the determination of more than 300 pesticide residues in food. *Food.*1: 101-110.

OLIVEIRA, F. A. D. S., PEREIRA, E. N. C., GOBBI, J. M., SOTO-BLANCO, B. & MELO, M. M. 2018. Multiresidue method for detection of pesticides in beef meat using liquid chromatography coupled to mass spectrometry detection (LC-MS) after QuEChERS extraction. *Food Additives & Contaminants: Part A.* 35: 94 -109.

O'SULLIVAN, G. 2013. *Environmental forensics for persistent organic pollutants.* Calgary /Alberta: Newnes Publishing.

OWAGO, O. J., QI, S., XIN, X., YUAN, Z. & SYLVIE, M. A. 2009. Residues of organochlorine pesticides in vegetables from Deyang and Yanting areas of the Chengdu economic region, Sichuan Province, China. *Journal of American Science.*5: 91-100.

PAGLIUCA, G., SERRAINO, A., GAZZOTTI, T., ZIRONI, E., BORSARI, A. & ROSMINI, R. 2006. Organophosphorus pesticides residues in Italian raw milk. *Journal of Dairy Research.* 73: 340-344.

PAL, A., HE, Y., JEKEL, M., REINHARD, M. & GIN, K. Y.-H. 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. *Environment International.*71: 46-62.

PANG, G. F. 2018. *Analysis of Pesticide in Tea: Chromatography-mass Spectrometry Methodology,* Amsterdam: Chemical Industry Prress and Gerhard Andre Wolff.

PANG, G.F., CAO, Y.Z., ZHANG, J.J., FAN, C.L., LIU, Y.M., LI, X.-M., JIA, G.Q., LI, Z.Y., SHI, Y.Q. & WU, Y.-P. 2006. Validation study on 660 pesticide residues in animal tissues by gel permeation chromatography cleanup/gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A.*1125: 1-30.

PANSERI, S., BIONDI, P., VIGO, D., COMMUNOD, R. & CHIESA, L. 2013. Occurrence of organochlorine pesticides residues in animal feed and fatty bovine tissue. *In*: Muzzalupo, I. *Food Industry.* pp 13-283. London: Intech Open.

PARAMASIVAM, M., NAIK, R. H. & CHANDRASEKARAN, S. 2011. QuEChERS method for determination of some chlorinated hydrocarbon and synthetic pyrethroid residues in sheep meat by gas chromatography-electron capture detector. *Madras Agricultural Journal.* 98: 282-285.

PARDÍO, V., MARTÍNEZ, D., FLORES, A., ROMERO, D., SUÁREZ, V., LÓPEZ, K. & USCANGA, R. 2012. Human health risk of dietary intake of organochlorine pesticide residues in bovine meat and tissues from Veracruz, México. *Food Chemistry.* 135: 1873-1893.

PARK, J.W., EL-ATY, A. A., LEE, M.H., SONG, S.O. & SHIM, J.H. 2006. Residue analysis of organophosphorus and organochlorine pesticides in fatty matrices by gas chromatography coupled with electron-capture detection. *Zeitschrift für Naturforschung C.* 61: 341-346.

PENG, G., HE, Q., LU, Y., MMEREKI, D. & ZHONG, Z. 2016. Determination of organophosphorus pesticides and their major degradation product residues in food samples by HPLC-UV. *Environmental Science and Pollution Research.* 23: 19409-19416.

PEREIRA, R. C., MARTÍNEZ, M. M., CORTÍZAS, A. M. & MACIAS, F. 2010. Analysis of composition, distribution and origin of hexachlorocyclohexane residues in agricultural soils from NW Spain. *Science of the Total Environment.* 408: 5583-5591.

PERELLÓ, G., MARTÍ-CID, R., CASTELL, V., LLOBET, J. M. & DOMINGO, J. L. 2010. Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods. *Food Control.* 21: 178-185.

PERIS-VICENTE, J., CARDA-BROCH, S., ESTEVE-ROMERO, J., RAMBLA-ALEGRE, M., BOSE, D., BELTRÁN-MARTINAVARRO, B., MARCO-PEIRÓ, S., MARTINAVARRO-DOMÍNGUEZ, A., OCHOA-ARANDA, E. & CHIN-CHEN, M. 2013. Validation of micellar LC-based methods applied to analyze foodstuffs. *Bioanalysis.*5: 481-494.

PICÓ, Y., FONT, G., JOSÉ RUIZ, M. & FERNÁNDEZ, M. 2006. Control of pesticide residues by liquid chromatography-mass spectrometry to ensure food safety. *Mass Spectrometry Reviews.* 25: 917-960.

PRASAD, K. & TIWARI, S. 2017. Therapeutic interventions for advanced glycation-end products and its receptor-mediated cardiovascular disease. *Current Pharmaceutical Design.* 23: 937-943.

PURI, P. 2014. Current trends in extraction methodologies for pesticide residues in food matrices. *International Journal of Agriculture Environment and Biotechnology.* 7: 331-342.

RAJASHEKAR, K., KONDAL REDDY, K., NARASIMHA REDDY, K. & SUDHAKAR REDDY, K. 2007. Effect of processing of milk into products on the residue levels of certain pesticides. *Journal of Food Science and Technology –Mysore.* 44: 551-552.

RAJPUT, S., KUMARI, A., ARORA, S. & KAUR, R. 2018. Multi-residue pesticides analysis in water samples using reverse phase high performance liquid chromatography (RP-HPLC). *MethodsX.* 5: 744-751.

RAMBLA-ALEGRE, M., ESTEVE-ROMERO, J. & CARDA-BROCH, S. 2012. Is it really necessary to validate an analytical method or not? That is the question. *Journal of Chromatography A.* 1232: 101-109.

RANI, M., SHANKER, U. & JASSAL, V. 2017. Recent strategies for removal and degradation of persistent & toxic organochlorine pesticides using nanoparticles: a review. *Journal of environmental management.* 190: 208-222.

RATHOD, A. L. & GARG, R. 2017. Chlorpyrifos poisoning and its implications in human fatal cases: A forensic perspective with reference to Indian scenario. *Journal of Forensic and Legal Medicine.* 47: 29-34.

RATHORE, H. S. & NOLLET, L. M. 2012. *Pesticides: evaluation of environmental pollution,* Boca Raton/Florida: CRC Press.

RAWN, D. F., BREAKELL, K., VERIGIN, V., TITTLEMIER, S. A., DEL GOBBO, L., DIAMOND, M., VANDERLINDEN, L. & SIT, D. 2013. Impacts of cooking technique on polychlorinated biphenyl and polychlorinated dioxins/furan concentrations in fish and fish products with intake estimates. *Journal of Agricultural and food Chemistry.* 61: 989-997.

REJI, K. K., MATHEW, V., ZACHARIAH, A., PATIL, A. K. B., HANSDAK, S. G., RALPH, R. & PETER, J. V. 2016. Extrapyramidal effects of acute organophosphate poisoning. *Clinical Toxicology.* 54: 259-265.

RISHER, J. F., TODD, G. D., MEYER, D. & ZUNKER, C. L. 2010. The elderly as a sensitive population in environmental exposures: making the case. *Reviews of Environmental Contamination and Toxicology.* 207: 95-157.

ROBERTS, G. W. 2008. *Chemical reactions and chemical reactors.* Oxford: Wiley.

RUTKOWSKA, E., ŁOZOWICKA, B. & KACZYŃSKI, P. 2018. Modification of multiresidue QuEChERS protocol to minimize matrix effect and improve recoveries for determination

of pesticide residues in dried herbs followed by GC-MS/MS. *Food Analytical Methods.*11: 709-724.

SABARWAL, A., KUMAR, K. & SINGH, R. P. 2018. Hazardous effects of chemical pesticides on human health–Cancer and other associated disorders. *Environmental Toxicology and Pharmacology.* 63: 103-114.

SAHU, R. K. & NELAPATI, K. 2018. Method validation for analysis of pesticide residues in Labeo rohita fish through GC-MS/MS. *International Journal of Chemical Studies.* 6: 1448-1452.

SALLAM, K. I. & MORSHEDY, A. E. M. A. 2008. Organochlorine pesticide residues in camel, cattle kand sheep carcasses slaughtered in Sharkia Province, Egypt. *Food Chemistry.*108: 154-164.

SANDS, B., MGIDISWA, N., NYAMUKONDIWA, C. & WALL, R. 2018. Environmental consequences of deltamethrin residues in cattle feces in an African agricultural landscape. *Ecology and Evolution.* 8 : 2938-2946.

SARTARELLI, N. C., DE MACEDO, A. N., DE SOUSA, J. P., NOGUEIRA, A. R. D. A. & BRONDI, S. H. G. 2012. Determination of chlorfenvinphos, fipronil, and cypermethrin residues in meat and bovine fat using QuEChERS method and gas chromatography-mass spectrometry. *Journal of Liquid Chromatography & Related Technologies.*35:1895-1908.

SARWAR, M. 2015. The dangers of pesticides associated with public health and preventing of the risks. *International Journal of Bioinformatics and Biomedical Engineering.*1: 130-136.

SHARMA, A., YADAV, B., ROHATGI, S. & YADAV, B. 2018. Cypermethrin Toxicity: A Review. *Jounal of Foensic Sciences and Ciminal Investigation.* 9: 001-003.

SHURUBOR, Y. I., D'AURELIO, M., CLARK-MATOTT, J., ISAKOVA, E. P., DERYABINA, Y. I., BEAL, M. F., COOPER, A. J. & KRASNIKOV, B. F. 2017. Determination of coenzyme A and acetyl-coenzyme A in biological samples using HPLC with UV detection. *Molecules.* 22 : 2-13.

SILVA, J. F., MATTOS, I. E., LUZ, L. L., CARMO, C. N. & AYDOS, R. D. 2016. Exposure to pesticides and prostate cancer: systematic review of the literature. *Reviews on Environmental Health.* 31: 311-327

SINGH, A., NAG, S. K. & SINGH, S. 2015. Persistent organochlorine pesticide residue in tissues and blood of goat. *Journal of Applied Animal Research.* 43: 366 -371.

SINGH, N. S., SHARMA, R., PARWEEN, T. & PATANJALI, P. 2018. Pesticide contamination and human health risk factor.*In :* Mohammad, O., Mohammad, Z.K, & Iqbal, M.I. *Modern Age Environmental Problems and their Remediation.* pp 49-68. New Delhi: Springer International Publishing.

SINGH, S. 2017. *Studies on the effect of different processing methods on the levels of pesticide residues in milk, meat and their products.* Ph.D thesis of Veterinary Science. Telangana Veterinary University, Telangana/India.

SINGH, S., KRISHNAIAH, N., RAO, T. M., PUSHPA, R. & REDDY, G. 2017. Effect of heat processing on degradation of organophosphorus compounds in milk. *The Pharma Innovation.*6 : 209-2012.

SLAGER, R. E., SIMPSON, S. L., LEVAN, T. D., POOLE, J. A., SANDLER, D. P. & HOPPIN, J. A. 2010. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *Journal of Toxicology and Environmental Health, Part A.* 73: 1382-1393.

SMITH, I. 2013. *Chromatography.* Dordrecht: Kluwer Academic publisher.

SNYDER, L. R., KIRKLAND, J. J. & DOLAN, J. W. 2011. *Introduction to Modern Liquid Chromatography.*New Jersey: John Wiley & Sons.

SOARES, W. L. & DE SOUZA PORTO, M. F. 2009. Estimating the social cost of pesticide use: An assessment from acute poisoning in Brazil. *Ecological Economics.* 68: 2721-2728.

SOBRAL, M. M. C., CUNHA, S. C., FARIA, M. A. & FERREIRA, I. M. 2018. Domestic cooking of muscle foods: impact on composition of nutrients and contaminants. *Comprehensive Reviews in Food Science and Food Safety.*17: 309-333.

