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Detection of Antiparasite Residues in Meat and Edible parts of Sheep in Karbala Province

A Thesis Submitted to the Council of the Faculty of Veterinary Medicine / University of Kerbala in Partial Fulfillment of the Requirement for the Master Degree of Science in Veterinary Medicine /Veterinary Public Health.

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بسم الله الرحمن الرحيم

شَهِدَ ٱللَّهُ أَنَّهُۥ لَآ إِلَٰهَ إِلَّا هُوَ وَٱلۡمَلَٰٓئِكَةُ وَأُوْلُواْ
ٱلۡعِلۡمِ قَآئِمَۢا بِٱلۡقِسۡطِۚ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡعَزِيزُ ٱلۡحَكِيمُ

صدق الله العظيم

[آل عمران: ١٨]

Supervisor certificate

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Dedication

Creator of the soul and the pen, Creator of the atom and the breeze and Creator of all things from nothing, to the one who conveyed the message and fulfilled the mission ... to the Prophet of Mercy and the Light of the worlds, to the pure masters and their mission. The most reliable support ... the people of the House of Prophecy.

For the desire of my heart and closer to my soul, hidden from sight and peeping in the eye of discernment, to the rest of God, the Almighty ... Owner of Age and Time (God Almighty eagerly awaits his appearance)

To the one who taught me that the world is a war... and that its weapon is knowledge. To the one who didn't stop me from doing anything. To those who fought for my comfort and success. The greatest and most loved person in the universe... my dear father.

To this very dear pure-hearted woman, to whom the Merciful One advised me to show justice and goodness, to whom she fought and suffered for me, to whom her prayers were the secret of my success... dear mother.

To our beloved supervisors, Dr. Kadhim Saleh Kadhim and Dr. Ali Redha Abid, thank you for the dedication and sincerity you have shown in every phase of our work. You have had a positive impact on our journey through your wisdom and sound advice.

To those who share my moments... to those who rejoice in my success as theirs... to my brothers and friends, with all my love, I offer you my humble effort.

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Summary

By using precise methods to detect the smaller levels of these residues. Antiparasitic residue study was performed on 125 samples (50 samples of sheep meat and 75 samples of edible parts) collected from different areas of the butcher shops. This study aims to determine the residues of anti-parasitic drugs (ivermectin and cypermethrin) in meat and edible parts (liver, kidneys and tail fat) of sheep in five different areas in Karbala Province / Iraq, by using high-performance liquid chromatography (HPLC) technique. From October 2023 to January 2024.

The analysis showed that 96% of the meat and 95% of the edible parts were contaminated above the maximum residue limits with cypermethrin, as for the ivermectin residue, 90% in all samples was (meat and edible parts) positive for ivermectin (IVM) residue in samples. Statistical analysis of the data found there are significant differences ($P<0.05$) for antiparasitic agents in sheep meat and its products. The effect rate of cypermethrin was higher than that of ivermectin, as the rate of cypermethrin was (2.66) and ivermectin was (0.63).

As for the edible parts, the contamination rate of ivermectin was higher than that of cypermethrin in the liver at a rate of (1.49); cyperemthrin was (0.96), and in the kidneys the percentage of contamination with cypermethrin was higher at a rate of (1.99), and ivermectin was (1.76) , and ivermectin was (1.29) . Likewise, for tail fat, the contamination with cypermethrin was higher at a rate of (2.64). The results showed that the samples were more contaminated with cypermethrin than ivermectin. Pesticide residue concentrations were in the order: Meat > tail fat > kidneys > Liver. As for the percentage of impact on the region, the percentage of residues of both cypermethrin and ivermectin in sheep meat according to each region was as the following: Al-Husseinah (0.56), Al-Hur (1.43), Tauweraij (3.00), Center (0.64), and Aeen tumer (2.57), as it was found that the Tauweraij area has higher pollution compared to other areas. As for tail fat, the percentages were Al-Hussaina (0.39), AL Hur (1.06), Tauweraij (1.12), center (2.92), Aeen tumer (4.31), where the area of Aeen Al-Tumer was the highest in pollution. The levels of contamination in the liver were as following: Al-Hussaina (2.75), AL Hur (2.12), Tauweraij (0.35), center (0.62), Aeen tumer (0.29) the Al-Hussaina area had the highest pollution. As for the kidneys, it was as following: Al-Hussaina (3.74), AL Hur (0.27), Tauweraij (0.50), center (3.89), Aeen tumer (0.98) it was found that the city center has the highest pollution.

As for contamination of samples with cypermethrin, the contamination rates in meat were according to each region, (0.85, 1.82, 5.17, 1.04 and 2.40), and tail fat was as following:(0.46, 0.25, 0.42, 2.27 and 3.78), liver, (3.62, 0.50, 0.19, 0.12 and 0.36) and kidneys: (3.97, 0.17, 0.18, 5.35 and 0.28). Contamination of samples with ivermectin the contamination rates in meat were according to each region (0.26, 1.05, 0.82, 0.25 and 0.75) and tail fat (0.31, 1.87, 1.83, 0.58 and 1.85), liver (1.87, 3.74, 0.52, 1.13 and 0.21) and kidney: (3.51, 0.37, 0.82, 2.44 and 1.68).

According to the maximum limits of residues allowed before the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) showed that the residues of antiparasitic agents (cypermethrin and ivermectin) in sheep meat and its products were higher than the maximum limits for residues. This indicates the incorrect use of parasiticides during use, as well as failure to follow the veterinarian’s instructions.

List of Contents

List of Tables

Table of Figures

List of abbreviations

Abbreviation	Meaning
ACN	Acetonitrile
ADI	Acceptable daily intake
CFHF	Crimean-Congo hemorrhagic fever
CNS	Central nervous system
CYP	Cypermethrin
DLM	Deltamethrin
DW	Deionized water
EC	European Commission

FAO	Food and Agriculture Organization
GABA	gamma-aminobutyric acid
GMPs	Good Manufacturing Practices
GVP	Good Veterinary Practices
HPLC	High Performance Liquid Chromatography
IVM	Ivermectin
MRLs	Maximum residue limits
OC	organochlorine
OP	organophosphates
PCV	packed cell volume
PNS	peripheral nervous system
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
SLE	solid-liquid extraction
TCA	Trichloro acetic acid
Vd	volumes of distribution
WHO	World Health Organization
WP	withdrawal Period

Chapter one

Introduction

1.1. Introduction

Hemorrhagic fever has spread in Iraq. It is a contagious viral disease that affects both humans and animals, spreading rapidly between them. The transmission of the disease depends on the presence of the vector, which is typically ticks (Bell-Sakyi *et al*., 2012). This disease is transmitted to humans through tick bites or through direct contact with the blood or tissue of a viral animal (Whitehouse, 2004). Ectoparasites, including lice, ticks, and mange, have been reported to cause a variety of health problems such as mechanical damage to tissues due to reduced quality of wool, meat, and milk production, resulting in serious economic losses to farms (Bisha *et al*., 2014).

The control of ectoparasites is therefore of great importance due to their impact on the profitability of livestock and the health status of the animals. Chemicals used to control ectoparasites can be classified according to the parasites they control (Tiensin, 2016). Pesticides are used as animal sterilization to combat ectoparasites (Casida, 2009). Many management strategies are used to prevent or reduce production losses; however, the use of antiparasitic drugs remains the main tool available against parasitic diseases in animals (Vercruysse and Claerebout, 2001). The most commonly used veterinary drugs are antiparasitic (ivermectin and cypermethrin).

Widespread use of these antiparasitic medications may pose a potential risk to the consumer if the residues enter the food chain. Thus, controlling its residues in meat and others animal by-products used for human consumption are important task. National and international public health agencies around the world are deeply concerned about the presence of drug residues in the edible meat and intestines of food-producing animals (Wang *et al*., 2011).

Drug residues remaining in edible tissue may enter the human diet as a result of the farmer's negligence in observing the discontinuation period for the drug and/or incorrect application of the drugs to the animal (Tajick and Shohreh, 2006). The possibility and potential for drug residues to remain in the edible product human health problems associated with exposure to these wastes are a source of concern for public safety (Foote, 2000). Many residual veterinary drugs may accumulate in human organs for a long time, and if it exceeds a certain range, they will show clear clinical symptoms (Li *et al*., 2020).

To reduce the side effects of this residue of veterinary medicines on human health pharmacologically active ingredients sorted with respect to Maximum residue limits (MRLs) in animal products the source is mapped and monitored. Hence it is necessary to identify concentrations of these residues in animal-derived foods, including meat (Chicoine *et al*., 2020).

Rationally, no product comes from a processed animal, it is consumed unless all of the medication is disposed off. This concept is called zero tolerance, which is equivalent in reality the idea of the complete absence of residual quantities. However, because improved analytical techniques, which means that value the zero became smaller and smaller, depicting the corresponding boundaries for sensitivities parts per million (ppm), parts per billion (ppb) and parts per trillion (ppt). As a result, using high effectiveness analytical methods, for example, uses a high-performance liquid chromatography. We can conclude that there is almost always detectable residues, but these residues are at a very low level concentration and it is not inevitably toxic (Rico, 1985).

1.2. Aims of Study

1- Residual percentage of anti-parasitic in meat and edible parts at karbala province.

2- Sutiablitiy of meat and ediple parts for human consumption

3- Application of HPLC technology to detect antiparasitic drugs in animal products.

Chapter Two

Review of the Related literature

2. Review of the related literature

2.1. The importance of mutton

Sheep farming is an important area of the economy, as it provides valuable products extracted from sheep, including products that use natural resources that humans rarely have access to. Incorporating sheep into a farming operation can contribute to economic and environmental sustainability of the entire farm. Relatively a small investment is required, and the size of the herd increases gradually sheep production is a good option for small or part-time farmers. For the established farmer seeking diversification, sheep offer a number of benefits (Petrović *et al.*, 2011).

Sheep are one of the major farm animals raised in Iraq (6,633,904 heads) (Faostat, 2017). That it the main source of livestock, and a major source of meat production, secondary source of milk and wool. Meat Produced from sheep is the main quality and desirable for Iraqi consumption compared to the meat of other animals or even imported live and frozen meat (Hermiz and Alkass, 2018). Sheep are found in intense and the production systems are large scale and from cold to hot environments, the exact line of descent between local sheep and their wild ancestors are unclear (Hiendleder *et al.*, 2002). Sheep play an important economic role and contribute significantly to local and export markets by providing food (meat, milk) and non-food products, manure, hides and wool (Duguma *et al.*,2011).

Although sheep play an important role in the country's economy, today the benefits of these livestock animals are still affected by various restrictions. Animal diseases are considered one of the crucial technical obstacles impeding trade in animals and animal products (Grasso *et al*., 2014).

However, an important characteristic of sheep is that they can live and produce on land that is unfavorable to other forms of agriculture. Numerous single- and dual-purpose sheep breeds have been developed that can achieve high production levels in appropriate environments and management systems. The use of sheep varies in many countries, but in many other countries sheep produce more than one product. Animals are slaughtered primarily for their meat. Therefore, meat represents the main product, while all other ovals become by-products (Morris, 2017).

These by-products are divided into edible and non-edible parts. By-products account for approximately 60 to 70% of slaughtered carcasses, of which approximately 40% are edible and 20% are inedible (Bhaskar *et al*., 2007). The use of by-products can also make the meat industry more economical. Some by-products of sheep slaughter are also considered edible in most developing countries from their use as sausage casings in developed countries (Khan, 2021).

On average, proteins associated with meat industry by-products account for more than one-eighth total protein in lean meat (Webster *et al*., 1982). Of course, the nutritional composition depends on each specific type the byproduct and the animal species from which it is obtained (Honikel, 2011). Meat and meat products are a class of food products that are commonly included in the human diet. Due to the intake of good quality nutrients and diverse presentation formats, they are greatly appreciated sensory properties (Grasso *et al*., 2014).

2.2. The importance of meat and meat products in human nutrition

Many societies have paid attention to the consumer's desire, demands, and taste for eating red meat, since the majority of consumers in these countries consider sheep meat to be rich in fat (Kempster *et al*., 1982). Meat is one of the most important and preferred nutrients available, which helps meet the greatest possible body requirements. Meat is an important source of high-quality protein, minerals, vitamins and micronutrients needed for good health throughout life (Arshad, 2018). Red meat is also a rich source of protein and other nutrients that are important for a balanced diet. (Abbassian *et al*., 2018).

Animal by-products are the parts (other than the carcass) released from a slaughtered animal after slaughter and may be edible or inedible depending on their use as food. Edible by-products are the products that humans can consume as food (Marti *et al*., 2011). By-products are generally more microbially perishable than muscle tissue because they have higher glycogen content and require additional handling in recovery. Therefore, edible by-products on the harvest floor should be removed, trimmed, washed, chilled drained and quickly cooled after slaughter, handled hygienically and cooked as quickly as possible (Ockerman and Basu, 2014).

Another classification of animal by-products are primary and secondary by-products (Sharma and Sharma, 2011). The nutritional value of edible animal by-products is of impressive quality. In some cases, the nutritional value of by- products is greater than that of meat and contains more amino acids, vitamins, minerals, fatty acids, etc. The carbohydrate content is higher in some offal, such as liver and kidneys, than in solid meat (Devatkal *et al*., 2004). The legs, tail and liver of animals contain a protein content close to that of lean meat (Unsal and Aktas, 2003).

2.3. Nutritional importance of edible parts

An animal byproduct can be defined as each part of the slaughtered animals, except for the prepared slaughtered animal (also it is called the offal or fifth quarter). Edible by-products are those products that can be consumed by humans as food organisms generally include the liver, kidneys, heart, and brain. Intestines, tongue, spleen, etc. They are also called various meats Edible meat by-products contain essential nutrients. Some by-products of meat are often edible they are used as medical treatments because they contain special substances nutrients such as amino acids, hormones, minerals, vitamins, or fatty acids (Irshad and Sharma, 2015).

Liver is perhaps the most consumed byproduct worldwide. Liver is a very good source of vitamins A, C, and D, B vitamins, and nutrients such as iron, zinc and copper (Ünsal and Aktaş, 2003). In fact, liver is used as a special source of vitamins B12 and A as well as iron. Liver can be vacuum packed to extend storage time and can also be stored frozen. However, this is not recommended, as it will become softer. Liver is the main ingredient in the preparation of pies and liver sausages. Other uses include chopping and cooking using various techniques or chopping and incorporating them into various dishes, baking, spreads, and sausages. Kidneys can be used as an ingredient in stews, casseroles, or meat pies (Marti *et al*., 2011).

Liver and kidneys contain a higher amount of riboflavin (1697-3630 mg/100 g), which is 5 to 7 times higher than lean meat. Niacin, vitamin B6, B12, A, and folacin are found primarily in the liver of animals. Kidneys are an excellent source of vitamin B complex. A good source of manganese (0.128 to 0.344 mg/100 g) is liver (Devatkal *et al*., 2004).

Sheep tail fat is an important production trait, as it is stored as an energy source in harsh conditions or when feed resources are scarce (Alizadeh *et al*., 2013). Tail fat is mainly composed of 79.1% fat, and is free of trans fatty acids, making it suitable for human consumption (Karabacak *et al*., 2014). Lamb tail fat also contains fat-soluble vitamins and conjugated linoleic acid, which is beneficial to health. The main fatty acids in tail fat are oleic acid (C18:1n-9c), hexadecanoic acid (C16:0), and stearic acid (C18:0). Lamb tail fat can be used in the food and confectionery industry due to its high nutritional value and energy (Kashan *et al*., 2005).

As a result of technological advances, the use of animals has come into play by-products as raw materials and auxiliary products different regions led to a significant increase in value final products. Today, there are more than twenty industrial branches animal by-products that have been used extensively (Scaria, 1989). The by-products of sheep and lamb can be utilized in four various fields, such as human food, animal feed, medicines, and industrial materials. The most important slaughter products for sheep and lamb and common ways to benefit from them (Ibarra, 1988). Offal from carcasses that is not used directly for human food is often processed materials used in animal food (Pearl, 2004).

Lamb and sheep offal are excellent sources of protein; minerals such as zinc, phosphorus, calcium and selenium; and B complex vitamins such as niacin, thiamine, and riboflavin. This red meat is lean and lower in cholesterol than other animal proteins. Lamb is also a good source of iron, providing twice as much iron as chicken or pork and six times as much iron as fish. Therefore, it is recommended as part of a healthy diet for young children and the elderly (Ukut *et al*., 2010).

2.4. Contamination of Sheep meat and edible by-products

Meat, including also meat products, is one of the major food groups in a variety of human diets. Its regular/frequent consumption represents an important protein contribution and a number of essential micronutrients (Fe, Zn, vitamin B12, etc.), as well as an important energy contribution (Quintavalla and Vicini, 2002). Despite its recognized benefits, sheep meat and offal can accumulate large amounts of toxic heavy metals, which can pose potential risks to human health (Abd-Elghany *et al*., 2020). By-products are generally more microbially perishable than muscle tissue because they have higher glycogen content and require additional handling in recovery.

Recently, public awareness of foodborne illnesses, particularly those related to meat and animal products, has increased significantly. Meat has been found to play an important role in the transmission of foodborne pathogens to people around the world. Foodborne illnesses in humans can be caused by microbial contamination of meat (Darwish *et al*., 2023). Therefore, continuous microbiological monitoring of carcasses by veterinary public health authorities is essential to prevent contamination and the occurrence of meat-related foodborne illnesses (Ncoko *et al*., 2020).

Bacterial spoilage of meat depends on the initial number of microorganisms, time/temperature combination of storage conditions and physicochemical properties of meat (Doulgeraki *et al*., 2010). Mostly, contamination occurs due to inadequate hygienic conditions and handling in slaughterhouses (Schlegelova *et al*., 2004), moreover the attachment properties and the biofilm formation of bacteria on surfaces facilitate cross-contamination (Koo *et al*., 2013). Many diseases are transmitted through food and have even resulted in death (Soepranianondo and Wardhana, 2019).

Likewise, Meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs (Hammer, 2004). Although healthy animal tissues are usually sterile, they are biodegradable contaminated during slaughtering, cutting, handling and transportation, and storage. As meat provides an ideal environment for its growth microorganisms being a rich source of nutrients, so meat is highiy affected by microbes and can cause food poisoning (Ukut *et al*., 2010).

A number of studies have reported outbreaks infections due to eating contaminated foods poor hygiene, in most cases, data is loose based on laboratory isolates that do not reflect the actual percentage of food-borne infections. However, a few community reports provide evidence of many of these cases outbreaks caused by *salmonella*, *shigella*, and *E. coli Listeria spps* in different parts of the world (Zweifer *et al*., 2008).

One of the most important diseases transmitted by ticks is hemorrhagic fever; Ticks are particularly suitable as vectors of pathogens, first because of their hematogenous behavior (including the relatively long duration of blood feeding), and secondly because of the great diversity of vertebrates that can be used as hosts (Estrada-Peña *et al*., 2015).Chemicals used to control ectoparasites can be classified according to the parasites they control (Taylor, 2001). Cypermethrin (CYP) is an active synthetic pesticide widely used in households, agricultural industrial fields and to control many insect pests (Farag *et al*., 2021).

2.5. Hemorrhagic fever and ticks

Crimean-Congo hemorrhagic fever (CFHF) is a zoonotic disease caused by a Nairovirus of the family Bunyaviridae and transmitted by ticks (Anagnostou and Papa 2009).This disease is transmitted to humans through tick bites or through direct contact with the blood or tissue of a viremic animal (Ergönül, 2006). Sheep are considered the most important native host of the virus in nature. Livestock and other hosts can transmit CCHFV to humans during the viremic period (Chinikar *et al*. 2008). Spreading of the disease depends on the presence of the vector; Ticks of the genus Hyalomma (Bell-Sakyi *et al*., 2012).

Ticks infect various vertebrates, including wild and domestic mammals, often without noticeable symptoms in animals. They serve as reservoirs for virus amplification, with the virus persisting in ticks throughout their lives and being transmitted to other ticks. Ticks are significant vectors for diseases affecting both humans and animals (Serretiello *et al*., 2020). Tick-borne diseases like anaplasmosis, babesiosis, and theileriosis pose a serious threat to livestock health, especially in tropical regions like Iraq (Bilgic *et al*., 2017), causing substantial economic losses. The recent spread of hemorrhagic fever in Iraq has led to increased pesticide use to control ticks, which also cause damage to livestock, reducing wool, meat, and milk production(Beyecha *et al*., 2014).

2.6. Pesticides

Pesticides are used as suppressants to control ectoparasites (FA *et al*., 2019). Pesticides are often used to protect animals from pests. However, it is important to remember that any pesticide should be considered an active poison. Although pesticides are developed through very strict regulatory processes to work with reasonable safety and to have animal effects on human health and the environment, serious concerns have been raised about health risks arising from occupational exposure and residues in food and drinking water. The use of pesticides has raised serious concerns not only because of the potential impact on human health, but also because of the impact on wildlife and delicate ecosystems (Jawale *et al*., 2017).

Pesticides must be effective, selective and safe. The benefits of pest control must outweigh the economic, health and environmental costs (Casida, 2009). In addition to their function, pesticides vary in structure and are sometimes grouped by chemical relationships. In addition, users often use several pesticides at the same time. These characteristics make designing and conducting epidemiological studies on their health effects difficult and costly (Schinasi and Leon, 2014).

Pesticides are divided into 4 main groups: organochlorine (OC), organophosphates (OP), carbamates and synthetic pyrethroids (SYP). The application of veterinary pesticides, including synthetic pyrethroids help maintain animal health by reducing parasitic burdens (Woodward, 2012). While pesticides offer many advantages, it is necessary to consider the potential risks associated with their misuse, including their toxicity to animals and humans. Responsible application and monitoring are critical to mitigate these risks and ensure the safety of treated animals (Saif *et al*., 2020).

2.7. Synthetic pyrethroids

Synthetic pyrethroid insecticides are derived from naturally occurring compounds (pyrethrins) isolated from the plant genus Chrysanthemum (Kumar *et al*., 2010). Synthetic pyrethroid is one of the main classes of insecticides widely used in agriculture. Pyrethroids are preferred over organophosphates, carbamates and organochlorines because they have high efficiency, low toxicity and easy biodegradability (Sharaf *et al*., 2010). Due to their lipophilicity, pyrethroid insecticides favor absorption through the gastrointestinal and respiratory tracts and also confer preferential distribution in lipid-rich internal tissues (Fetoui *et al*., 2009).

Pyrethroids are synthetic organic insecticides that are divided into two different groups (Type I and Type II). Type I pyrethroids have no cyano moiety and type II pyrethroids have an alpha-cyano group. Deltamethrin (DLM), Cypermethrin (CYP) and lambda-cyhalothrin are synthetic forms of pyrethroid insecticides that are widely used worldwide (Kumar *et al*., 2011).

Sheep are affected by a variety of ectoparasites (scabies mites, flies, ticks and lice) and can be treated by “dipping,” which is immersion in a mixture of water and an insecticidal formulation. The control of ectoparasites is therefore of great importance due to their impact on the profitability of livestock and the health status of the animals. Especially when pathological mutationsoccur, ectoparasites transmit several diseases, such as hemorrhagic fever (Boucard *et al*., 2009).

2.7.1. Cypermethrin (CYP)

Cypermethrin was first manufactured in 1974, and began being marketed and used in 1977 as a highly active synthetic pyrethroid an insecticide effective against a wide range of pests in agriculture, public health and animal husbandry (WHO, 1989). Cypermethrin is the common name,for(±)Cyano3phenoxybenzyl(±)cis,trans3(2,2dichlorovinyl)2,2dimet hylcyclopropae carboxylate. The chemical structure of cypermethrin is shown in figure (2-1). Cypermethrin is a synthetic pyrethroid insecticide that has been used for several years against many pests, particularly butterflies, cockroaches and termites (Elbetieha *et al*., 2001). The family of natural pyrethrin comes from the flowers of the chrysanthemum; contains about 25% pyrethrin (Harlod *et al*., 2003).

Cypermethrin (CYP) (type II synthetic pyrethroid), lipophilic in nature, is considered less toxic due to its rapid insecticidal properties and low toxicity to mammalian tissues (Aslam *et al*., 2010). Sheep are usually dipped or cured insecticides every year for prevention and control external parasites. Cypermethrin is increasingly used as the active ingredient in many solutions used for the prevention and treatment of ticks, lice, and mange in sheep. (Khan *et al*., 2012).

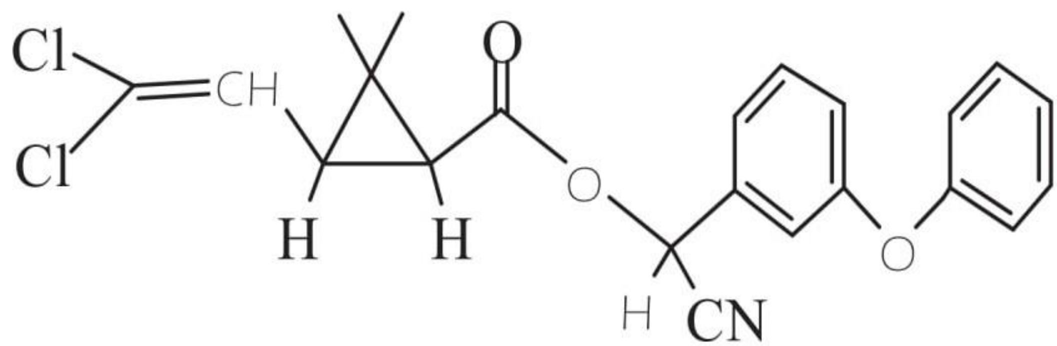


Fig (2-1). Chemical structure of cypermethrin.

2.7.1.1. Mode of action

Cypermethrin is a class II synthetic pyrethroid insecticide that crosses the blood-brain barrier and has a harmful effect on the nervous system on the central nervous system. It also causes motor failure. Pyrethroids, including cypermethrin, dilate sodium channels in the central nervous system, causing hypopolarization and hyperexcitation of neurons. Cypermethrin-induced shortening is often discussed with an excess of the central nervous system (Kumar Singh *et al*., 2012).Additionally, cypermethrin induces neurotoxicity by modulating the level of gamma-aminobutyric acid (GABA) (Manna *et al*., 2005).