SODERLUND, D. M. 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. *Archives of Toxicology.* 86: 165-181.

SOGORB, M. A. & VILANOVA, E. 2002. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. *Toxicology Letters.*128: 215-228.

STAREK-ŚWIECHOWICZ, B., BUDZISZEWSKA, B. & STAREK, A. 2017. Hexachlorobenzene as a persistent organic pollutant: toxicity and molecular mechanism of action. *Pharmacological Reports.* 69: 1232-1239.

STEFANELLI, P., SANTILIO, A., CATALDI, L. & DOMMARCO, R. 2009. Multiresidue analysis of organochlorine and pyrethroid pesticides in ground beef meat by gas chromatography-mass spectrometry. *Journal of Environmental Science and Health Part B.* 44: 350-356.

STENERSEN, J. 2004. *Chemical pesticides mode of action and toxicology.* Florida: CRC press.

STUBBINGS, G. & BIGWOOD, T. 2009. The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC–MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) approach. *Analytica Chimica Acta.* 637: 68-78.

STURARO, A., DE MARCHI, M., MASI, A. & CASSANDRO, M. 2016. Quantification of whey proteins by reversed phase-HPLC and effectiveness of mid-infrared spectroscopy for their rapid prediction in sweet whey. *Journal of Dairy Science.* 99: 68-76.

SUÁREZ, A. & ELLIS, A. 2002. *Alpha-Cypermethrin and Cypermethrin. FAO FNP 41/16. FAO- FNP(Food and Nutrition Paper).*Washington: FAO FNP.

SUGANTHY, M. & KUTTALAM, S. 2003. Organochlorine and chlorpyriphos insecticide residues in market samples of meat. *Pestology.* 27: 51-4.

SUGANTHY, M., KUTTALAM, S. & CHANDRASEKARAN, S. 2009. Residues of chlorinated hydrocarbon insecticides and chlorpyriphos in market samples of mutton. *Madras Agricultural Journal.* 96: 221-224.

SUN, D.-W. 2016. *Thermal food processing: new technologies and quality issues.* Boca Raton: CRC Press.

SURMA, M., PISKUŁA, M., WICZKOWSKI, W. & ZIELIŃSKI, H. 2017. The perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonates (PFSAs) contamination level in spices. *European Food Research and Technology.* 243: 297-307.

TADEO, J. L., SÁNCHEZ-BRUNETE, C. & GONZÁLEZ, L. 2008. Pesticides: Classification and Properties. *In:* Tadeo, J.L. *Analysis of pesticides in food and environmental samples.* pp 13-46. London, New York, Boca raton: CRC Press, Taylor and Francis Group.

TARLA, D., TCHAMBA, N., BALEGUEL, N., FONTEM, D., BALEGUEL, P. & HANS, D. 2014. Inventory of obsolete pesticide stockpiles in Cameroon. *Scholers Journal of Agricalture and Veterinary Scinces.* 4: 43-50.

THERDTEPPITAK, A. & YAMMENG, K. 2003. Determiantion of organochlorine pesticides in commercical fish by Gas Chromatograpy with electron captue detector and confirmation by Gas Chromatogrraphy-Mass Spectrometry. *Science Asia.* 29: 127-134.

TILAHUN, A., JAMBARE, L., TESHALE, A. & GETACHEW, A. 2016. Review on Chemical and Drug Residue in Meat. *World Journal of Agricultural Sciences.*12: 196-204.

TONGO, I. & EZEMONYE, L. 2015. Human health risks associated with residual pesticide levels in edible tissues of slaughtered cattle in Benin City, Southern Nigeria. *Toxicology Reports.*2: 1117-1135.

TOR, A., AYDIN, M. E. & ÖZCAN, S. 2006. Ultrasonic solvent extraction of organochlorine pesticides from soil. *Analytica Chimica Acta.*559 : 173-180.

TSIPLAKOU, E., ANAGNOSTOPOULOS, C., LIAPIS, K., HAROUTOUNIAN, S. & ZERVAS, G. 2010. Pesticides residues in milks and feedstuff of farm animals drawn from Greece. *Chemosphere.*80: 504-512.

TURYAHIKAYO, E. 2013. *Assessing the levels of pesticide residues in livestock products and water around Lake Mburo National Park, South Western Uganda.* Ph.D thesis in agriculture and environmental Sciences, Makerere University, Uganda.

TUZIMSKI, T. 2016. Multiclass, Multiresidue Methods (MRMs) for the Determination of Pesticides by Modern Extraction and Detection Techniques: The Combination of QuEChERS and High-Resolution Mass Spectrometry (HRMS). *Journal of AOAC International.* 99: 1381-1382.

UCLÉS, S., LOZANO, A., SOSA, A., VÁZQUEZ, P. P., VALVERDE, A. & FERNÁNDEZ-ALBA, A. 2017. Matrix interference evaluation employing GC and LC coupled to triple quadrupole tandem mass spectrometry. *Talanta.*174 : 72-81.

UPADHAYAY, J., RANA, M., JUYAL, V., BISHT, S. S. & JOSHI, R. 2020. *Impact of Pesticide Exposure and Associated Health Effects. In:* Srivastava, P.K. *Pesticides in Crop Production. Physiological and Biochemical Action.* pp 69 -78. Chennai: John Wiley and Sons.

USEPA (United States Enviomental Protection Agency). 2015a. *Fourth Unregulated Contaminant Monitoring Rule.* [Online] Availbale from.< **Error! Hyperlink reference not valid.**. >. Accessed 6 th July 2019.

USEPA (United States Enviomental Protection Agency). 2015b. *Summary of Nominations for the Fourth Contaminant Candidate List*. EPA 815-R-15e001. draft ccl list. U.S. Washington: USEPA.

USEPA (United States Enviomental Protection Agency). 2018. *Basic Information about Pesticide Ingredients*. [Online] Availbale from.< https://www.epa.gov/ingredients-used-pesticide-products/basic-information-about-pesticide-ingredients>. Accessed 5th July 2019.

USEPA (United States Enviomental Protection Agency). 2019. *About Pesticide Registration*.Washington: USEPA.

UTAMA, D. T., BAEK, K. H., JEONG, H. S., YOON, S. K., JOO, S.T. & LEE, S. K. 2018. Effects of cooking method and final core-temperature on cooking loss, lipid oxidation, nucleotide-related compounds and aroma volatiles of Hanwoo brisket. *Asian-Australasian Journal of Animal Sciences.*31:1-8.

UTAMA, D. T., LEE, S. G., BAEK, K. H., KIM, H.K., CHO, C.Y., LEE, C.K. & LEE, S. K. 2016. Correlation between antioxidant enzyme activity, free iron content and lipid oxidation in four lines of Korean native chicken meat. *Korean Journal for Food Science of Animal Resources.*36: 44-50.

VACLAVIK, V. A. & CHRISTIAN, E. W. 2008. *Essentials of Food Science.* New York: Springer-Verlag.

VANLOON, G. W. & DUFFY, S. J. 2017. *Environmental chemistry: a global perspective,* Oxford: Oxford university press.

VELKOSKA-MARKOVSKA, L. & PETANOVSKA-ILIEVSKA, B. 2019. Development and Validation of Rp-Hplc Method For Determination of Some Pesticide Residues in Water Samples. *Contributions. Section of Natural, Mathematical & Biotechnical Sciences.* 40: 169-180.

VENTURA, C., NIETO, M. R. R., BOURGUIGNON, N., LUX-LANTOS, V., RODRIGUEZ, H., CAO, G., RANDI, A., COCCA, C. & NÚÑEZ, M. 2016. Pesticide chlorpyrifos acts

as an endocrine disruptor in adult rats causing changes in mammary gland and hormonal balance. *The Journal of Steroid Biochemistry and Molecular Biology.*156: 1- 9.

VIEL, J.-F., WAREMBOURG, C., LE MANER-IDRISSI, G., LACROIX, A., LIMON, G., ROUGET, F., MONFORT, C., DURAND, G., CORDIER, S. & CHEVRIER, C. 2015. Pyrethroid insecticide exposure and cognitive developmental disabilities in children: the PELAGIE mother–child cohort. *Environment International.* 82: 69-75.

VITHA, M. F. 2016. *Chromatography: principles and instrumentation.*Washington: John Wiley & Sons.

VOGEL, A. I., TATCHELL, A., FURNIS, B., HANNAFORD, A. & SMITH, P. 1989. *Vogel's textbook of practical organic chemistry.* New York: John Wiley & Sons, Inc. .

VU-DUC, N., NGUYEN-QUANG, T., LE-MINH, T., NGUYEN-THI, X., TRAN, T. M., VU, H. A., NGUYEN, L.-A., DOAN-DUY, T., VAN HOI, B. & VU, C.-T. 2019. Multiresidue Pesticides Analysis of Vegetables in Vietnam by Ultrahigh-Performance Liquid Chromatography in Combination with High-Resolution Mass Spectrometry (UPLC-Orbitrap MS). *Journal of Analytical Methods in Chemistry.* 2019: 1-13.

WALISZEWSKI, S., GOMEZ-ARROYO, S., INFANZON, R., VILLALOBOS-PIETRINI, R. & MAXWELL HART, M. 2003. Comparison of organochlorine pesticide levels between abdominal and breast adipose tissue. *Bulletin of Environmental Contamination and Toxicology.*71: 156-162.

WANG, H., DING, J. & REN, N. 2016. Recent advances in microwave-assisted extraction of trace organic pollutants from food and environmental samples. *TrAC Trends in Analytical Chemistry*, 75. 197-208.

WARE, G. W. 2000. *The pesticide book* . Fresno/California: Thomson Publications.

WHO (Word Health Organisation). 2016. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.*Geneva: WHO Library Cataloguing-in-Publication Data

WILKOWSKA, A. & BIZIUK, M. 2011. Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chemistry.*125: 803 - 812.

WILLIAMSON, C., FOSTER, R., STANNER, S. & BUTTRISS, J. 2005. Red meat in the diet. *Nutrition Bulleti* .30: 323 -355.

WITCZAK, A. 2009. Effect of heat treatment on organochlorine pesticide residues in selected fish species. *Polish Journal of Food and Nutrition Sciences.*59: 231-235.

XIANG, L., WU, H., CUI, Z. & TANG, J. 2019. Indirect Competitive Aptamer-Based Enzyme-Linked Immunosorbent Assay (apt-ELISA) for the Specific and Sensitive Detection of Isocarbophos Residues. *Analytical Letters.*52: 1966-1975.

XIAO, Z., HE, M., CHEN, B. & HU, B. 2016. Polydimethylsiloxane/metal-organic frameworks coated stir bar sorptive extraction coupled to gas chromatography-flame photometric detection for the determination of organophosphorus pesticides in environmental water samples. *Talanta.*156: 126-133.

YADAV, I. C. & DEVI, N. L. 2017. Pesticides classification and its impact on human and environment. *Environmental Science and Engineering.*6 : 140-158.

YEO, H.-G., CHOI, M. & SUNWOO, Y. 2004. Seasonal variations in atmospheric concentrations of organochlorine pesticides in urban and rural areas of Korea. *Atmospheric Environment.* 38: 4779-4788.

YU, Y., YANG, Y., ZHAO, X., LIU, X., XUE, J., ZHANG, J. & YANG, A. 2017. Exposure to the mixture of organophosphorus pesticides is embryotoxic and teratogenic on gestational rats during the sensitive period. *Environmental Toxicology.* 32: 139-146.

YUN-SANG, C., HWANG, E., JEONG, J., KIM, B., JEON, H., KIM, E., SUNG, J., KIM, H. & KIM, C. 2016. Comparative study on the effects of boiling, steaming, grilling, microwaving and superheated steaming on quality characteristics of marinated chicken steak. *Korean Journal for Food Science of Animal Resources.*36: 1-7.