2.7.1.2. Cypermethrin Use

Cypermethrin is increasingly being used as an active ingredient in many sauces to prevent and treat ticks, lice and mange in sheep (Khan *et al*., 2012). However, CYP is widely used in various insecticide applications, both domestic and agricultural. Pyrethroids are known to affect the nervous system, particularly the synaptic membranes, and potential toxic effects are expected in insects, mammals and especially humans (Saillenfait *et al*., 2015).In addition, cypermethrin has also found use in pest control as a public health agent (Palmquist *et al*., 2012). If these pesticides are used in excessive doses, they enter the food chain and cause a number of hematological diseases (Hussain *et al*., 2011).

2.7.1.3. Cypermethrin Toxicity

Cypermethrin (CYP) is classified as a Class II toxicity substance (Al-Autaish, 2010) and poses risks to mammals, causing issues like muscle tremors, ataxia, and respiratory depression (Shuklan *et al*., 2023). Despite its safety margin, CYP toxicity can occur in livestock through exposure via spraying, dipping, ingestion of sprayed crops, or feed. The severity of toxicity depends on the exposure route, dosage, and vehicle used (Ahmad *et al*., 2009), with dermal or oral exposure being particularly hazardous (G El-Shemi *et al*., 2015).

Cypermethrin (CYP) is moderately toxic when absorbed through the skin or ingested (Al-Autaish, 2010). Toxicity signs appear within hours after oral exposure, with survivors typically recovering in 3 days (Yilmaz *et al*., 2004). High doses can cause ataxia, tremors, seizures, and even death, along with symptoms like drooling, walking deformities, and loss of coordination. This toxicity disrupts sodium and potassium balance in neurons, leading to muscle spasms and central nervous system dysfunction (Shah *et al*., 2007).

Cypermethrin (CYP) causes significant physiological changes, including alterations in blood parameters (e.g., blood sugar, hemoglobin, white and red blood cells) and structural damage to organs like the liver, kidneys, and intestines, affecting health (Sharmin *et al*., 2015). Respiratory symptoms such as cough, pulmonary edema, and dyspnea have been observed. In sheep, high doses can lead to hyperesthesia, muscle twitching, uncoordinated behavior, and even death. CYP also affects the central and peripheral nervous systems, causing symptoms like fatigue, head shaking, convulsions, and coma (Khan *et al*., 2009).

Cypermethrin (CYP) is used in various formulations for environmental, animal, and agricultural health (Pham *et al*., 2011). These insecticides can cause both acute and chronic toxicity in humans and non-target species through therapeutic, intentional, or accidental exposure, such as ingestion of contaminated food, water, or feed (Anadón *et al*., 2009). Pesticide workers are at risk of chronic toxicity from direct exposure (Li & You, 2015). Many insecticide formulations combine multiple active ingredients, but their harmful effects on humans and animals are not always well-evaluated (Eraslan *et al*., 2015). Chronic exposure to CYP may impact sheep health, particularly reproductive and metabolic functions (Rodríguez *et al*., 2017).

Despite its low toxicity, pyrethroid insecticides like cypermethrin can persist in mammalian tissues, posing dangers. Cypermethrin acts as a neurotoxin and suppresses the immune system in mammals, causing various clinical and behavioral anomalies (Raj *et al*., 2013). It is also hepatotoxic (Sheikh *et al*., 2014) and can disrupt the food chain if used in excessive doses, leading to blood disorders and abnormalities in the respiratory, nervous, immune, and endocrine systems (Hussain *et al*., 2011).

.2.71.4. Cypermethrin absorption

Recent research highlights on cypermethrin absorption in sheep its efficacy in controlling ectoparasites and its impact on animal health. Cypermethrin, a synthetic pyrethroid, has been shown to effectively prevent blowfly strikes and control lice infestations, while also raising concerns regarding its residues in animal tissues. A validated method for extracting cypermethrin residues from animal tissues showed high recovery rates, emphasizing the need for monitoring its presence in meat, fat, and liver. The study indicated that cypermethrin can accumulate in animal tissues, raising concerns about food safety and environmental impact (Chaparro *et al*., 2014).

2.7.1.5. Cypermethrin residues

The severity of the toxic effects of pesticides is determined mainly by the type of compound, dose, and duration of exposure, as well as the type and age of the animals, and other environmental factors. (Dunnick *et al*., 1984). Farm animals have been contaminated in a number of ways, which can affect the animal's growth and production. Sheep are usually dipped or treated with pesticides every year for prevention and control external parasites. Cypermethrin is being increasingly used as an active ingredient in many dips that are used to prevent and treat ticks, lice and mange on sheep. Cypermethrin residues can arise in animal foods origin (meat or meat products), either from topical application to livestock for control from environmental parasites or from livestock ration residues (WHO, 1989). The majority of insecticides used in veterinary medicine are ectoparasiticides. It treats insects that live on the skin of mammals and is not absorbed through the skin but it can be absorbed through the intestine and pulmonary membrane, leading to the formation of concern that the widespread use of these compounds increases the risk of infection (Khan *et al*., 2012).

It is lipophilic and almost insoluble in water. Therefore they tend to accumulate in the fatty tissue if repeated applications are made. They also reported that waste tends to be are higher in lean animals following the same treatment and that residue concentrations higher in renal fat than in subcutaneous back fat. This trend accumulation of cypermethrin residues in edible adipose tissue has been confirmed from detecting persistent residues in livestock through waste (Akre, 2016).

2.7.2. Ivermectin (IVM)

Ivermectin (mixture of 22,23 dihydroavermectin B1a 9 and 22,23-dihydroavermectin B1b 10. The molar ratio is not less than 80:20) (Gaisser *et al*., 2003), is a synthetic derivative of a broad-spectrum antiparasitic class of macrocyclic lactones known as avermectins. Avermectin was first isolated by soil fermentation Microorganism, Streptomyces avermitilis. Ivermectin has a structure similar to that of macrolide antibiotics, but without antibacterial activity. The chemical structure of ivermectin (IVM) is shown in figure (2-2) (Dourmishev *et al*., 2005). monitoring and control programmes (Akre and MacNeil, 2006). Ivermectin (IVM) is a broad-spectrum antiparasitic drug that belongs to the macrocyclic lactone family and is widely used to control endoparasites and ectoparasites. IVM can be administered to sheep either subcutaneously or orally (Lloberas *et al*., 2012). Ivermectin was identified in the late 1970s and was first approved for use in animals in 1981 (Heidary and Gharebaghi, 2020).

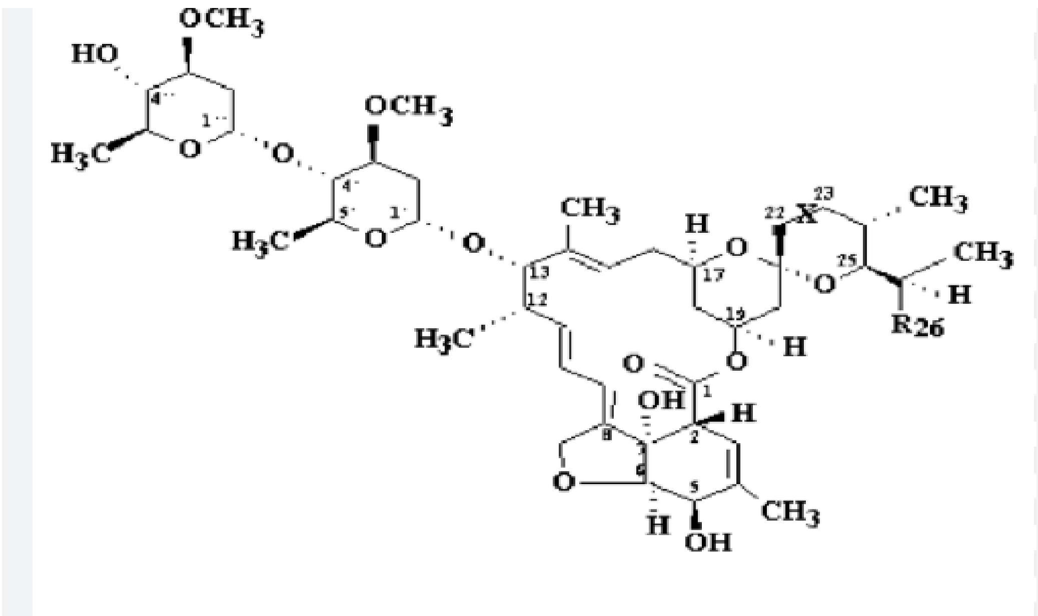


Figure: (2-2). The structure of ivermectin.

2.7.2.1. Ivermectin use

Ivermectin (IVM) is an antiparasitic drug that has been widely used in veterinary medicine around the world since it was introduced to the market in 1981.It is used in all control methods and all types of domestic animals for the treatment of internal and external parasites (Villar and Schaeffer, 2023). It is effective against internal parasites such as nematodes in the gastrointestinal tract and lungs. And external parasites such as scabies mites and blood lice (Laura *et al*., 2020).

2.7.2.2. Mode of Action

Ivermectin was originally thought to be an agonist of neurotransmitter function due to its rapid and specific antiparasitic and anthelmintic effects. Ivermectin works by selectively binding to specific neurotransmitter receptors that function in the peripheral motor synapses of the parasites. It has an endectocidal effect (against endo- and ectoparasites at the same time) and leads to paralysis of nematodes, arthropods and insects by suppressing the conduction of nerve impulses in the internal neurons (intermediate neurons) of nematodes and the nerve-muscle synapses of arthropods insects (Dourmishev *et al*., 2005).

2.7.2.3. Ivermectin toxicity

Ivermectin is widely used in sheep for its anti-parasitic properties. However, its use can lead to toxic effects, especially under certain conditions Studies indicate that ivermectin can cause a significant decrease in erythrocyte count, hemoglobin concentration, and packed cell volume (PCV) in treated sheep, which may lead to anemia (El-Ghoneimy and EL-Gazzar, 2007). Toxicity is often associated with overdoses, but even at therapeutic doses, certain strains may show increased susceptibility to ivermectin toxicity (González-Canga *et al*., 2010).

While ivermectin is effective against various parasites, its potential toxic effects require careful management and monitoring in sheep to ensure animal welfare and environmental safety. On the contrary, some studies highlight that when used correctly, ivermectin can significantly improve animal health and productivity, indicating the need for a balanced approach in its application (Kabir *et al*., 2022).

2.7.2.4. Ivermectin absorption

Estimating relative bioavailability is useful for comparing the absorption of different drug formulations of the same active ingredient. Assuming a relationship between plasma concentration of the active moiety and clinical efficacy, knowledge of the bioavailability and elimination kinetics of the active compound will be particularly useful in developing dosage forms and comparing routes of administration/formulations. Ivermectin (IVM) absorption in sheep has been the subject of recent research, revealing significant insights into its pharmacokinetics and the effects of various formulations. Studies indicate that the absorption and disposition of IVM can vary based on the formulation used and the physiological state of the sheep (Suárez *et al*., 2013).

2.7.2.5. Ivermectin Residue

Ivermectin is one of the most widely used medications in pharmaceutical treatment of parasitic diseases in food production animals due to a wide spectrum of activity and high effectiveness. There is a wide marginof safety for the target species as in the condition of any therapeutic agent administered for food production ivermectin residues in animal tissues are a major safety concern for animal food consumers The majority of these residue studies have shown that this tissue distribution of residues and total proportions of total residue markers were generally similar across most species, and the highest concentrations of ivermectin residue were found in fat and liver with high levels also detected in injection site muscle. (Pisanello and pisanello, 2014). Residue levels were consistently low in muscles other than the injection site (Escribano *et al*., 2012) which should be taken into consideration when implementing waste control strategies. Extremely lipophilic character Ivermectin and different fat percentages in muscle the reason could be the uneven distribution of waste. Ivermectin residues are responsible for many health ailments risks it may cause a mild mazotic reaction, including fever, itching, Arthralgia, myalgia, postural hypotension, edema, lymphadenopathy, hence the gastrointestinal symptoms, sore throat, cough, and headache become necessary for waste to be strictly regulated from a food safety point of view (Koesukwiwat *et al*.,2007).

2.8. Antiparasitic residues

Human health is directly linked to the environment (Esworthy and Bearden, 2015) especially the nature and quality of food (Ames, 1983). The quality of the food animal products is a widespread concern for public health agencies the world since veterinary medicines have played an important role in the field of animal husbandry and agricultural industry, and increase occurrence of residues and resistance become interesting issues (Rokka *et al*., 2005). The international movement of animal products may constitute a risk to public and animal health (Tiensin, 2016). One type of potential risk to public health is residues of veterinary medicines, which are widely used in animal production for disease prevention and treatment (Beyene, 2016).

Veterinary drug residue can be defined as quantity the drug, its metabolites and manufacturing impurities that remains in products produced from treated animals (BRASIL *et al*., 1999). Pesticides or other chemicals used in agriculture or food production as well as veterinary drugs may be present as residues in foods of animal origin from treated animals and therefore represent a potential health problem for the public. Antiparasitic agents represent an important class in veterinary medicine (Delatour and Parish, 1986).

After administration of a drug to an animal, the drug is usually metabolized to promote its excretion and largely its detoxification. However, there are still public health concerns related to the use of veterinary medicines in livestock. It has been shown that regular ingestion of small amounts of the same substance can lead to toxic symptoms over time (chronic exposure) and can promote the development of allergies (Ortelli *et al*., 2018).

Meat and its products are important for human nutrition and diet, however it is also one of the main ways humans consume pollutants, a lot environmental pollutants persist in the environment due to the high percentage of chemicals in it stability, often bioaccumulates in animal fauna depending on it for their love of fat. Human exposure to these environmental pollutants, as well as veterinary medicines, usually through foods of animal origin (Kim, 2012).

2.8.1. Maximum Residual Limits (MRLs) in the use of Antiparasitic

The maximum residual value (MRL) is the maximum usefulness of veterinary drug residues that can be beneficial to society when used legally and recognized in food. MRL expresses the maximum residue resulting from the use of veterinary drugs, is expressed in units of mg/kg or µg/kg on an adjusted weight basis, and is suitable for use in or on foods. (Beyene *et al.,* 2011). It mostly depends on the type and quantity of residue It is considered free of toxic risks to human health as expressed with helpful resources to use acceptable daily intake (ADI), or for short ADI that uses an additional security element (Ame *et al*., 2022).

After the antiparasitic formulation is given, the medication should be released from the vehicle in which it is formulated, and once dissolved at the site of administration, it is absorbed into the systemic circulation and must reach its site of action. Effectiveness Antiparasitic drugs are related to their pharmacokinetic and pharmacodynamic behavior in the body of the treated animal (Imperiale and Lanusse, 2021).

They are an indicator of if pesticides were used correctly. This means that there are risks to consumers if maximum residue limits are in place exceeding scientifically incorrect. MRLs contain a large number of embedded elements Safety factors, with the value set way below the level at which It would cause harm to human health. The development of human toxicity and exposure data, including the Acceptable Daily Intake (ADI), should be considered MRLs. Furthermore, for this particular pesticide, the amount of product consumed should not exceed the regulated amount that the consumer can take (Handford *et al*. 2015).

Table (2-1): Maximum Residue Level (MRL) and Acceptable Daily Intake (ADI) percentages for both Cypermethrin and Ivermectin in the organs.

Animal product	Maximum Residue Level (MRL) for Cypermethrin	Maximum Residue Level (MRL) for ivermectin	Acceptable Daily Intake (ADI) for Cypermethrin	Acceptable Daily Intake (ADI) for ivermectin
Liver	0.05	0.06	0-2 µg/kg bw	0-1 µg/kg bw
Kidney	0.05	0.02	0-2 µg/kg bw	0-1 µg/kg bw
Meat	0.05	0.03	0-2 µg/kg bw	0-1 µg/kg bw
Tail fat	0.2	0.1	0-2 µg/kg bw	0-1 µg/kg bw

2.8.2. Withdrawal period

The withdrawal period is the fixation to protect the person from its exposure to food laced with drugs. Withdrawal timing is appropriate for human administration and target tissues. Most often, they are liver or kidney as they are they are the basic disposal devices and usually display are mains for the longest period. that time required for residual toxicity to occur expend or reduce fixed interest as defined in a useful resource for using tolerances or time a little of the final dose given is passed to the animal. At the same time that the eye of the remains is inside tissues: muscle, liver, kidney, skin/fat (Mohamed *et al*., 2009).

The withdrawal period is determined at the same time as the tolerance limits on the remaining interest is equal to or less than the allowable interest (Haile, 2017). The public health importance of veterinary medicines National and global public health implications around the world companies have a deep case regarding the presence of drug residues in meat and in the form of human ingestion of food-producing animal entrails (Tilahun *et al*., 2016). Residues of medications that remain in their form when consumed by human tissue may also be inserted in the healthy eating plan for humans because of the farms negligence to take into account the withdrawal period for the medication incorrect use of drugs for the animal (Alhaji *et al*., 2018).

Drugs used in animal feed can have an effect on overall health due to its secretion in form for animal tissues ingested by humans and intended for human consumption. There are several channels through which drug residues can lead to negative effects on humans, including the development of resistant pathogens that can be transmitted directly from animals to humans. The consequences of an allergic reaction on the intestinal microbiota and the immune system are important (Beyene *et al*., 2016).

2.8.3. Antiparasitic residues risk factors

Chemical antiparasitic drugs are mainly used to control parasitic diseases. They are crucial in animal husbandry, development, and animal health safety, but the most anti-parasitic drugs have low bioavailability due to their insolubility and short half-life. Therefore, treatment of parasitic diseases requires repeated doses for a long period of time long life cycles of parasites. Repeated treatment may cause animal stress, high labor intensity of farms and drugs resistance (Vercruysse *et al*., 2007).

The waiting period is the time after treatment it is administered during which no food can be offered from the treated animal sold. In the processes of absorption, diffusion, metabolism, and excretion of waste, and the specific waiting period takes into account Pharmacokinetic variation between individual animals (active ingredients and metabolites). These processes are affected by the animal physiological condition as well as genetic traits that affect metabolism or secretion. Since these differences affect the kinetics of the residue when it is medical when giving products to animals, the waiting time may need to be adjusted. Such differences are not taken into account at this stage of veterinary medicine production (EFSA, 2012).

2.8.4. Effect of residue on public health

It depends on the drug, but after it is given to the animals, the tissue containing it, removed the highest drug residues that are usually in the liver, kidneys, or fat, with residues found in meat being too low. Provided that the drugs are used in animals in accordance with the good veterinary practices (GVP), the risk to public health is minimal following consumption of any edible tissue. However, due to many cases of misuse such as additional use of the label or non-compliance with withdrawal Period (WP), much higher residue levels may be seen in edible animal products after taking the medication. Such residues are in meat and/or edible tissues that may produce harmful effects on people who consume these foodstuffs (Moreno and Lanusse, 2017).

The main reasons for meat or edible tissue with residual levels exceeding the permissible limit/maximum limit associated with, the illegal sale of veterinary medicines, Off-label drug use, Cross-contamination in animal feed due to poor Good Manufacturing Practices (GMPs), Failure to follow good animal husbandry practices, Misuse of drugs in therapeutic animal feed, marketing of processed/processed animals intended for presentation purposes converting it to slaughter for human consumption (KuKanich *et al*.,2005).

One of the most serious objections is presence of drug residues in the intended foods for human consumption arises as a result of human health considerations. With the widespread use of drugs in animal production, residues of the original are eliminated rugs and/or metabolites have a high potential to be present in edible animal products. The public health significance of such fraud in the food supply is mainly determined by the level of residues and the individual drugs they contain originated (Botsoglou and Fletouris, 2000).

Therefore, the residues of veterinary medicines were food contamination which is a global challenge (Pavliček and Lugomer, 2019) and these wastes may result from inappropriate food contamination or off-label drug use and failure to maintain medication Periods of withdrawal or poor animal production Practices (Tajick and Shohreh, 2006). In general, health effects of drugs chemical residues include acting as a mutagen, carcinogenic, teratogenic agent and reducing in reproductive performance, drug sensitivity, and Acute toxicity in humans (Singh *et al* ., 2014). In addition to the drug dose, residual levels also depend on the withdrawal period. Although most drugs present relatively low risks to the general public when used responsibly and in accordance with instructions previously approved by veterinary drug manufacturing laboratories, it is important to consider these factors. (Lozano and Trujillo, 2012).

In general, veterinary medicines and medicines intended for humans are the same, but the method they are regulated differently, especially for drugs used in food production animals. Since humans can be chronically exposed to veterinary waste through diet and veterinary medications residues in food are evaluated for effects due to chronic exposure, so the Acceptable Daily Intake (ADI) must be determined (Joint *et al*., 2017). After establishing the ADI, maximum residual limits (MRLs) are determined for individual food commodities are identified. (Lee *et al*., 2021).

2.8.5. The importance of drug residue control programs

Monitoring drug residues in foods of animal origin is useful to ensure that banned substances (due to their toxicity) are not used and that approved substances do not exceed maximum residue levels by adhering to the withdrawal times between treatment and slaughter. Residue monitoring consists of taking food samples to determine trends in use of veterinary drugs, so more targeted monitoring can be carried out. Veterinary medicines for inclusion in these programs they are selected based on their risk profiles. Only take local waste samples the program includes steps to address the occurrence of illegal waste in animals producing food on the farm (Ortelli *et al*., 2018).

Local waste sampling programs are also available a trade condition, either mandatory or as an expectation of import countries that allow animal-derived foodstuffs to reach markets. Import residue the sampling program isprimarily a verification program to determine that the local waste sampling program in the exporting country is working effectively. These programs consist of two main components, monitoring and control.

Residue monitoring programs take random samples of animal tissue in slaughter. Tissue samples are examined for residues of veterinary drugs and pesticides and environmental pollutants, and the residua is evaluated to ensure compliance with them applicable maximum residue limit or environmental standard. In return, surveillance programs take tissue samples animals suspected of having offending residue on the basis of clinical signs or herd history (Joint *et al*., 1996).

If monitoring reveals a potential waste problem, the action taken will vary in accordance with the policies of the specific jurisdiction. Usually, source of the symptom is tracked and action is taken to avoid further incidents. Work may include confiscation and disposal of products, and additional testing for residues at expense product, quarantine the farm, and even prevent the sale of the products, it was found that the commodity is fit for consumption and is fit for sale in both cases local markets and export markets. Audit users and operators, and request feedback from distributors, industry codes of practice can also be used to implement them increased residue monitoring (Mohsina *et al*., 2015).

2.8.6. Method of detecting and identifying drug residues

Absolutely exclusive strategies for drug residue control identity in the components of the animal foundation together. The screening approach must be ready for what is coming through the residues in the roads facing the maximum drug residues and it should additionally be reduced, to a significant minimum (Foster and Beecroft, 2014).

Verification strategies are strategies that provide complete verification data that permits the substance to be definitely considered and if vital is quantified in the desired quantity. These styles are designed to meet the overall style performance standards that can be established in the course for verification required before using it for legal monitoring (Ame *et al*., 2022).

2.9. High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a separation technique that can be applied for analysis compounds with different properties from low to very high molecular mass material. This chromatographic separation involves passing a dissolved mixture in a mobile phase through another substance called the stationary substance phase, and both the mobile and stationary phases are immiscible. Depending on nature, chemical composition and molecular weight of the analytes, we can select the type of liquid chromatography. In this sense, different types of HPLC They appeared to allow qualitative and quantitative information regarding the individual components of the sample under study (Lozano-Sánchez *et al*., 2018).

The primary advantages of HPLC take a short time (a few minutes/pattern) to obtain results, has excessive reagent-dependent sensitivity and specificity and excessive automation leading to excessive throughput. Hence the ability to receive quite a few statistics of spectra once the diode array detector is misused. The downside includes excessive initial investment (equipment), need for experience, and desire for style preparation (extraction, filtration, addition of internal standard, etc.) and column charges (Toldra and Reig, 2006). High universality of the general performance of liquid chromatography (HPLC) is obtaining advanced use in apex laboratories due to the possibility of examining multiple residues at the same time in a sample in a very fast time. Recent trends in the increased frequency of HPLC can reduce sample processing and evaluation time (Torre *et al*., 2015).

Chapter Three

Methodology

3. Methodology

3.1. Materials:

3.1.1. Equipment and Instruments

Table 3.1: Equipment and Instruments

No.	Equipment and Instruments	Company and Country
1	Centrifuge	Hettich EBA 20 centrifuge (Germany)
2	Magnetic stirrer	Lab tech, (Korea)
3	Homogenizer	Silver crest (Germany)
4	Water bath	Memmert (Germany)
5	High Performance Liquid Chromatography (HPLC)	Shimadzu corporation (Japan)
6	Micropipette different size	Shaanxi, China
7	Fume Hood	Lab tech, (Korea)
8	Beakers in different size	Shaanxi, China
9	Syringes filter	CHMLAB GROUP, S.L. (Barcelona, Spain)
10	Syringes in different size	Golden Gate Iraq
11	Poly propylene Centrifuge tube	IWAKI (Japan)

12	Capsule Magnetic Stirrer Beads	ALRK India
13	Amber glass bottle	SATYA India
14	Sensitive Balance	Denver (Germany)
16	Pipette	Dragon Lab (India)
17	Boxes	Iraq

3.1.2. Chemicals

Table 3.2: Chemicals used and their manufacturer company

No.	Chemicals	Manufacturer company
1	Acetonitrile 99.9% (ACN)	LOBA CHEMIE India
2	1-Methylimidazole	Fluka, Switzerland
3	Trichloro acetic acid (TCA)	SDFCL, India
4	Solution composed of: A-5ml from 1-Methylimidazole and 5ml from acetonitrile 99.9%	Public health laboratory Veterinary Medicine / Karbala
5	Solution composed of: B-10ml from TCA and 5ml from ACN	Public health laboratory Veterinary Medicine / Karbala

6	Cypermethrin (CYP)	Mobedco, Jordan
7	Ivermectin	kipro, holland
8	HPLC Grade water	SIDDHI LABS, India
9	Methanol HPLC grade	LAB ALLEY (Austin, USA)
10	Distill water	Iraq (Al-Kafeel Company)

3.2. Study design

One hundred and twenty-five samples of sheep meat and edible parts (liver, kidney and fat tail), 50 samples of sheep meat and 75 represent samples of edible parts (25 liver, 25 kidney and 25 fat tail). It was collected randomly from butcher shops in five different areas (Al-Hussaina, AL Hur, Tauweraij, City center, Aeen tumer) in Karbala Governorate. An extraction process was performed on the samples using organic solvents to extract the antiparasitic residues. After the extraction process, the resulting extract is analyzed to determine the antiparasitic residues (cypermethrin and ivermectin) using a high-performance liquid chromatography (HPLC) device.