YUSÀ, V., COSCOLLÀ, C. & MILLET, M. 2014. New screening approach for risk assessment of pesticides in ambient air. *Atmospheric Environment.* 96: 322 - 330.

ZAMARIOLA, N., TOLEDO NETTO, P., DA SILVA FRANCHI, C. A., DE CAMARGO, J. L. V. & DE MARCHI, M. R. R. 2017. QuEChERS-based method for pesticides analysis in adipose tissue associated with rat ovaries. *Journal of Analytical Toxicology.* 41: 399-406.

ZAMORA-SEQUEIRA, R., STARBIRD-PÉREZ, R., ROJAS-CARILLO, O. & VARGAS-VILLALOBOS, S. 2019. What are the main sensor methods for quantifying pesticides in agricultural activities? A review. *Molecules.* 24: 1-26.

ZARE, S., BEHZADI, M., TARZANAN, M., MOHAMADI, M. B., OMIDI, L., HEYDARABADI, A. B. & KAZEMI, S. 2015. The impacts of pesticides on the health of farmers in Fasa, Iran. *Electronic Physician* .7: 1168-1173.

ZEIGENBAUM, J. & STONE, P. 2009. *An application kit for multi-residue screening of pesticides using LC/TOF or Q-TOF with a pesticide personal compound database*, Agilent Technologies – Application note. Wilmington/North Carolina: Agilent Technologies.

ZENG, L., PENG, L., WU, D. & YANG, B. 2018. Electrochemical Sensors for Food Safety. *In:* Mozsik, G & Figler, M. *Nutrition in Health and Disease-Our Challenges Now and Forthcoming Time*. pp 1-11. London: IntechOpen.

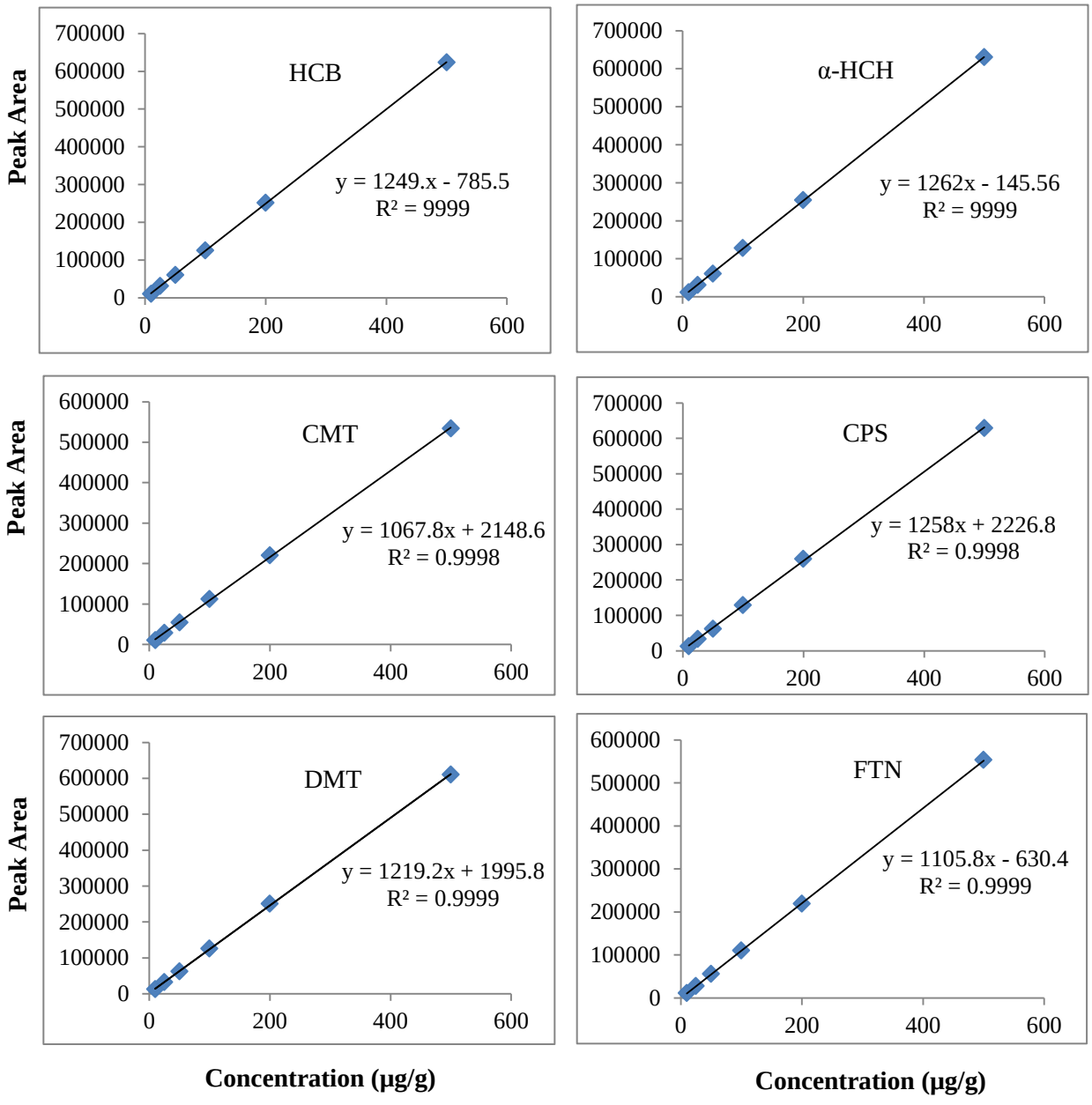
ZHANG, C., HU, R., SHI, G., JIN, Y., ROBSON, M. G. & HUANG, X. 2015. Overuse or underuse? An observation of pesticide use in China. *Science of the Total Environment.* 538: 1-6.

ZHOU, W., HUANG, X. & LIN, K. 2019. Analysis of polyhalogenated carbazoles in sediment using liquid chromatography–tandem mass spectrometry. *Ecotoxicology and Environmental Safety.* 170: 148-155.