3.3. Experimental Design

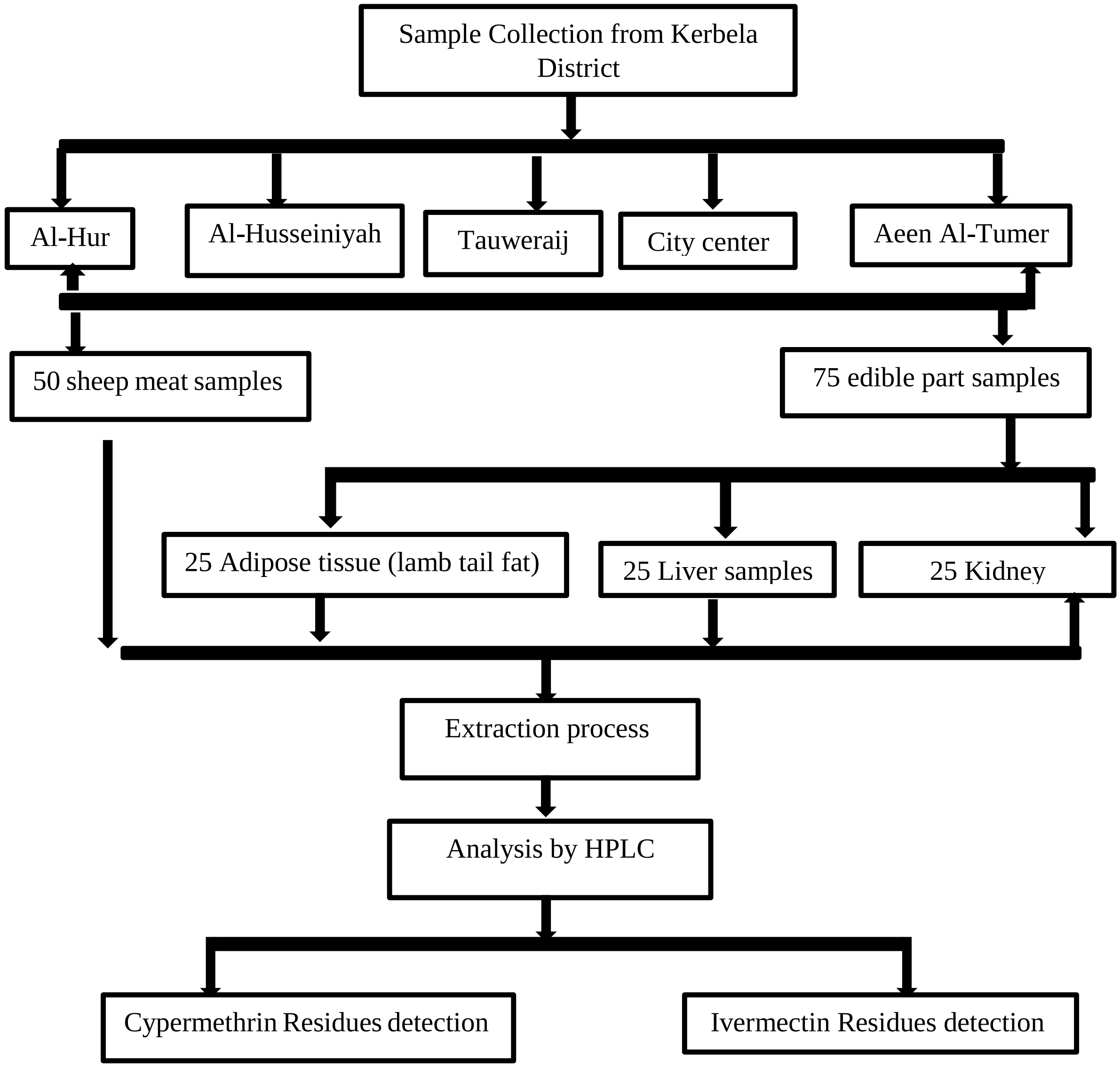
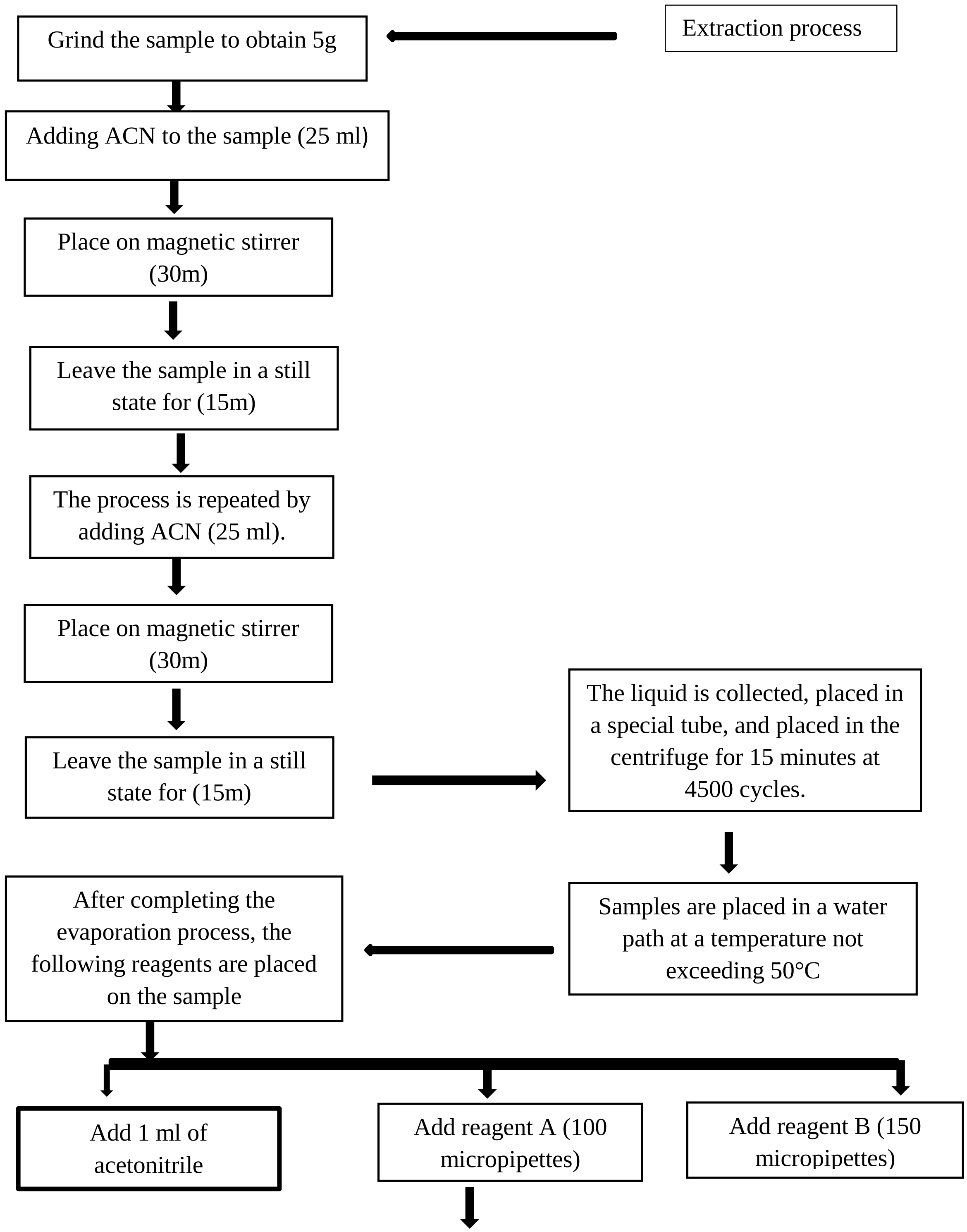


Figure 3.1: General experimental design for the current study to determine the antiparasitic drugs in sheep meat and edible parts.

3.4. Detailed Scheme for Extraction and Analysis of Samples to Detect Residues of Cypermethrin and IvermectinDetect Residues of Cypermethrin and Ivermectin



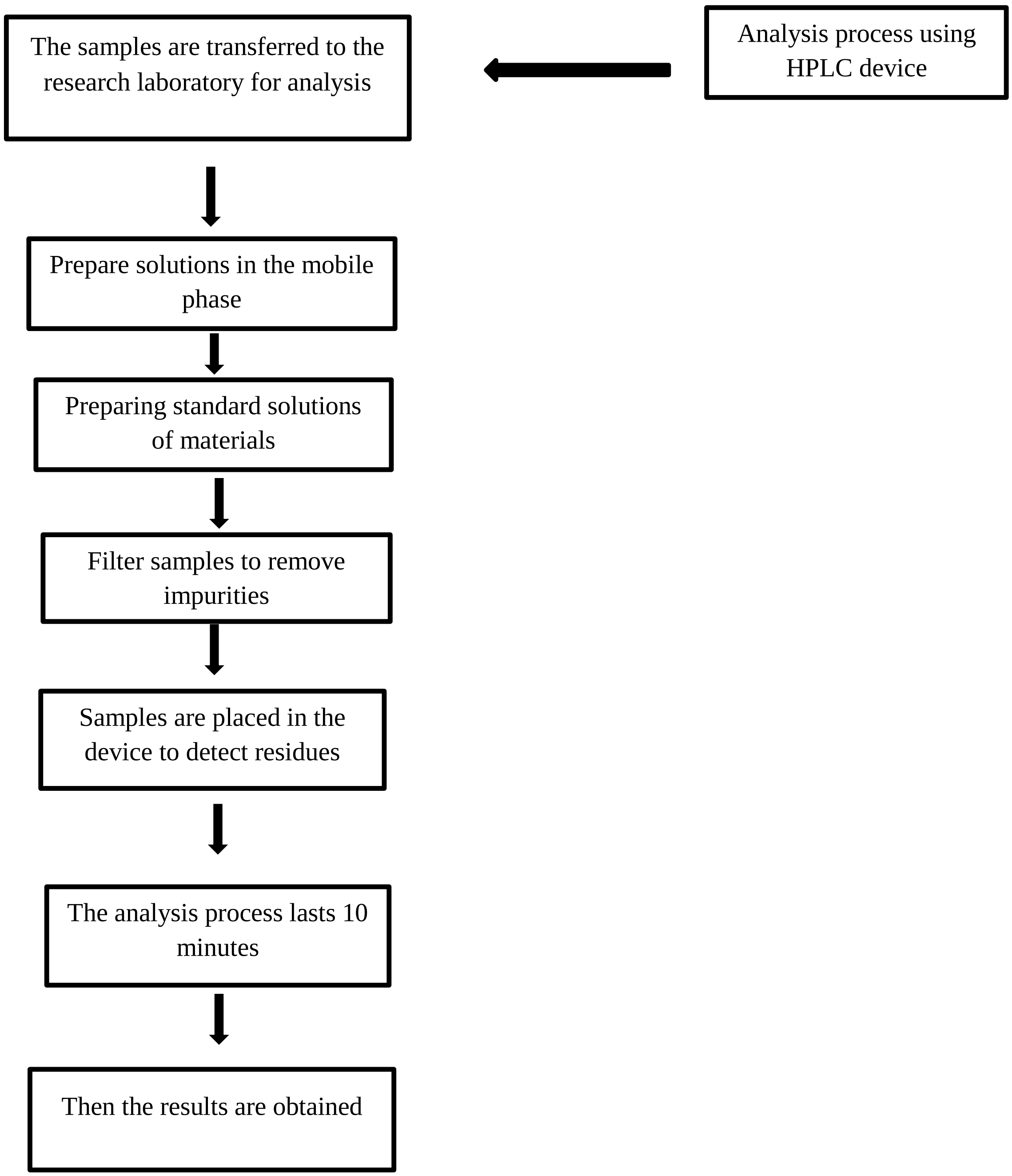


Figure (3.2): shows the extraction and analysis process for the studied samples.

3.4. Methods

3.4.1. Samples collection

All samples were collected in special clean containers from local markets in Karbala Governorate. It was collected twenty-four hours before the extraction process and placed in a special bag while being transported to the Public Health Laboratory /College of Veterinary Medicine/ University of Karbala. The High-Performance Liquid Chromatography (HPLC) analysis to determine the antiparasitic levels (cypermethrin and ivermectin) residues in in sheep meat and edible parts (liver, kidneys and fat tail).

3.4.2. Preparation of reagents

These are Acetonitrile 99.9%, 1-methylimidazole and trichloroacetic acid (TCA) and distilled water.

Preparation of reagents A: 5 mL of 1-methylimidazole and 5 mL of 99.9% acetonitrile were placed in an amber glass bottle. Thus, the first reagent A was prepared.

Preparation of reagents B: 0.1 g of trichloroacetic acid (TCA) was mixed with 100 ml of distilled water. 10 ml of the resulting solution was removed, placed in an amber glass bottle and 5 ml of acetonitrile 99.9% (ACN) was added, thus, the second reagent B was prepared.

3.4.3. Extraction process

All samples were conducted in the Nutrition and Public Health Laboratory at the College of Veterinary Medicine, University of Karbala. To prepare a deskill sample of ivermectin and cypermethrin in sheep tissue, solid-liquid extraction (SLE) was adopted on the basis initial report on this study, one of the most common methods of extracting antiparasitic from solid matrices is solid-liquid extraction, where the solid tissue is chopped up into small pieces and then homogenized with organic solvent.

The experimental sample was ground using a blender for 5 minutes to obtain an equal and uniform reaction. After completing the grinding, 5 grams of the grind sample were taken and placed in a beaker. Add 25 ml of acetonitrile to beaker containing the grind sample. After adding acetonitrile and mixing the samples, place the beaker on the magnetic stirrer for half an hour. This step aims to facilitate the effective extraction of drug substances. After the magnetic stirrer period ends, leave the samples for a quarter of an hour until they settle again. After that, separate the liquid (extracted) part again from the solid part.

Repeat the same steps of the previous process on the same sample, so that the liquid portion becomes approximately 50 ml. After separating the liquid fraction, transfer it to centrifuge tubes. Place the centrifuge tubes in the centrifuge and perform the centrifugation for a quarter of an hour at a cycle of about 4500. This step aims to concentrate the substance and separate other impurities. After extracting the concentrated liquid portion from the centrifuge, it is stored in a clean conical flask (250 ml) and evaporated to dryness in a water bath. Adjust dryness conditions appropriately, such as temperature not exceeding 50 Celsius according to approved procedures. After completing the drying process, the dried residue was reconstituted using 1 ml of acetonitrile, after washing the sample add the reagents that were previously prepared for detection, the first reagent A (100 micro pipette) consists of 5ml from 1-Methylimidazole and 5ml from acetonitrile 99.9% and adding the second reagent B (150 micro pipette) consists of 10ml from Trichloro acetic acid (TCA) and 5ml from Acetonitrile 99.9% (ACN).

3.4.4. Analysis of antiparasitic drug in samples

The samples were collected and prepared for quantitative analysis using HPLC (figure3.2).

1. The first solution is called the mobile phase, which was prepared from ACN: D.I (70: 30).
2. The stationary phase is the column for the column analysis, determined by the type of column is the C18, which are the components of the column of silica and carbon, and adjust the calibrator on the assigned phase with a rate of injection of 20 microliters for a period of 10 minutes.
3. Standard solutions of each cypermethrin and ivermectin, compound were prepared by dissolving 1 mg of the compound in ten ml of methanol to obtain a final concentration of 100 ppm. Stock standard solutions were packed in amber glass and stored at 20 ºC to prevent photo deterioration. Stock solutions were diluted with methanol to produce a series of working.

4. Standard solutions (3.3,5,2.5 µL) that were produced weekly (de Lima *et al*., 2016; Shaheed & Dhahir, 2020).
5. Standard solutions (3.3,5,2.5 µL) that were produced weekly (de Lima *et al*., 2016; Shaheed & Dhahir, 2020).
6. Chromatographic separation condition: The gradient elution by using a mixture of ACN (mobile phase) and deionized water was applied (de Lima *et al*., 2016; Shaheed & Dhahir, 2020).

a- The chromatographic column (cypermethrin): Mobile phase = methanol: D.W (70:30), Column = C18-ODS (25 cm * 4.6 mm) Detector (UV-Vis) at 245,,,or 220 , Flow rate = 1.0 ml / min Injection volume: 100µL(Shaheed & Dhahir, 2020).

b- The chromatographic column (ivermectin): The mobile phase was acetonitrile flow A =deionized water /10 mM ammonium acetate B: acetonitrile flow rate at 1.0mL/min, the column was C18 – ODS (25 cm * 4.6 mm), and the detector (UV- 240 nm) Injection volume: 100µl (de Lima *et al*., 2016).

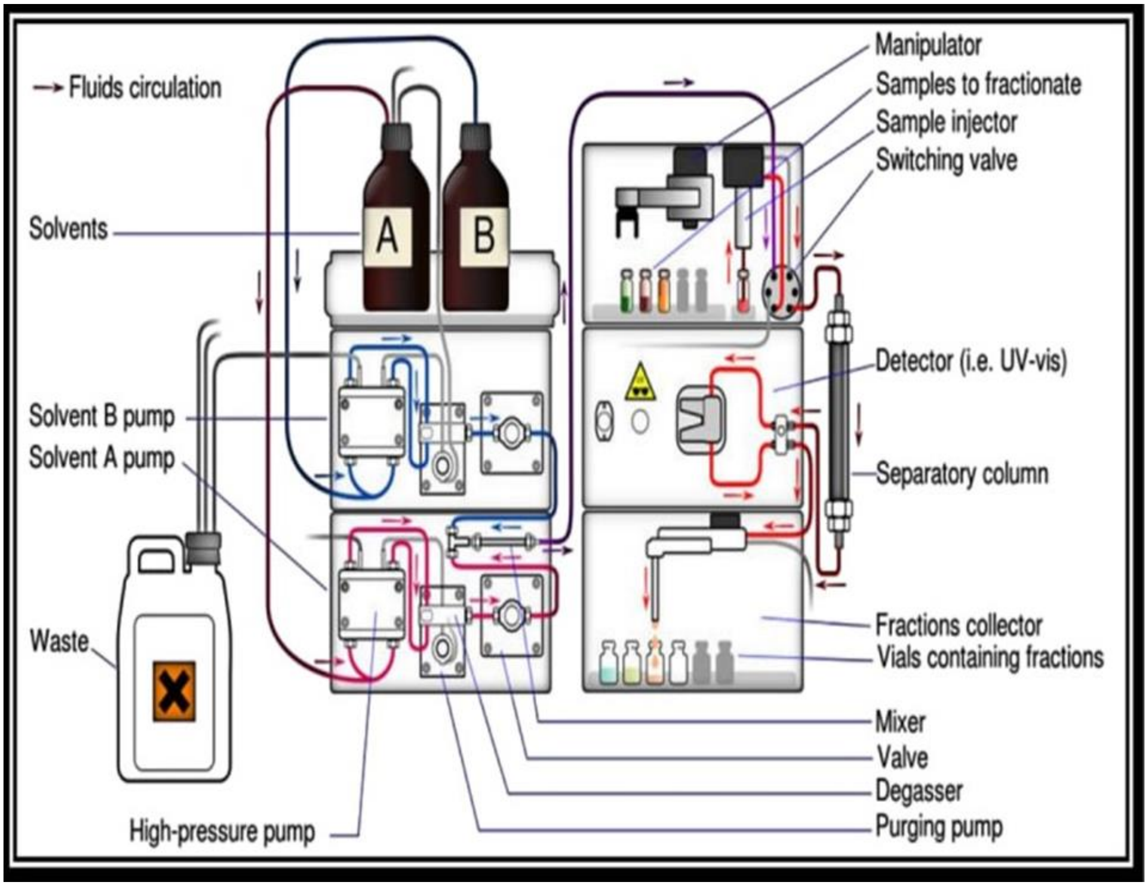


Figure 3.3: Operational diagram of HPLC (Najim and Al- kurashi, 2017).

3.5. Statistical analysis

The data were analyzed using SAS software (SAS, 2018), and the results were compared using the least significant difference (LSD) value at the probability level of 0.05.

This was done using the Arc Gis map v10.7 program to prepare the experiment maps.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Antiparasitic residues in sheep meat and edible parts

Through the current study, which was conducted on 125 samples of sheep meat and edible parts (liver, kidney, and tail fat), positive results were found for antiparasitic contaminants (cypermethrin and ivermectin), and in comparison with the stander residue of 2023 World Health Organization. It was found that there is a significant difference between the natural residue and the residue that was obtained. The results obtained also showed a high level of contamination of meat and edible parts that it may be due to incorrect use of antiparasitic agents to treat animals.

4.1.1. Levels of cypermethrin residues in sheep meat

Table (4.1) and figure (4.1) show the percentages of cypermethrin residues in sheep meat and compared to the maximum limits of residues, where it was found that there is a significant difference between the results.

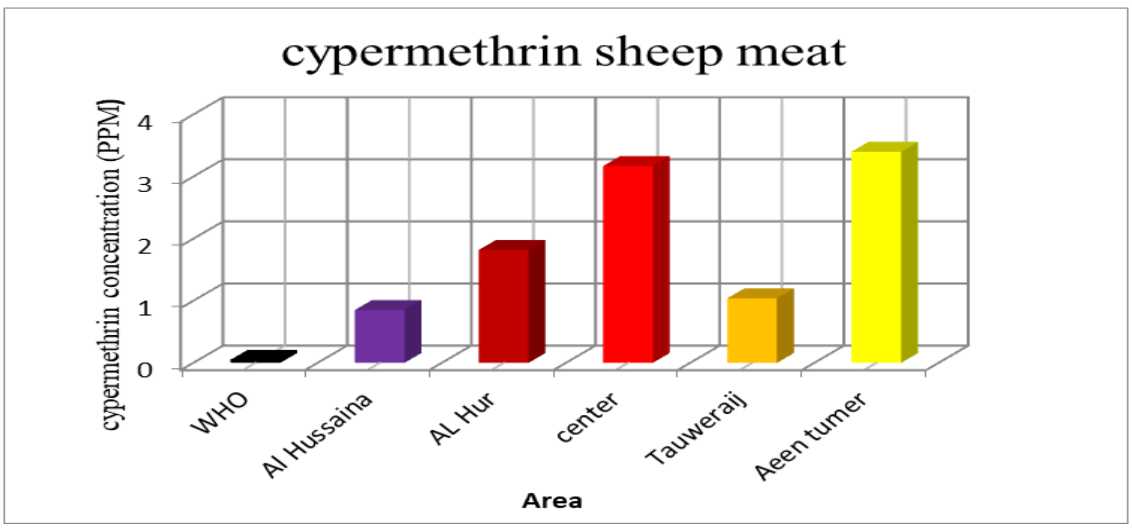


Figure (4.1): Cypermethrin concentrations (PPM) in sheep meat.

Table (4.1): Cypermethrin residues in sheep meat with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.05	0.85 ±0.003 e *	1.82 ±0.02 c **	3.17 ±0.01 b **	1.04 ±0.006 d ****	3.40 ±0.03 a *****	0.0624

Means with common or similar letters do not differ significantly.

(* Results obtained through HPLC results in appendix (V), ** Results in appendix (VI), *** Results in appendix (VII), **** Results in appendix (VIII), ***** Results in appendix (IX)).

By analyzing the data presented in the table and comparing it to different regions, it becomes clear that there are regions suffering from high levels of pollution. For example, the Aeen al-Tumer area records the highest concentration of pollution at (3.40), followed by the city center area, which records a concentration of (3.17). When these values are compared to the maximum permissible limits for the level of pollution, which are set at (0.05), it clearly appears that there is a huge difference between the results recorded in the samples and the accepted natural values. This large difference is a clear indication of non-compliance with the required safety standards when using this material, which warns of potential health and environmental risks. The situation requires immediate action to ensure the safety of citizens and the environment.

4.1.2. Levels of cypermethrin residues in liver

As shown in table (4.2) and figure (4.2), the data display the percentages of cypermethrin residues found in sheep liver samples, along with a comparative analysis of these residues across different regions. The results indicate that there is a large discrepancy between the percentages of detected residues and the permissible limits for these materials. It is worth noting that the Al Hussaina area showed the highest level of contamination with cypermethrin compared to the other areas examined.

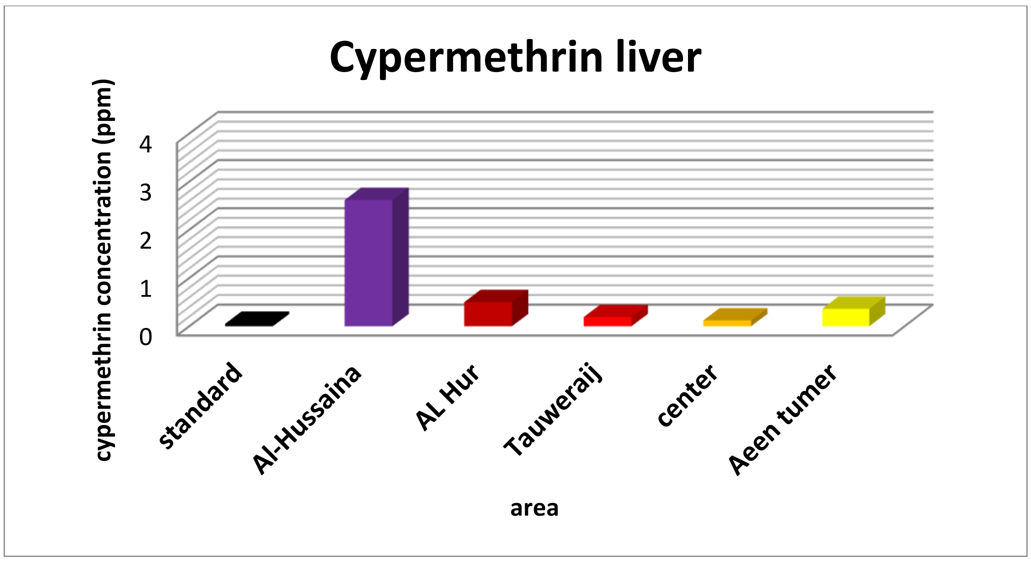


Figure (4.2) Cypermethrin concentrations (PPM) in sheep liver

Table (4.2): Cypermethrin residues in sheep liver with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.05	2.62 ±0.0002 a	0.50 ±0.0002 b	0.12 ±0.005 e	0.19 ±0.04 d	0.36 ±0.005 c	0.0592

Means with common or similar letters do not differ significantly.