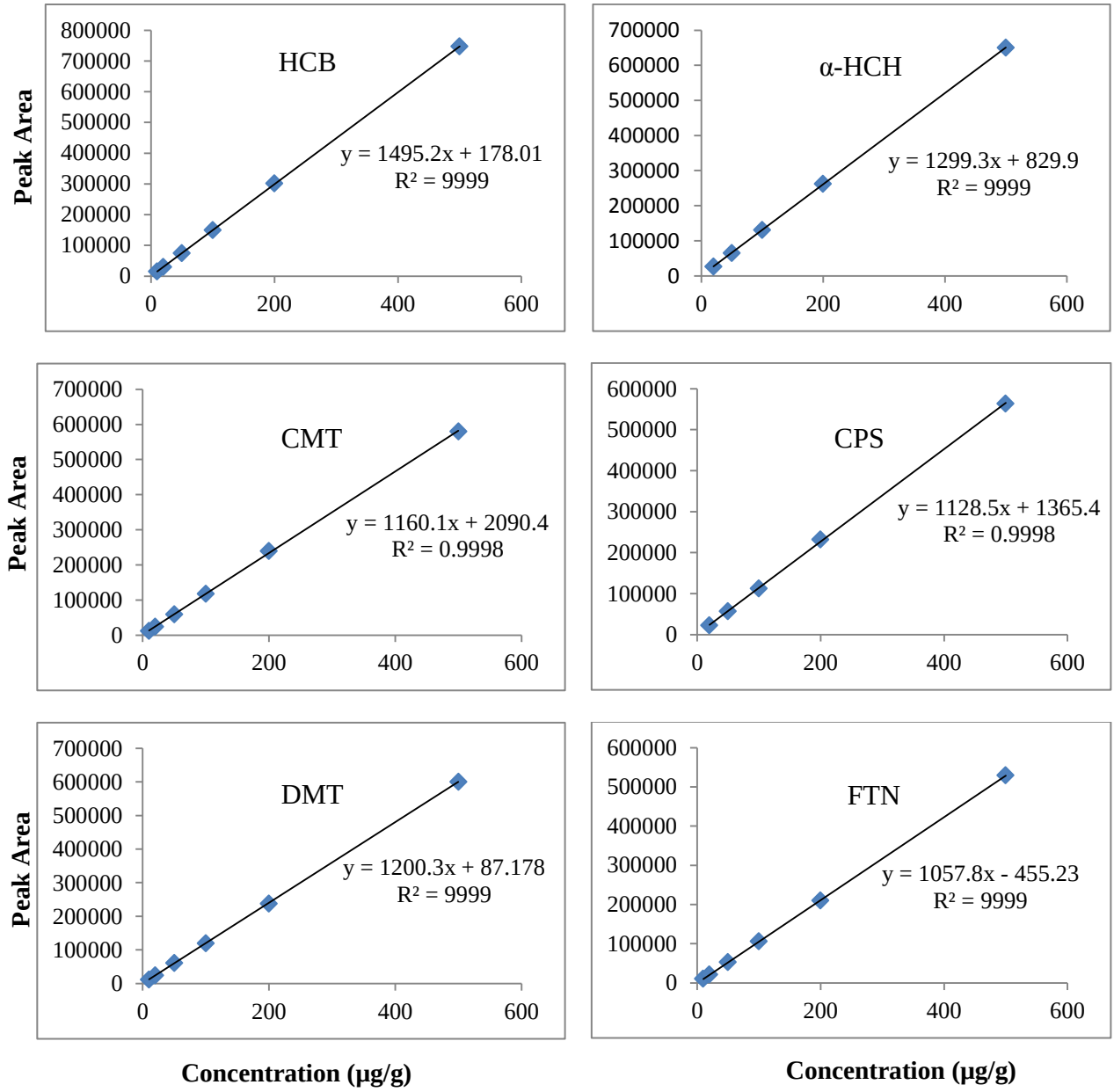
APPENDICES

Appendix A. Linearities

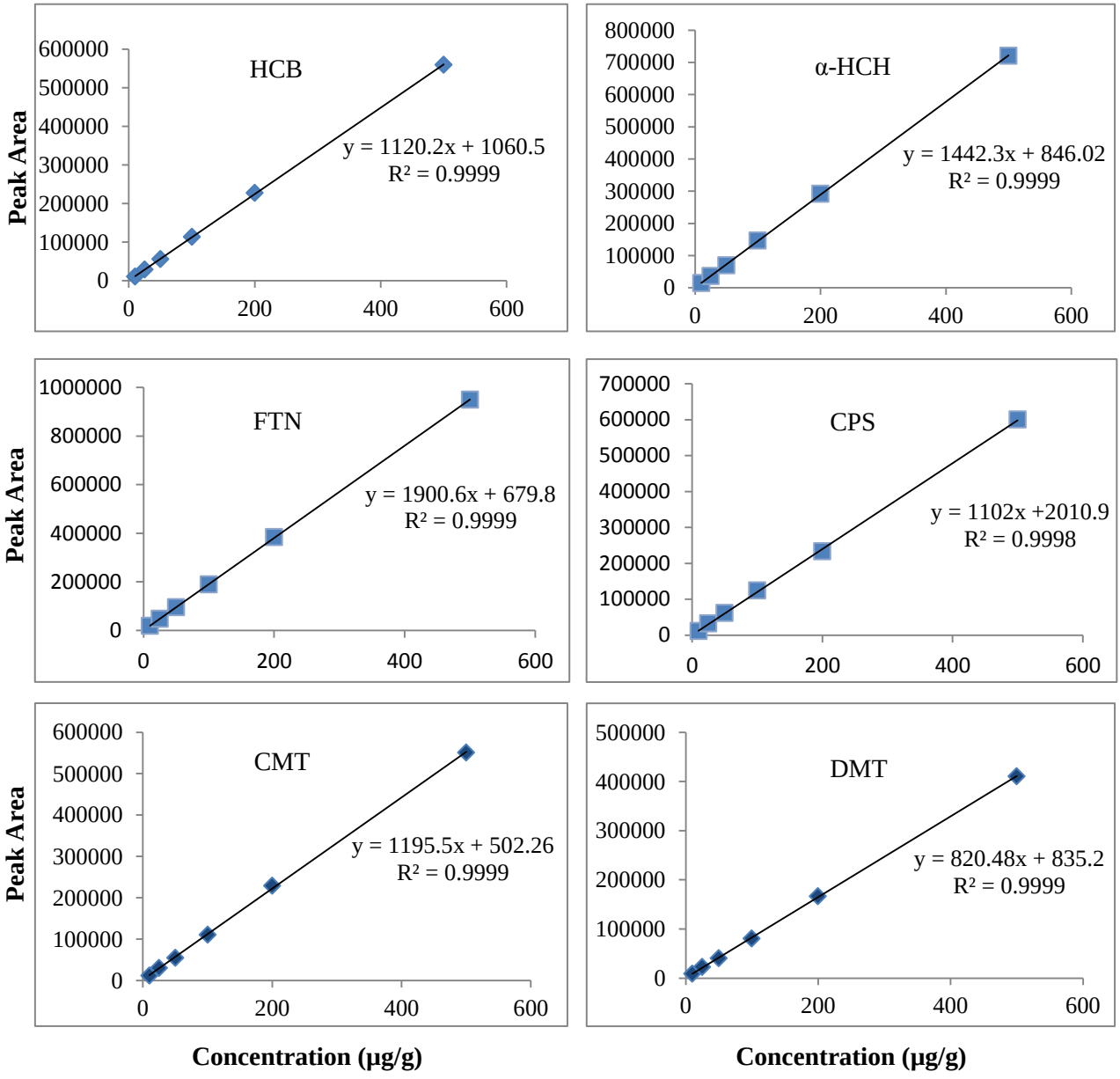
Appenxix A1. Plot linearities of matrix- matched calibrration in meat samples used for sensitivity test in HPLC-UV analysis.



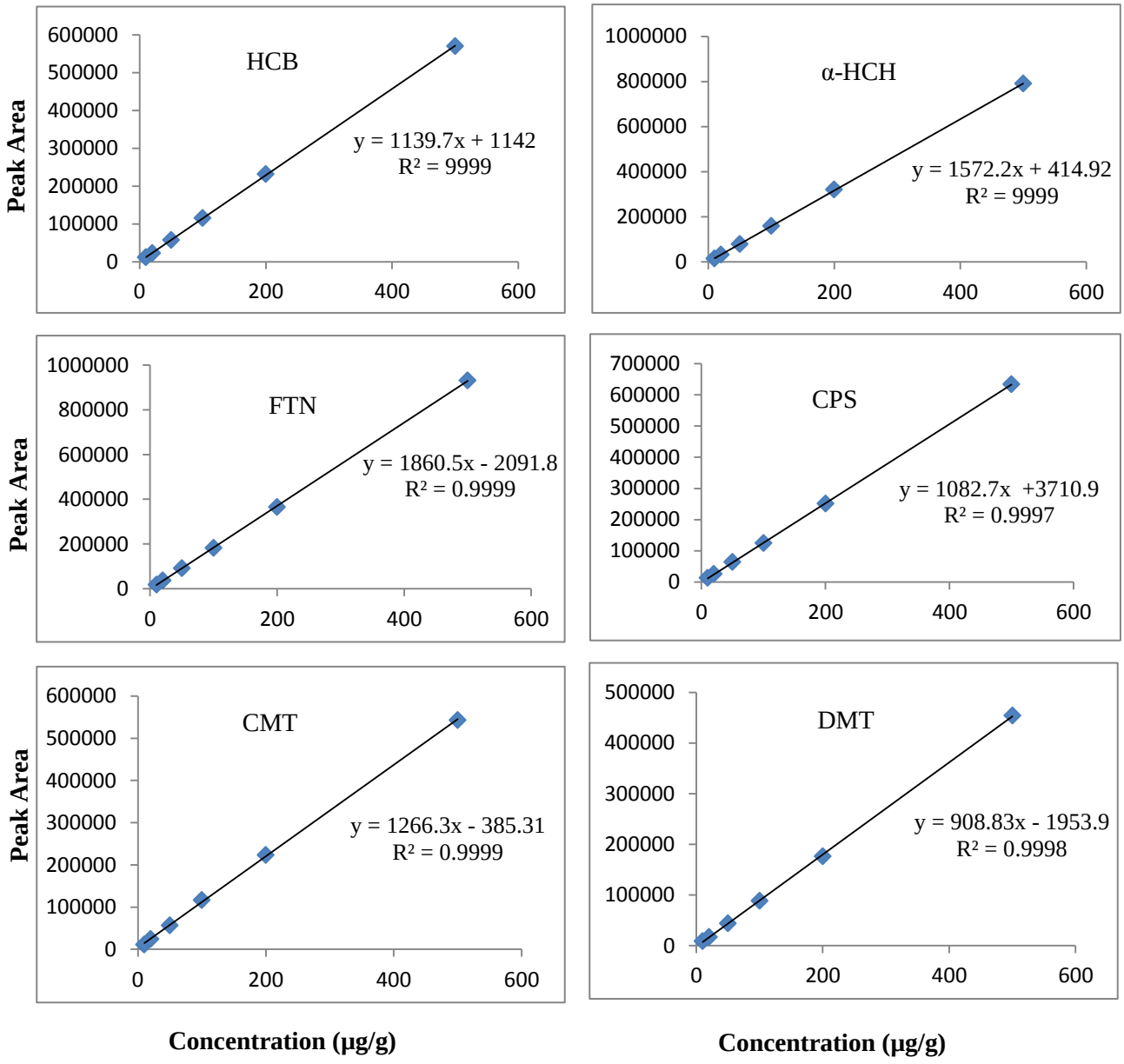
Appendix A2. Plot linearities of matrix- matched calibrration in fat samples used for sensitivity test in HPLC-UV analysis.



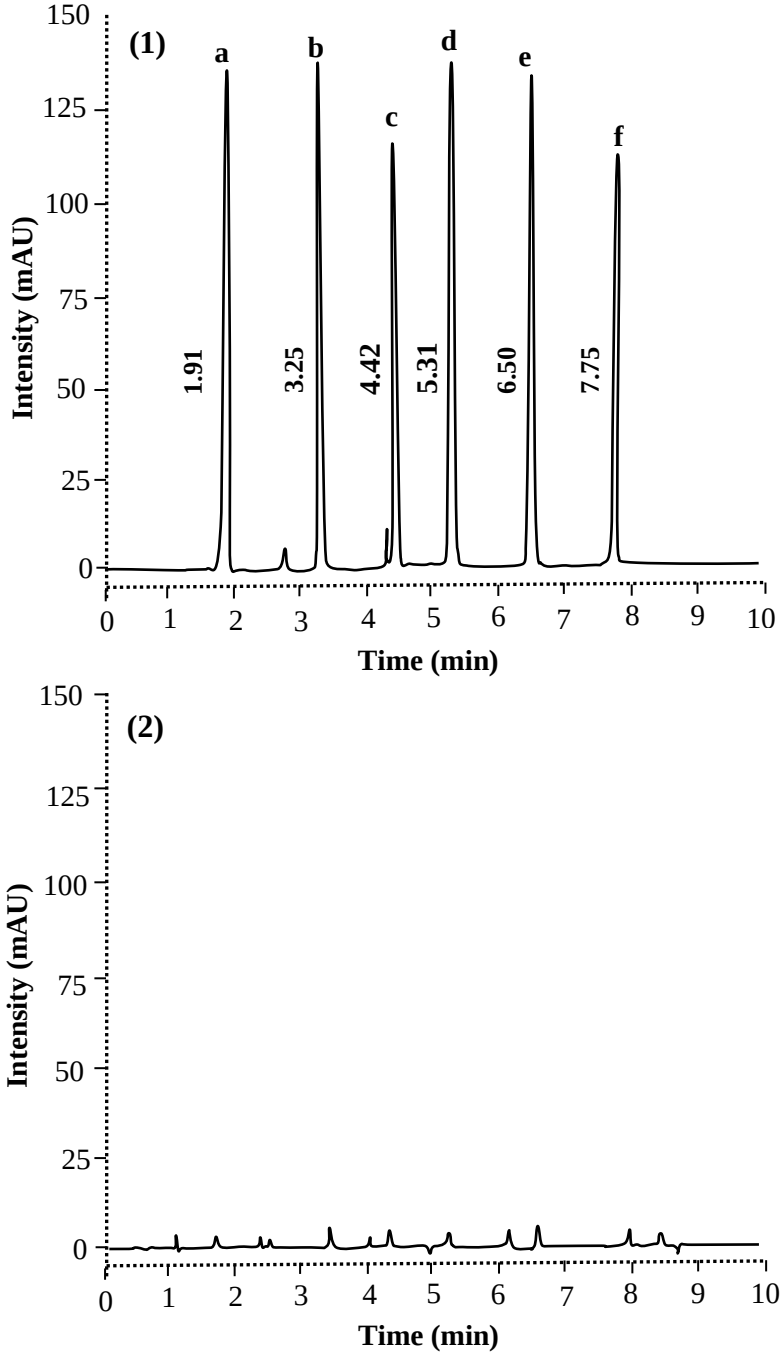
Appenndix A3. Plot linearities of matrix- matched calibrration in meat samples used for sensitivity test in GC-MS analysis



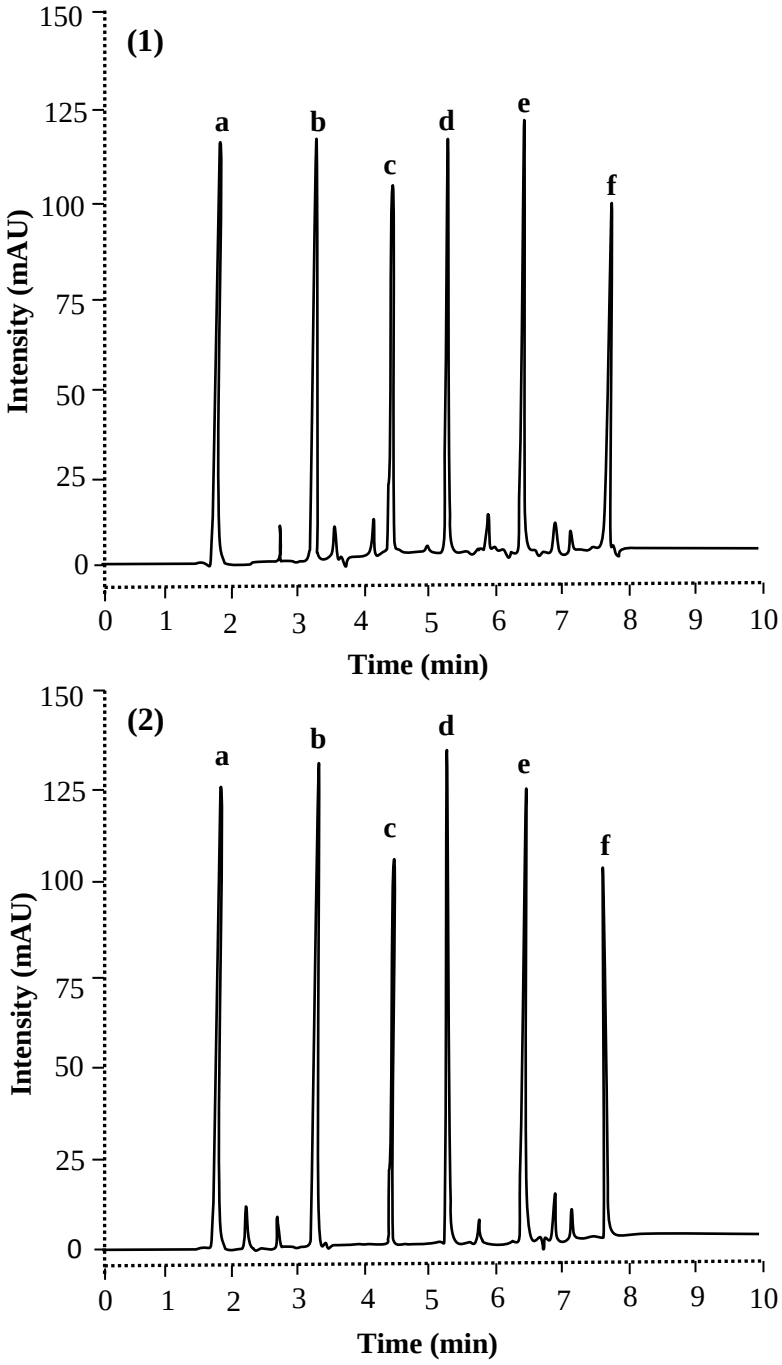
Appendix A4. Plot linearities of matrix- matched calibrration in fat samples used for sensitivity test in GC-MS analysis



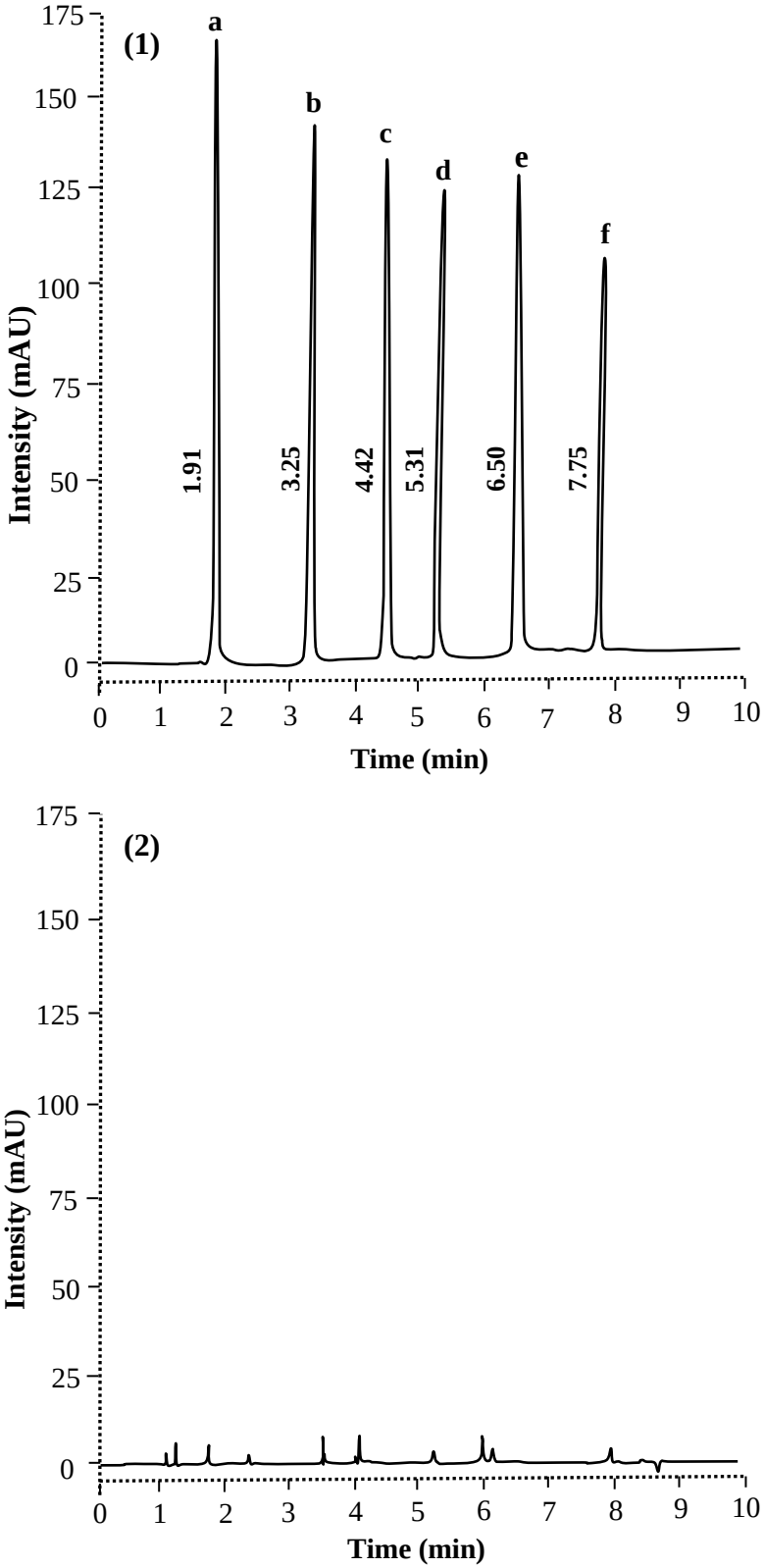
Appendix B. Chromatographs



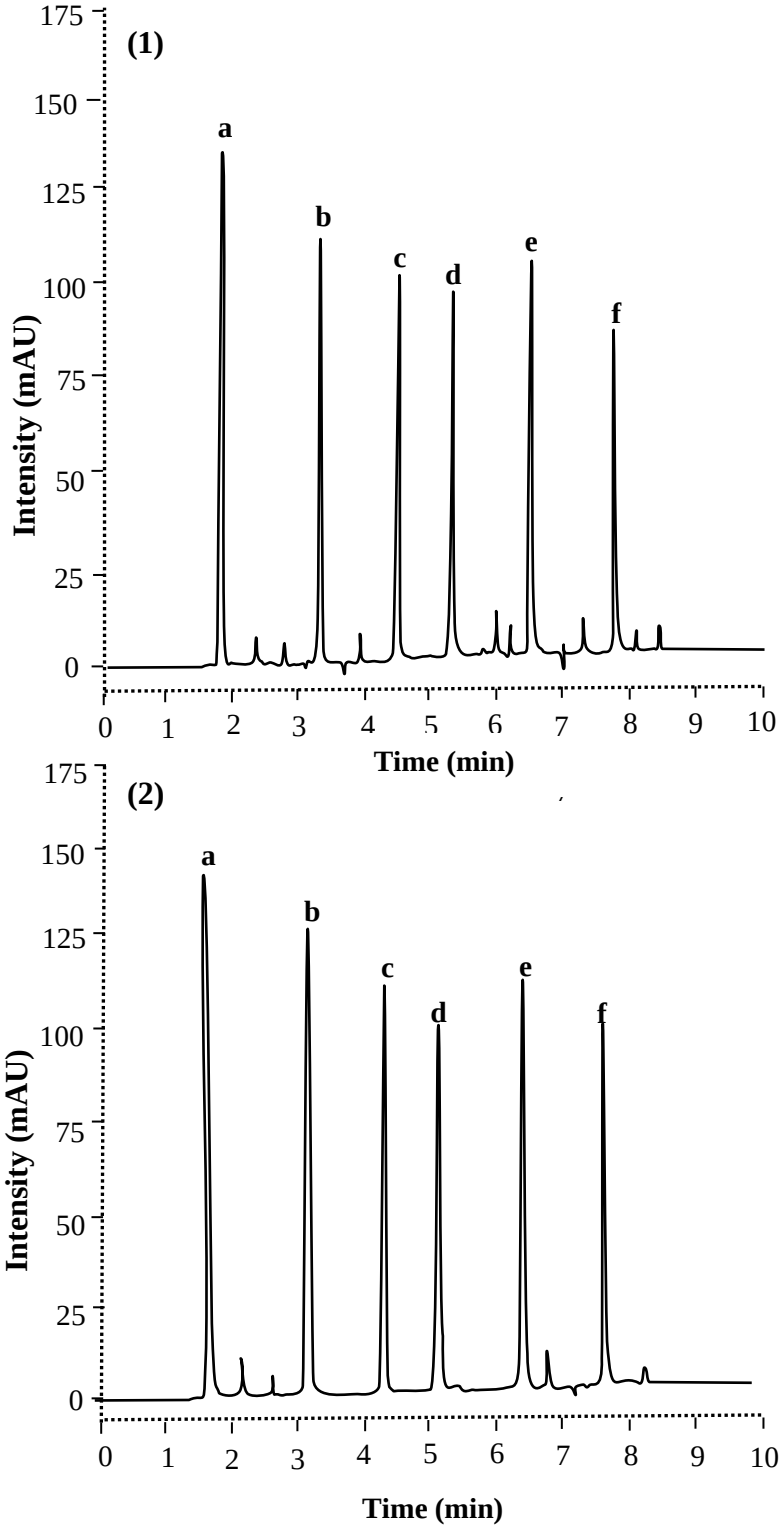
Appendix B1. HPLC-UV chromatograms: (1) Multistandard solutions (10 mg/L); (2) Blank meat samples. **a**. Hexachlorobenzene; **b**. α-Hexachlorocyclohexane,;**c**. Cypermethrin; **d**. Chlorpyrifos; **e**. Deltamethrin; **f**. Fenitrothion.



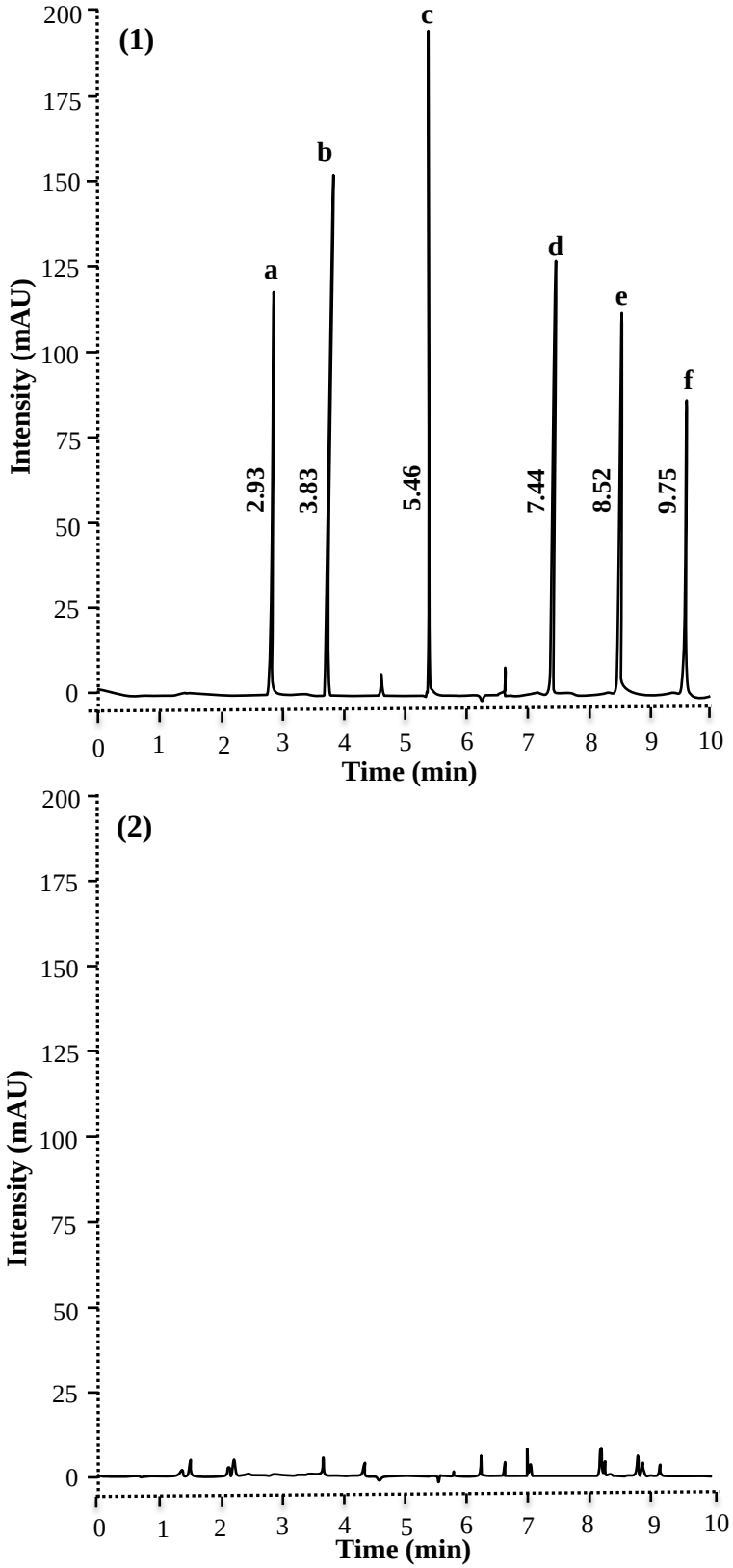
Appendix B2. HPLC-UV chromatograms: (1) Spiked meat samples before extraction (0.1 mg/kg); (2). Spiked meat sample after extraction (0.1 mg/kg). **a**. Hexachlorobenzene; **b**. α-Hexachlorocyclohexane; **c**. Cypermethrin; **d**. Chlorpyrifos; **e**. Deltamethrin; **f**. Fenitrothion.