4.1.3. Levels of cypermethrin residues in kidney

The analysis was conducted on 25 samples of sheep kidneys, and the results showed that there was a high percentage of contamination of the samples with cypermethrin, exceeding the normal permissible limit. In comparison with the percentage of residue concentrations in the regions, it was found that the city center area had the highest concentration, followed by the Al Hussaina area, respectively, as shown in table (4.3) and figure (4.3).

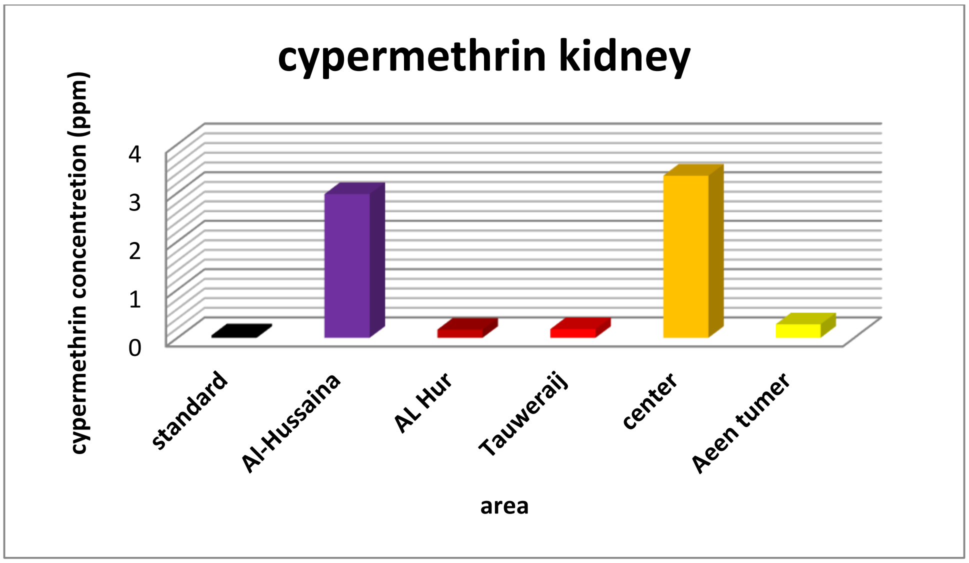


Figure (4.3) Cypermethrin concentrations (PPM) in sheep kidney

Table (4.3): Cypermethrin residues in sheep kidney with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
result	0.05	2.97 ±0.0004 b	0.17 ±0.009 e	3.35 ±0.008 a	0.18 ±0.06 d	0.28 ±0.001 c	0.0899

Means with common or similar letters do not differ significantly.

4.1.4. Levels of cypermethrin residues in sheep tail fat

The analysis was conducted on 25 samples of sheep tail fat, and the of analyzes showed positive results for cypermethrin contaminants in the samples studied. In comparison with the maximum limits of the residues, it was found that there was a significant difference between the residues in the samples and the natural residues. When a comparison was made between the areas from which samples were taken, it was found that the Aeen al-Tumer area, followed by the city center area, had a high rate of pollution compared to the rest of the areas, as shown in table (4.4) and figure (4.4).

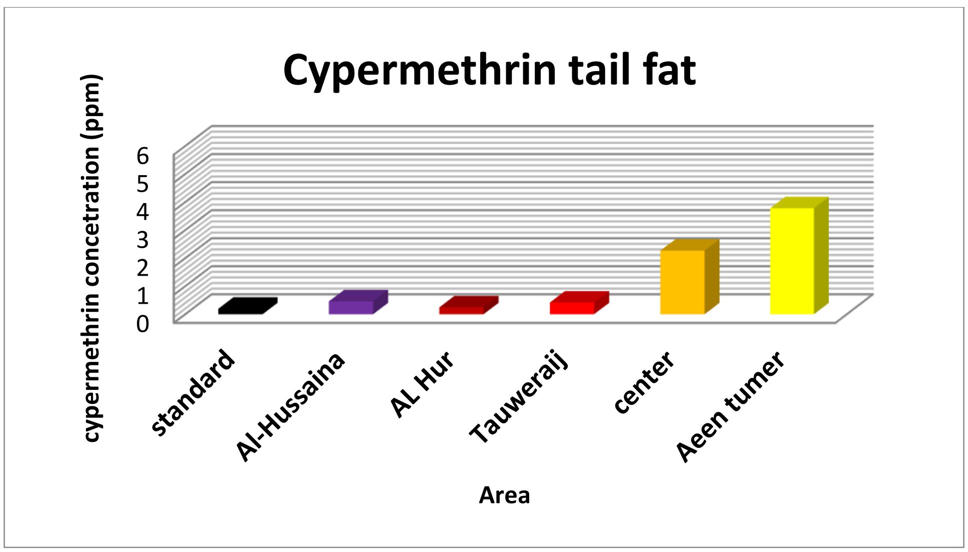


Figure (4.4): Cypermethrin concentrations (PPM) in sheep tail fat

Table (4.4): Cypermethrin residues in sheep tail fat with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.2	0.46 ±0.003 c	0.25 ±0.001 d	2.27 ±0.01 b	0.42 ±0.005 c	3.78 ±0.005 a	0.0496

Means with common or similar letters do not differ significantly.

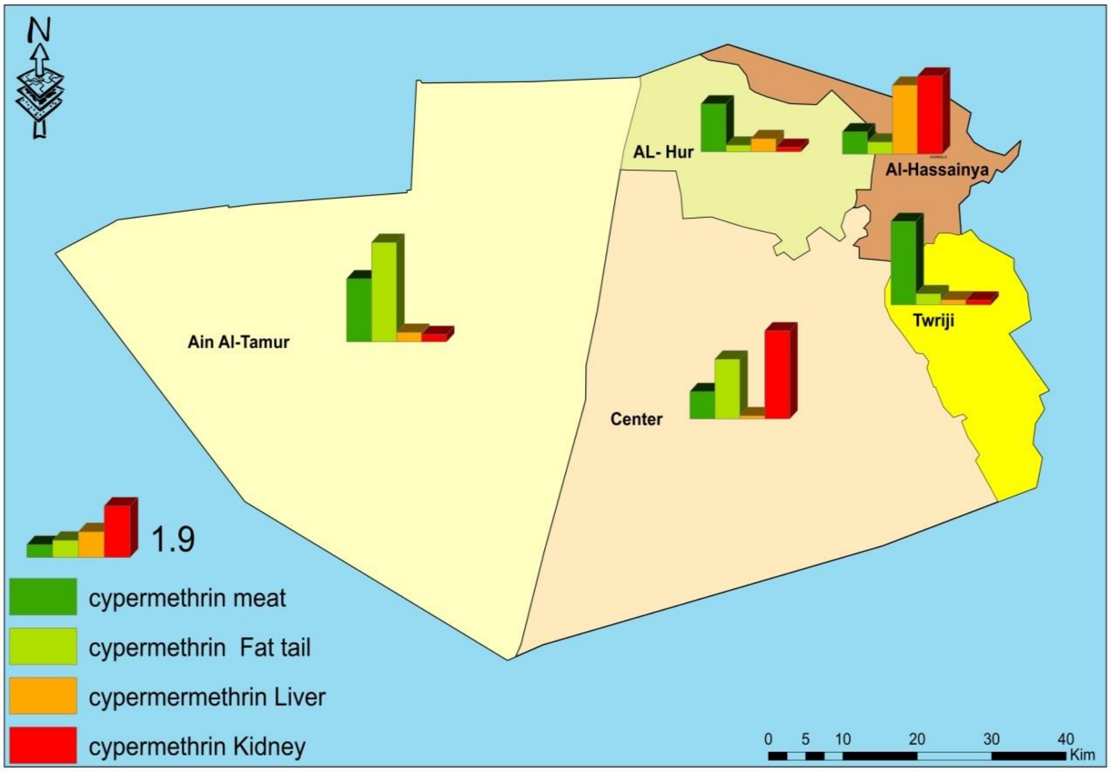


Figure (4.5): Spatial distribution map showing samples that exceed the maximum values for Cypermethrin residues obtained from different areas in Karbala, Iraq.

*Distances between the studied areas in appendix XV

Cypermethrin residues can arise in foods of animal origin (meat or meat products), either from topical application to livestock to control environmental parasites or from residues in livestock rations (RAM *et al*., 2010). Presence of veterinary drugs in meat and edible tissues that exceed standards can impact food trade and human health (Moreno and Lanusse, 2017). Meat and edible tissue samples are monitored in official food control programs. When samples show drug residue levels above MRLs/tolerances, food may be confiscated. (Brazzil, 2010).

The current study demonstrated presence of residues higher than the normal limit, which is not consistent with (Meligy *et al*., 2019). In conclusion, pesticide residues (cypermethrin) were detected in meat and liver tissues of sheep, and the detected pesticide residues were well below the maximum residue limits. Therefore there are no risks associated with their human consumption. This is consistent with (FA *et al*., 2019). It was found that the samples collected were liver, muscle, kidneys, fat pesticide residues were represented by cypermethrin which it was detected in most samples at a value lower than the maximum residue limit according to the European Commission (EC) (2005).

Pyrethroids is commonly used because it is safe for animals and humans because it breaks down hydrophobically, but overdose causes serious problems for humans and animals. Through the study, it was found that cypermethrin residues in meat were higher than the normal limit, and this does not agree with. The proposed method was applied to analyze pyrethroid (cypermethrin) residues in 687 meat samples. Mainly from ruminants. No pyrethroid residues were detected in any of these samples (Niewiadowska *et al*., 2010).

As for the edible parts, such as the liver and kidneys, traces of cypermethrin were found at high levels, and this is consistent with (G EL-SHEMI *et al*., 2015). Residue analysis revealed higher levels of cypermethrin in kidneys and muscles of the liver. It can be concluded from this study that prolonged exposure of farm animals to cypermethrin will affect their physiological and biochemical characteristics. With the resulting negative effects on animal health and production. Moreover, presence of a large amount of tissue residues of this compound in animal tissues waste can affect human health as well. On the other hand, results showed that the cypermethrin residues in the treated tissues the animals revealed that cypermethrin was found in everything tissue samples from treated animals. Moreover, the present results showed that the levels of residue Cypermethrin was in the following descending order, Kidney > Muscle > Liver .

Crusher *et al*. (1985) found that cypermethrin residues detected in the liver and kidneys. Crawford and Hutson (1977) also reported that residuals Cypermethrin has been detected in muscle, liver, and kidneys from sheep. Cypermethrin showed that residues in muscle, liver, and kidney were 0.005 mg/kg in tissue and less than 0.01 mg/kg in fat. (Bosio, 1979).

The maximum residue is limited, and therefore there are no risks associated with their human consumption. This is consistent with (FA *et al*., 2019). It was found that the samples collected were liver, muscle, kidneys, fat pesticide residues were represented by cypermethrin it was detected in most samples at a value lower than the maximum residue limit according to the European Commission (EC) (2005).

4.1.5. Levels of ivermectin residues in sheep meat

Extensive analysis was performed on a total of 50 sheep meat samples to evaluate presence of ivermectin residues. The results showed positive detections of ivermectin, at levels exceeding the maximum permissible limits for these residues. This finding raises significant concerns about food safety and potential health effects on consumers. When examining the contamination rates in the various regions, it was clear that Aeen tumer region showed the highest residual levels of ivermectin, exceeding those found in other regions. Immediately afterwards, Al-Hur area also showed a noticeable level of pollution. This trend is clearly shown in table (4.5) and illustrated in figure (4.5).

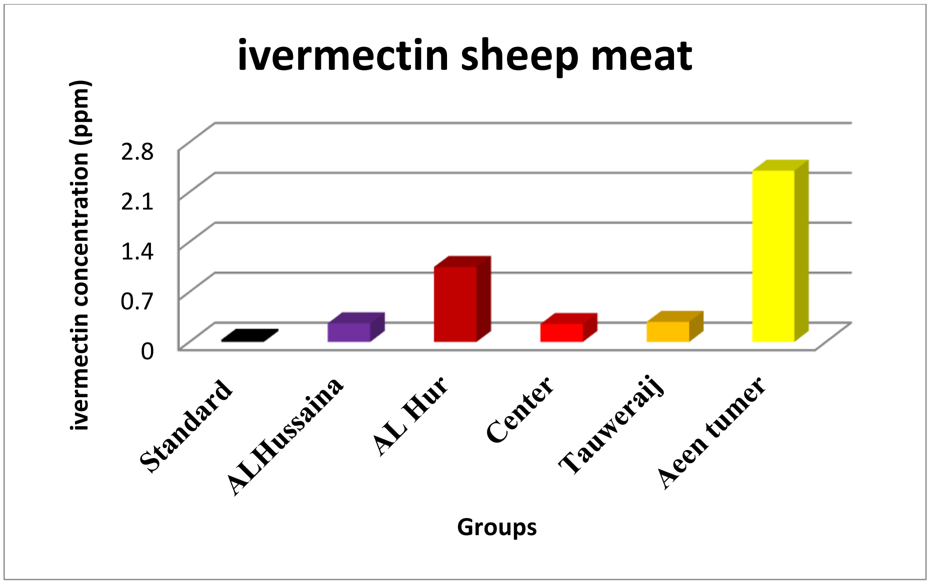


Figure (4.6): ivermectin concentrations (PPM) in sheep meat

Table (4.5): Ivermectin residues in sheep meat with maximum residue limits (ppm)

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
result	0.03	0.26 ±0.002 d *	1.05 ±0.009 a **	0.25 ±0.009 e ***	0.82 ±0.003 c ****	2.40 ±0.007 b *****	0.0163

Means with common or similar letters do not differ significantly.