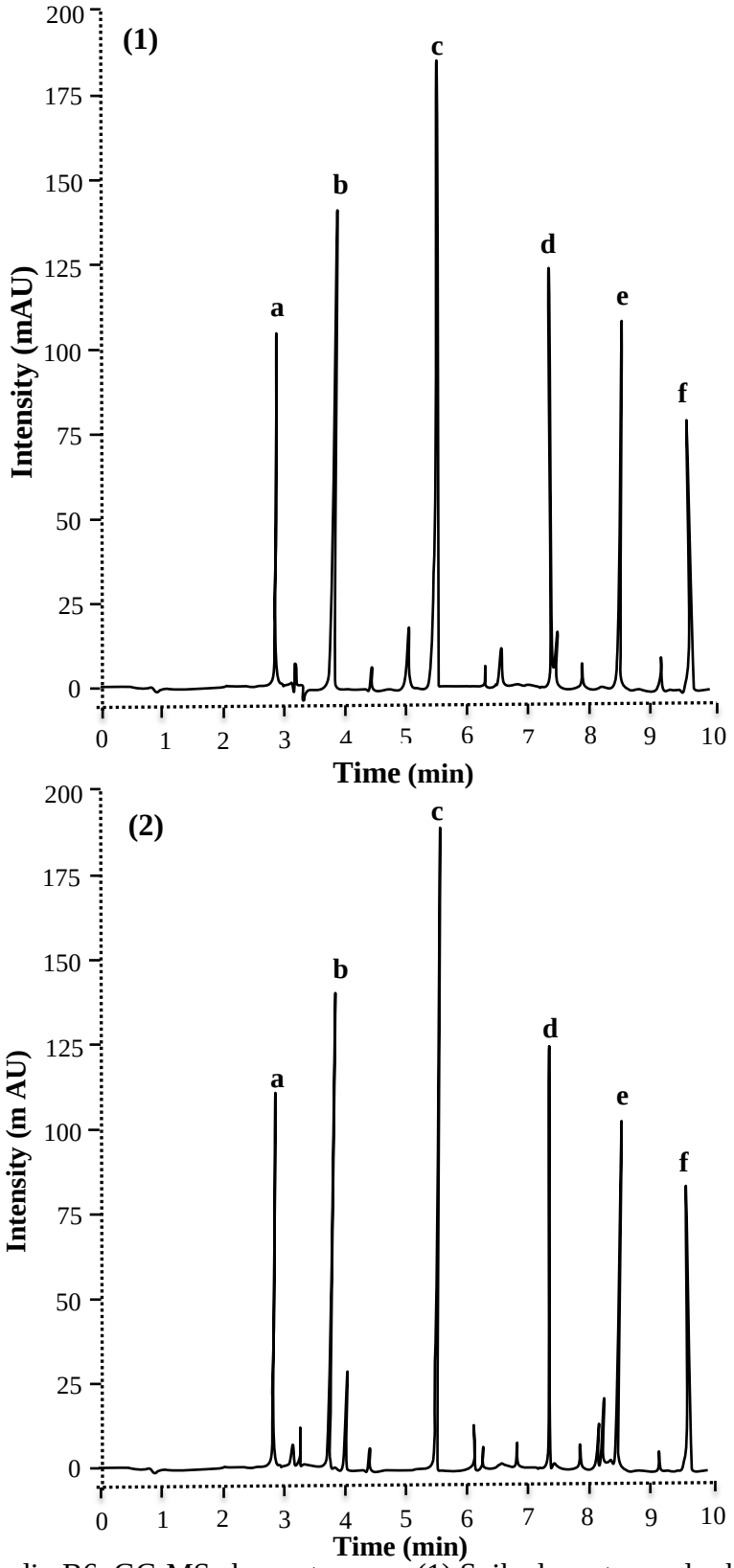
Appendix B3. HPLC-UV chromatograms: (1) Multi-standard solutions (10 mg/L); (2) Blank fat samples. **a**. Hexachlorobenzene; **b**. α-Hexachlorocyclohexane,;**c**. Cypermethrin; **d**. Chlorpyrifos; **e**. Deltamethrin; **f**. Fenitrothion.



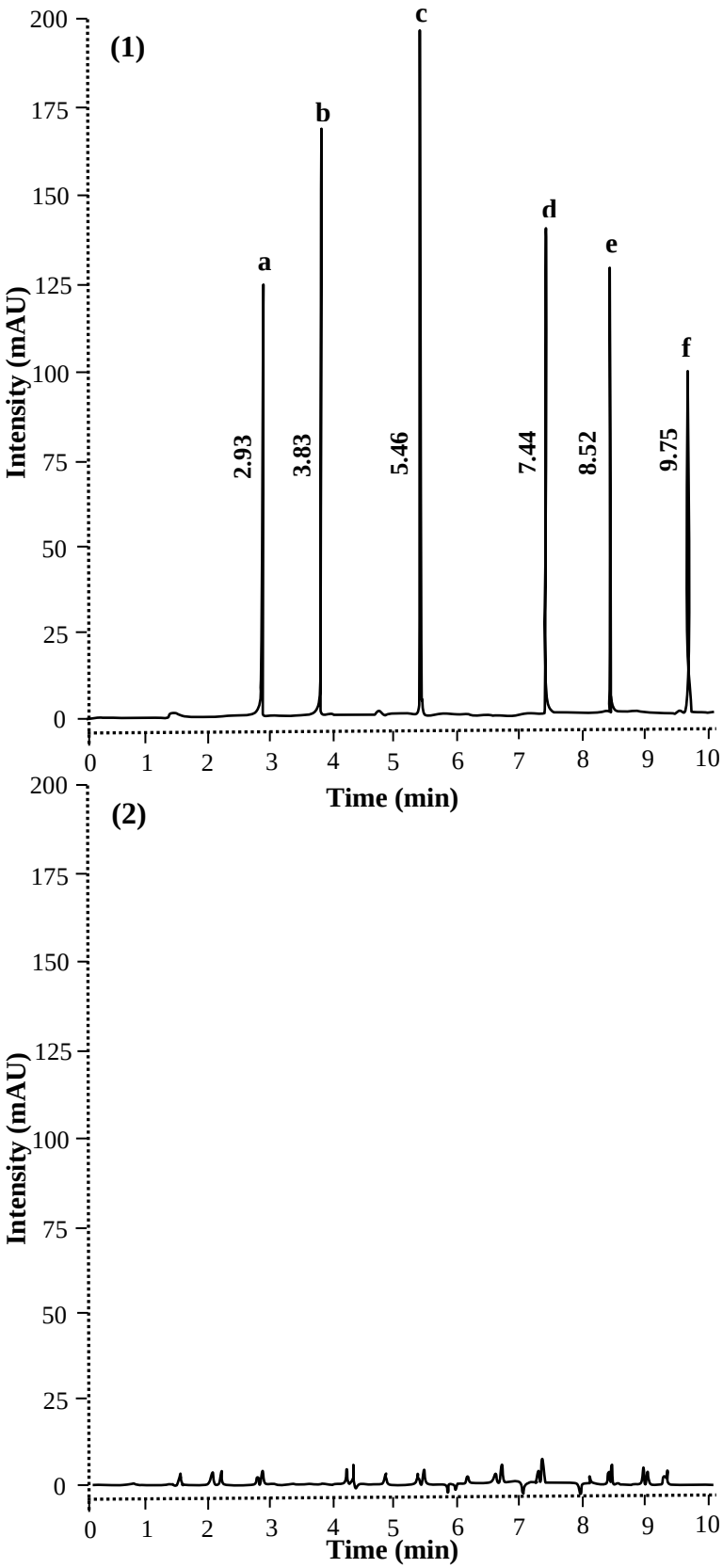
Appendix B4. HPLC-UV chromatograms: (1) Spiked fat samples before extraction (0.1 mg/kg); (2). Spiked fat sample after extraction (0.1 mg/kg). **a**. Hexachlorobenzene; **b**. α-Hexachlorocyclohexane; **c**. Cypermethrin; **d**. Chlorpyrifos; **e**. Deltamethrin; **f**. Fenitrothion.



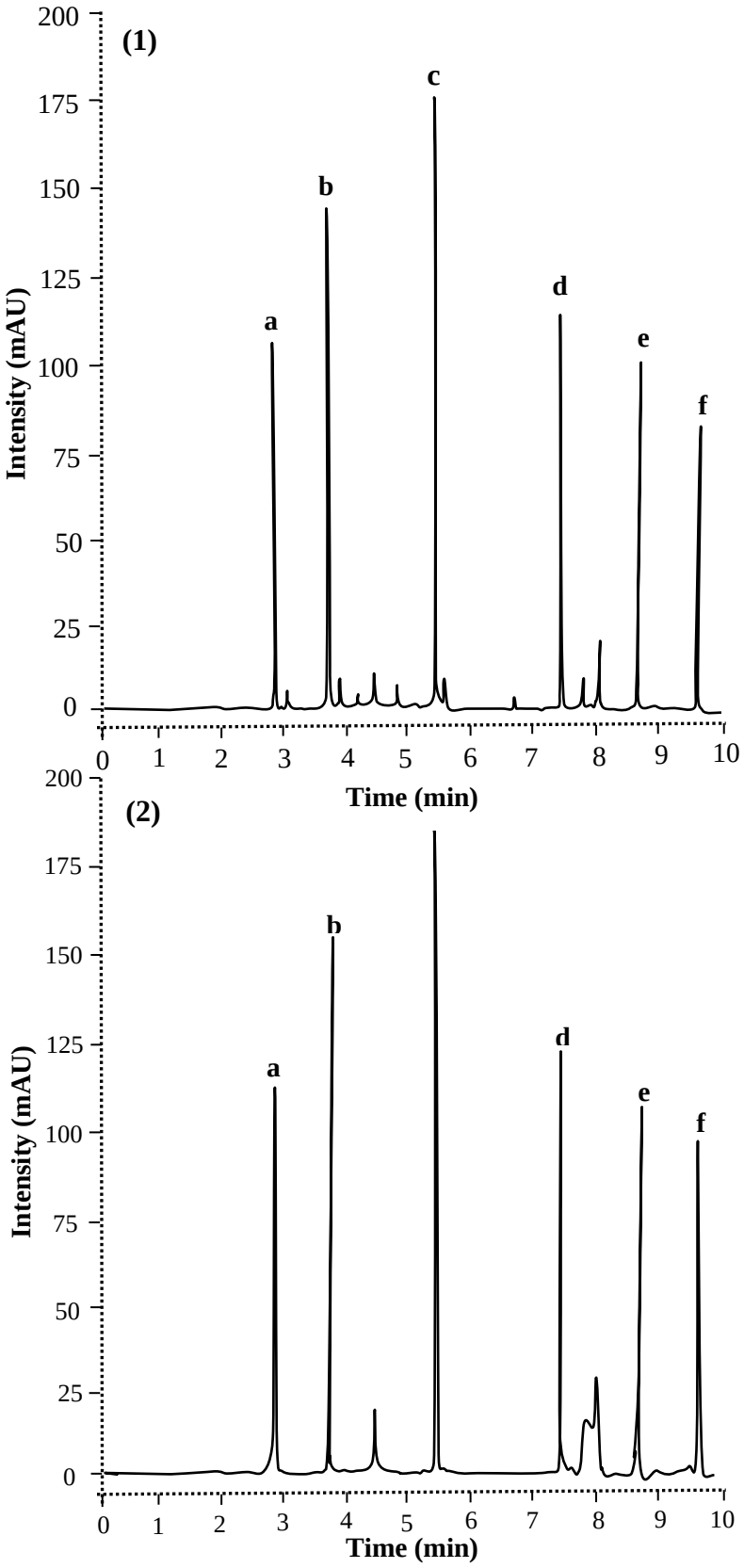
AppendixB5. GC-MS chromatograms (1) Mmultistandard solutions (10 mg/L); (2) Blank meat samples. **a**. Hexachlorobenzene; **b**. Hexachlorocyclohexane; **c**, Fenitrothion; **d**. Chlorpyrifos; **e**. Cypermethrin; **f**. Deltamethrin.



Appendix B6. GC-MS chromatograms (1) Spiked meat samples before extraction (0.1 mg/kg); (2) Spiked blank meat samples after extraction (0.1 mg/kg). **a**. Hexachlorobenzene; **b**. α-hexachlorocyclohexane; **c**, Fenitrothion; **d**. Chlorpyrifos; **e**. Cypermethrin; **f**. Deltamethrin



Appendix B7. GC-MS chromatograms (1) A multistandard solutions (10 mg/L); (2) Blank fat samples. **a**. Hexachlorobenzene; **b**. α-hexachlorocyclohexane; **c**, Fenitrothion; **d**. Chlorpyrifos; **e**. Cypermethrin; **f**. Deltamethrin



Appendix B8. GC-MS chromatograms (1) Spiked blank fat samples before extraction (0.1 mg/kg); (2) Spiked blank fat samples after extraction (0.1 mg/kg). **a**. Hexachlorobenzene; **b**. α-hexachlorocyclohexane; **c**, Fenitrothion; **d**. Chlorpyrifos; **e**. Cypermethrin; **f**. Deltamethrin

BIODATA OF THE STUDENT

Ahmad Yaseen Hamadamin is an assistant lecturer at the College of Veterinary Medicine, University of Sulaimani, Iraq. He was born in Erbil capital city of Kurdistan Region. He got a Bachelor degree in Veterinary Medicine and Surgery / University of Sulaimani, in 2010, and passed as excellence grade and first over the rank of 43 students.

He got scholarship in 2011, moved to United Kingdom and started his academic pre-sessional courses for 9 months in the same year, and finished the pre-sessional academic courses as a first grade over the rank of 15 students. He started his MSc on the title of "Nutrition and Food Sciences" in 2012 at School of Applied Sciences / University of Huddersfield-United Kingdom. In his MSc project, he developed a new methodology for extraction and cleanup proteins and compounds in products and analysis by Gel electrophoresis and Liquid chromatography. He got "distinction" and passed the MSc with first grade over the rank of 13 students in 7 different countries.

Prior his Ph.D, he published three journal articles about extraction and quantification of steroid hormones residue in meat, mycotoxins residue in chicken meat, and mycotoxin residue in milk. He obtained essential expericnes about extraction of contaminants residues in matrices and qauntification with chromatography.

Ahmed started his Ph.D. in 2016 and his research novelty is about development of QuEChERS method to extract pesticides in fatty and high fat matrices such as meat and fat, as well as effect of heat treatment on pesticide dissipation. During his Ph.D. study, he attended in several workshops and conferences and published two journal articles.

LIST OF PUBLICATIONS

Ahmad, Y. and Khulod, I., 2020. Development and methods of validation for measurement of pesticides in muscle tissue using gas chromatography based modified analytical QuEChERS approach. *Applied Ecology and Environmental Research*, 18 (1): .275-288.

Ahmad,Y. and Khulod,.I., 2020. Gas chromatography–mass spectrometry based sensitive analytical approach to detect and quantify non-polar pesticides accumulated in the fat tissues of domestic animals. *Saudi Journal of Biological Sciences*, 27(3): 887-893.

تقييم مخلفات المبيدات الحشريه فى بعض الأنسجة الحيوانية في محافظة السليمانية

اطروحة مقدمة إلى مجلس كلية الطب البيطرى في جامعة السليمانية كجزء من متطلبات ليل شهادة الدكتوراة في الطب البيطرى/ Meat inspection and hygiene

من قبل

أحمد ياسين حمد أمين

بـإشـــراف

الاستاذ المساعد الدكتورة خلود أبراهيم حسن

٢٠٢٠ م

الخلاصة

ان وجود مميزات وفوائد متعددة لبعض مركبات المبيدات وسعت كثيرا من نطاق ستخدامها في الزراعة والثروة الحيوانية مما ادى الى زيادة فرص تراكم تلك المركبات في الأنسجة الحيوانية والمنتجات الزراعية وبالتالي زيادة تاثيرها السلبي في صحة الانسان .وبانسبة لبعض المنتوجات الحيوانية كاللحوم فان تقييم مخاطر المبيدات الزراعية على صحة الانسان تعتمد على نسب وتركيزتلك المبيدات في اللحوم وان ادى بعض عمليات الطهي الى تقليل تلك النسب.ان عملية الكشف النوعي والكمي لبقايا المبيدات في بعض المواد الغذائية المعقدة كاللحوم والدهون يتطلب إجراء خطوات متعددة، وينبغي التحقق من تلك الخطوات وتقييمها استنادا على لتوجيهات منظمة المفوضية الأوروبية لمراقبة جودة المواد الغذائية وتحليل الاغذية.

وقد تم تصميم هذا المشروع لتطوير طريقة للكشف المتعدد والتقدير الكمي لستة مبيدات مستخدمة للاغراض الزراعية على نطاق واسع في السليمانية وذلك في اللحوم والأنسجة الدهنية للأبقار والأغنام والماعز .تم جمع عينات من اللحوم والدهون وذلك من مجازر اللحوم في خمس مناطق مختلفة في السليمانية وهي دربندخان، سيد صادق، ءربت، بازيان، وپيرەمگرون.

تم إجراء التحليل اعتمادا على استخلاص بقايا المبيدات من عينات اللحم والدهن باستخدام طريقة ال QuEChERSالمحورة ، بما في ذلك liquid-liquid partitioning(LLP) و dispersive solid (d-SPE) phase extraction. تم إجراء الكشف باستخدام كرموتوغرافيا السائل ذات الأداء العالي (performance liquid High chromatography) مقرونة بكاشف الاشعه فوق البنفسجية (HPLC-UV) ، و كرموتوغرافيا الغاز (chromatography Gas) مقترنة بكاشف طيف الكتلة Mass spectrophotometer (GC – MS).

في هذه الدراسة ، تم اختبار تأثير سلق (100 درجة مئوية ، لمدة 30دقيقة) والشوي (176 درجة مئوية ،لمدة 20 دقيقة) على مستوى المبيدات في عينات اللحوم والدهون ، وتمت مقارنة النتائج إحصائياً. تمت مقارنة أداء كرموتوغرافيا السائل ذات الأداء العالي(HPLC-UV) و كرموتوغرافيا الغاز(GC-MS).

اظهرت نتائج تقييم اداء تقانتي الاداء (HPLC و GC) المستخدمة في هذه الدراسة الحصول على قيم الاسترداد(recoveries values) المقبول لجميع المبيدات المستخدمة في هذه الدراسة وبجميع مستويات الاضافة(0.01 إلى 0.1 ملغم / كغم) من المبيد لعينات المقارنه (blank samples) في اللحوم والدهون. ففي تحليل ال HPLC ، بلغت

معامل الارتباط (r2) ≤ 0.9998 لعينات اللحوم والدهون. وتراوحت قيم اقل حدود الكشف للتقانة (Limits of detection) من 0.003 إلى 0.013 ومن 0.003 إلى 0.016 ملغم / كغم لعينات اللحوم والدهون على التوالي، في حين تراوحت قيم اقل حدود للتقديرالكمي(limits of quantification) من 0.011 إلى 0.039 ومن 0.010 إلى 0.048 ملغم / كغم لعينات اللحوم والدهون على التوالي . تراوحت قيم الاسترداد(recoveries value) لمستويات المبيد الدستخدمة في هذه الدراسة بهذه التقانة لجميع المستويات بين 78.08 إلى 101 ٪ و 77 إلى 106 ٪ لعينات اللحوم والدهون على التوالي مع الانحراف المعياري النسبي (RSD) من 0.5 إلى 15 ٪ للحوم و 0.23 إلى 12.9 ٪ لعينات الدهون.

في تحليل كرموتوغرافيا الغاز(GC) تم تقييم استجابة التقانة لتقدير كمية المبيدات المدروسة من خلال معامل الارتباط (r2) التي بلغ ≤ 0.9997 لعينات اللحوم والدهون . تراوحت وتراوحت قيم اقل حدود الكشف للتقانة (Limits of detection) و قيم اقل حدود للتقديرالكمي(limits of quantification) لها من 0.004 إلى 0.014 ومن 0.012 إلى 0.043 ملغم / كغم للحوم ومن 0.0052 إلى 0.014 ومن 0.015 إلى 0.044 ملغم / كغم لعينات الدهون على التوالي. اما قيم الاسترداد(recoveries value) لمستويات المبيد الدستخدمة في هذه الدراسة بهذه التقانة فتراوحت بين 79.2 إلى 104.3٪ وبمعدل انحراف معياري نسبي(RSD) مقبول تراوح من 0.32 إلى 14.6٪ لعينات اللحوم. وبالمثل ، تراوحت قيم الاسترداد(recoveries value) لمستويات المبيد الدستخدمة في هذه الدراسة بهذه التقانة من 81.5 إلى98.6٪ بالنسبة لعينات الدهون ، وبمعدل انحراف معياري نسبي مقبول(RSD) من 0.3 إلى 9.3٪.

تم تطبيق الطريقة المطورة في هذه الدراسة بنجاح على 300 عينة من اللحوم والدهون. وكان المبيد الاكثر تركيزا في عينات لحوم ودهون الأبقار هو ال hexachlorobenzene، بينما كان المبيد deltamethrrin الأكثر تركيزا في عينات لحوم ودهون الأغنام والماعز.وبالمقارنة بين محتوى عينات اللحوم والدهون للمبيدات المدروسة , كانت جميع تركيزات بقايا المبيدات أعلى في عينات الدهن من الأبقار والأغنام والماعز مقارنة بعينات اللحوم من نفس الحيوانات.

وبالمقارنة بين مناطق السليمانية في محتوى اللحوم من المبيدات لم تكن هناك فروق معنوية بين المناطق ولكن تم الحصول على اعلى مستويات بقايا المبيدات المدروسة في عينات لحوم ودهون الحيوانات الماخوذة من بازيان و پيرەمەگرون، في حين أن أدنى مستويات بقايا المبيدات المدروسة وجدت في العينات الماخوذة في دربنديخان.

وبالنسبة لتاثير المعاملات الحرارية في تقليل تراكيز المبيدات المدروسه في عينات اللحوم والدهون ، كان هناك فروق معنوية بين المعاملتين الحراريتين اذ تفوق تأثير الغليان (100 درجة مئوية ، لمدة 30دقيقة) بشكل كبير ($p<0.05$)

،في تقليل مستويات المبيدات المدروسة في حين أظهروالشوي (176 درجة مئوية ،لمدة 20 دقيقة) أقل فعالية في تقليل تلك المستويات كما لوحظ أعلى النسب المئوية لتخفيض مستوى المبيد وفي كلا المعاملتين الحراريتين كان لل pyrethroids، في حين أن أدنى النسب المئوية للتخفيض كان لمبيدات ال organophosphorus.

بالمقارنة بين تحليل HPLC و GC ، إحصائيًا ، لم تكن هناك فروق ذات دلالة بين تركيزات مبيدات الآفات، الموجودة في HPLC و GC لكل من عينات اللحوم والدهون ؛ ومع ذلك ، قدم HPLC حساسة أعلى قليلاً من GC.

هەڵسەنگاندنی جێماوە قڕکەرەکان لە شانە ئاژەڵیەکانی پارێزگای سلێمانی

ئەم نامەیە پێشکەشە بە کۆلیجی پزیشکیی ڤێتێرنەری زانکۆی سلێمانی وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی بڕوانامەی دکتۆرا لە بواری پزیشکی ڤێتێرنەری/ Meat inspection and Hygiene

لە لایەن

أحمد یاسین حمد أمین

بە سەرپەرشتی

پرۆفیسۆری یاریدەدەر د. خلود أبراهیم حسن

٢٧٢٠ک ٢٠٢٠ز

پوختە

تایبەتمەندیە شەبەنگی و بەربڵاوەکانی هەندی لە مادە مێروو کوژەکان(قڕکەرەکان)، وا یلێکردوون کە بەشێوەیەکی زۆر بەکاربهێندرێن لە بواری کشتوکاڵ و ئاژەڵداری. ئەمەش بووەتە هۆی زیادبوونی ئەگەری مانەوەی ئەم ماددانە لە شانەکانی لەشی ئاژەڵ و بەرهەمەکانیان، وە کاریگەری نەرێنی لەسەر تەندروستی مرۆڤ جێبێڵێت. لە بارەی هەڵسەنگاندنی کاریگەریەخراپەکانی ئەم مێروو کوژانە لە بەهەمە ئاژەڵیەکانی وەک گۆشت، لەسەر بنەمای هەڵسەنگاندنیانە لە ناوگۆشتەکە پێش کوڵاندنیان، هەرچەندە ڕێژەی زۆری گۆشت و بەرهەمەکانی دەکوڵێنرێن یان پرۆسێس دەکرێن پێش خواردنیان. دۆزینەوە و دیاریکردنی بڕی کۆمەڵێک مێروو کوژی هەمە جەشنە لە شانە ئاڵۆزەکانی وەک گۆشت و چەوریە شانەکان، پێویستی بە کۆمەڵە هانگاویک هەیە، وە ئەم هەنگاوانەش پێویستە سەلماندن یاخود ڕاستاندنیان بۆ بکرێت بەگوێرەی ڕێنماییەکانی کۆمەڵگەی ئەوروپی بۆ شیکاری کۆنترۆڵی جۆری و شیکردنەوەی خواردن و بەرهەمە خۆراکیەکان.

ئەم پرۆژەزانستیە دیزاین کرا بۆ بەرەو پێش بردنی هەنگاوکانی ڕێگایەک بۆ دۆزینەوە و دیاری کردنی ڕێژەی چەندێتی شەش جۆری لە مێروو کوژی بەکارهێنراو لە شانە ماسولکەییی و چەوریەکانی لەشی مانگا ومە ڕ و بزن. نموونەی شانە ماسولکەیی و چەوریەکان لە سەربڕخانەی نوێی سلێمانی کۆکرایەوە، لەلاشەی ئەو ئاژەڵانەی کە بەخێوکرابوون لە ناوجەکانی وەک دەربەندیخان، سەید سادق، عەربەت، بازیان، و پیرەمەگرون.

شیکردنەوەکە ئەنجام درا پشت بەست بە ڕێگەیەکی پێشکەوتووی QuEChESRS، کەتییایدا هەنگاوەکانی liquid-liquid partitioning (LLP) and dispersive solid-phase extraction (d-SPE) بەرجەستە کرابوو. دیاری کردنەکان ئەنجام درا بەهۆی بەکارهێنانی ئامێری High performance liquid chromatography (HPLC) بەستراو بە Ultraviolet detector (UV)، وە Gas Chromatography (GC) بەستراو بە Mass spectrometry(MS).

هەر لەم پرۆژە زانستیەدا، کاریگەری کولاندن لە پلەی گەرمی ١٠٠ سیلیزی بۆماوەی ٣٠ خولەک، هەروەها برژاندن لە پلەی گەرمی ١٧٦سیلیزی بۆماوەی ٢٠ خولەک تاقیکرایەوە بۆ زانینی ڕێژەی کەمبوونەوەی مێروو کوژەکان لەناو نموونەی ماسوولکە و چەوریە شانەکان. لە کۆتاییدا، توانای کارکردنی هەریەک لە ئامیری HPLC-UV and GC-MS بەراوورد کران بە شێوەیەکی ئاماری.

لە شێکردنەوەکان، کە ئەنجام درابوو بەهۆی ئامێیریHPLC-UV و GC-MS، ڕێژەی گە ڕاوەیی گونجاو بەدەستهات لەسەر ئاستی خەستی 0.01 تا 0.1 مگم/کگم، بۆ چوار ئاستی جیاواز لە زیاد کردنی مێروو کووژ بۆ شانە ماسولکەیی و چەرییەکان. لە شیکردنەوەکانی کە ئەنجام درابوو بە ئامێری HPLC، کاردانەوەی ڕاستێنراو بەدەستهات لە هاوکۆلکەی پەیوەندی (r^2) ≤ 0.9998 بۆ نموونەی گۆشت و چەوریەکان. بەهای سنووری دیاریکردنەکان بۆ هەموو مێروو کوژەکان لە مەودای 0.003 تا 0.013 ، وە 0.003 تا 0.016 مگم/کگ بوو، بۆ نموونەی گۆشت و چەورییەکان یەک بەدوای یەک. بەهای سنووری پێوانە کردنی بڕەکان لە مەودای 0.011 تا 0.039 , وە 0.010 تا 0.048 بوو، بۆنموونەی گۆشت و چەوریەکان، یەک بە دوای یەک. بەهای گە ڕاوەیی مێرووکوژە زیاد کراوەکان کە بەدەستهات کەوتە مەودای % 78.08 تا 101، وە % 77 تا 106 بۆ نمونەی گۆشت و چەوریەکان، یەک بەدوای یەک، هەروەها لادانە پێوانەییەکان کەوتە مەودای % 0.5 تا 15 بۆ نمونەی گۆشتەکان، وە % 0.23 تا 12.9 بۆ نمونەی چەوریەکان.

لە شیکردنەوەکانی کە ئەنجام دربوو بە ئامێری GC-MS، کاردانەوەی ڕاستێنراو بەدەستهات لە هاوکۆلکەی پەیوەندی (r^2) ≤ 0.9997 بۆ نموونەی گۆشت و چەوریەکان. بەهای سنووری دۆزینەوە و پێوانە کردنەکان لەمەودای 0.004 تا 0.014 وە 0.012 تا 0.043 مگم/کگم بوو، بۆ نموونەی گۆشتەکان، هەروەها مەودای 0.0052 تا 0.014، وە 0.015 تا 0.044 مگم/کگم بۆ نموونەی چەوریەکان. بەهەمان شێوە، بەهای گە ڕاوەیی مێروو کووژە زیاد کراوەکان کە بەدەستهات، کەوتە مەودای 79.2% تا 104.3 لەگەڵ لادانە پێوانەیی % 0.32 تا 14.6 بۆ نمونەی گۆشتەکان. هەروەها، بەهای گە ڕاوەیی مێروو کوژە زیاد کراوەکان کە بەدەستهات، کەوتە مەودای % 81.5 تا 98.6، لەگەل لادانە پێوانەیی % 0.3 تا 9.3 بۆ نموونەی جەوریەکان.

ئەو ڕێگەیەی پەرەی پێ سەندرا لەم پرۆژە زانستیە، توانرا بەکاربهێندرێت سەرکەوتوانە بۆ ٣٠٠ نموونەی گۆشت و چەوری . بەرزترین ڕێژەی مێروو کوژی دۆزراوە لەنموونەی شانە ماسولکەیی و چەوریەکانی مانگا، بریتی بوو لە hexachlorobenzene، کەچی لە نموونەی شانە ماسولکەیی و چەوریەکانی مە ڕو بزنەکان بریتی بوو لە deltamethrin. بە بەراوورد لە نێوان ماسوولکە و جەوریە شانەکان، هەموو ئەو مێروو کوژانەی دۆزرانەوە لە نموونەی چەوریەکانی مانگا و مە ڕ و بزن، ڕێژەکانیان زیاتر بووبە بەراوورد بەو ڕێژا نەی کە دۆزرانەوە لە نمونەی گۆشتەکان لەهەمان جۆری ئازەڵەکان.

لەبارەی جیاوازی ڕێژەی مێروو کوژە دۆزراوەکان بە گوێرەی ناوچەکان، بەرزترین ڕێژەی مێروو کوژ کە دۆزرایەوە، لە شانەکانی ئەو ئاژەڵانە بوو کە لە ناحیەی بازیان وپیرەمەگروون بەخێوکرابوون، بەڵام نزمترین ڕێژەی مێروو کوژ کە دۆزرایەوە لە لەو ئاژەڵانەی کە بەخێو کرابوون لە قەزای دەربەندیخان.

لەبارەی کاریگەری پرۆسەی گەرم کردنەکە لەسەر نموونەی گۆشت و چەوریەکان، پرۆسەی کولاندن توانی ڕێژەی مێروو کوژەکان کەم بکاتەوە بەشێوەیەکی بەرچاو ($P< 0.05$)، کەچی توانای پرۆسەی برژاندن بۆ کەم کردنەوەی مێروو کوژەکان زۆر کەم تر بوو بە بەراوورد بە پرۆسەی کوڵاندن .لە هەرردوو پرۆسەی کوڵاندن و برژاندنەکە، بەرزترین ڕێژەی کەم کردنی مێروو کوژەکان تێبینی کرا لە گرووپی **pyrethroids**، کەچی نزمترین ڕێژەی کەمبوونەوە تێبینی کرا لە گرووپی **organophosphor**us.

بە بەراوورد لە نێوان ئەنجامی شیکردنەوەکان بە هەردوو ئامێری HPLC و GC، بە شێوەیەکی ئاماری جیاوازی نەبوو لە نێوان ڕێژەی مێروو کوژە دۆزراوەکان بە ڕێگەی HPLC و GC بۆ هەردوو نموونەی گۆشت وچەوریە شانەکان، هەرچەندە ڕاددەی هەستیاری ئامێری HPLC کەمێك باڵا تر بوو لە GC.