(* Results obtained through HPLC results in appendix (X), ** Results in appendix (XI), *** Results in appendix (XII), **** Results in appendix (XIII), ***** Results in appendix (IX)

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4.1.6. Levels of ivermectin residues in sheep tail fat

Analysis was performed on 25 tail fat samples to evaluate the presence of ivermectin residue. The results revealed ivermectin levels, with residues exceeding the maximum permissible limits. This finding is alarming because it raises questions about the safety and quality of animal-derived products. When comparing contamination levels in different regions, it was noteworthy that all sampled regions showed equal concentration of ivermectin contamination. This uniformity in residue levels suggests that sources of pollution may be widespread. The data summarized in table (4.6) and shown in figure (4.6), which provides a clear visual

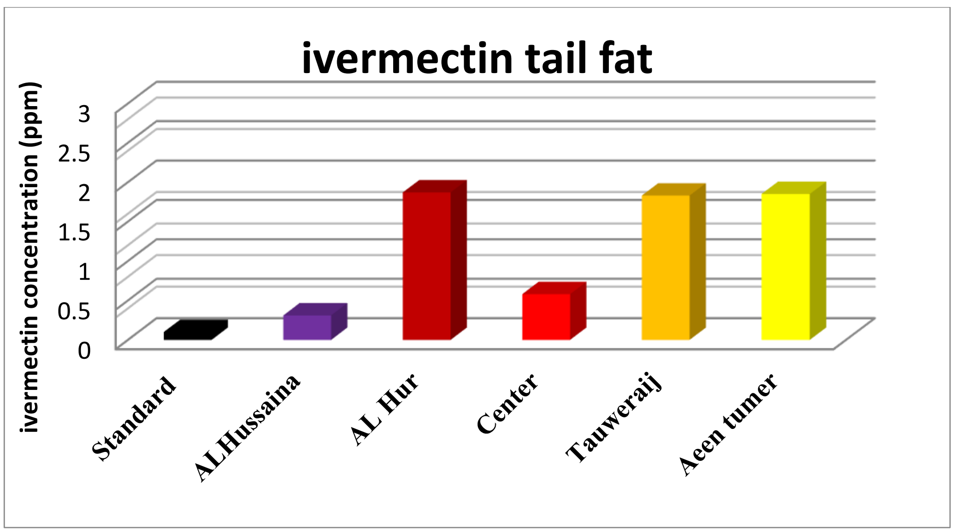

Figure (4.7): ivermectin concentrations (PPM) in sheep tail fat

Table (4.6): Ivermectin residues in sheep tail fat with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.1	0.31 ±0.002 d	1.87 ±0.007 a	0.58 ±0.004 c	1.83 ±0.01 b	1.85 ±0.02 ab	0.0366

Means with common or similar letters do not differ significantly.

4.1.7. Levels of ivermectin residues in sheep liver

Analysis was conducted on 25 samples of sheep liver to determine ivermectin residues in the samples, and the results showed the presence of ivermectin residues exceeding the maximum percentage of residues. In comparison with the percentage of pollution in the regions, it was found that the Al-Hur area had a concentration of (3.74), followed by Al Hussaina area with a concentration of (1.87), the city center area with a concentration of (1.13), the Tauweraij area with a concentration of (0.52), and the Aeen al-Tumer area with a concentration of (0.21), respectively, as shown in table (4.7) and figure (4.7).

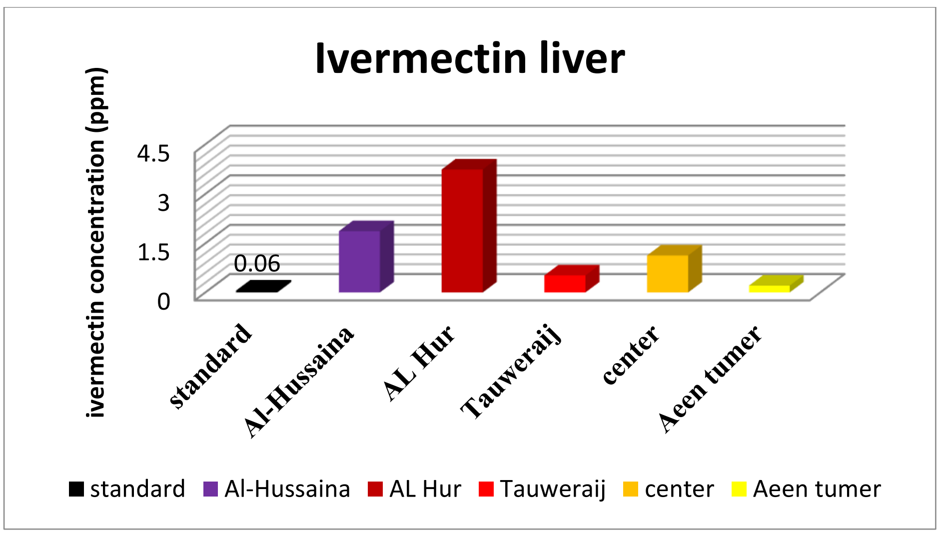

Figure (4.8): ivermectin concentrations (PPM) in sheep liver

Table (4.7): Ivermectin residues in sheep liver with maximum residue limits (ppm)

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.06	1.87 ±0.009 b	3.74 ±0.04 a	1.13 ±0.003 c	0.52 ±0.003 d	0.21 ±0.003 e	0.0567

Means with common or similar letters do not differ significantly.

4.1.8. Levels of ivermectin residues in sheep kidneys

Samples of sheep kidneys to determine ivermectin residues in the samples, and the results showed the presence of ivermectin residues exceeding the maximum percentage of residues. In comparison with the percentage of pollution in the regions, it was found that the Al Hussaina area had a concentration of (3.51), followed by the city center area with a concentration of (2.44), and the Aeen al-Tumer area with a concentration of (1.68), respectively, as shown in table (4.8) and figure (4.8).

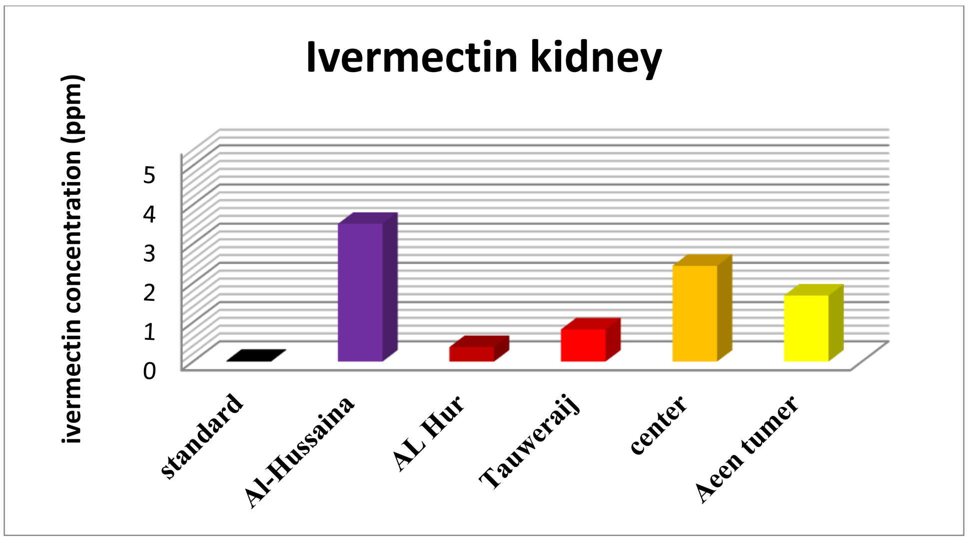

Figure (4.9): ivermectin concentrations (PPM) in sheep kidneys

Table (4.8): Ivermectin residues in sheep kidney with maximum residue limits (ppm).

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.02	3.51 ±0.01 a	0.37 ±0.002 e	2.44 ±0.01 b	0.82 ±0.003 d	1.68 ±0.008 c	0.0333

Means with common or similar letters do not differ significantly.

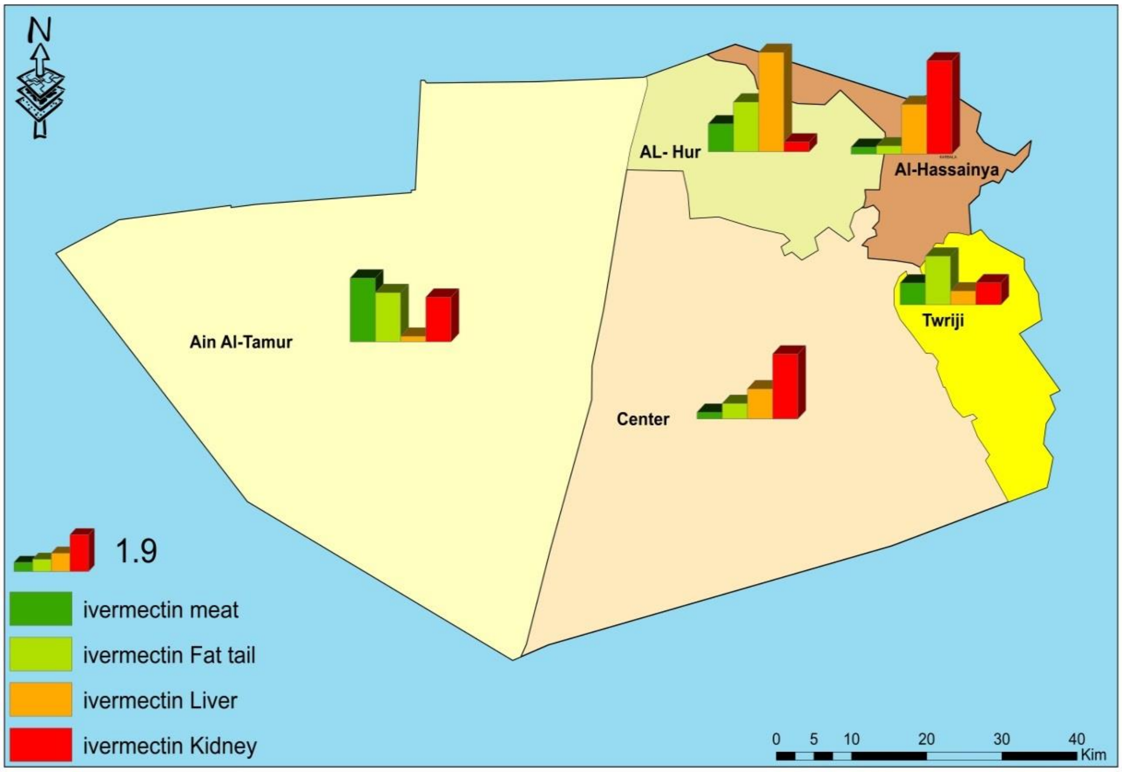

Figure (4-10): Spatial distribution map showing samples that exceed the maximum values for ivermectin residues obtained from different areas in Karbala, Iraq.

* Distances Between the Studied Areas in Appendix XV

Ivermectin is one of the most widely used medications in pharmaceutical treatment of parasitic diseases in food production animals due to a wide spectrum of activity, high effectiveness ; There is a wide margin of safety for the target species. As in if any therapeutic agent is administered to food-producing animals, ivermectin residues in animal tissues pose a major safety concern for animal food consumers. A study of ivermectin contamination in sheep meat and edible parts shows that there is a significant difference in each type of sample compared to the maximum limits of residues, and these obtained percentages pose a threat to the health of the consumer. This is similar to a study previously (Escribano *et al*., 2012) as the study showed that. Tissue distribution of residues and ratios of total marker to total residues were generally similar in most species, and the highest concentrations of ivermectin residues were found in fat and liver with high levels of residues. Levels were also detected in the muscles of the injection site.

This is due to the fact that it is a very lipophilic substance that dissolves in most of it reactant (Canga *et al*., 2009), this is similar to the (Prabhu *et al*., 1991). It showed that ivermectin distribution the pattern showed high levels in fat and liver and generally rapid depletion in all tissues. A high-performance liquid chromatography device was used to analyze the residues in the samples, as is the case in (Tway *et al*., 1981), an analytical procedure has been developed to isolate and identify the antiparasitic agent ivermectin in sheep tissue. The examination has a minimum of reliable measurement of 10 ppb and detection limit of 1-2 ppb. Recovery of ivermectin mutations average 83% for liver, kidney, muscle and fat.

In residue studies, it appears that the liver retains the residue the tallest, in sheep liver no residue was detected at the suggested withdrawal time of 14 days. In another study, (Mani and Al Araji, 2022) was monitored using HPLC a technique for determining the degree of ivermectin residue (IVE). The analysis showed that lamb meat was positive for residual IVM during the study period. The mutton results detected, were 100% in violation of the percentage Maximum residue limits in accordance with previously permitted maximum residue limits World Health Organization and Food and Agriculture Organization.

(Campbell *et al*., 1999), showed that ivermectin is not extensively metabolized in mammals, with 90% of the dose being excreted in feces and tissue debris occurring mainly in the original drug form. (Chiu *et al*., 1990), edible tissue (edible tissue refers to the major tissues/organs in the general population that consume meat, such as liver, kidney, etc. Muscle and fat. These are also edible tissues/organs that are specially monitored under the drug Residue Monitoring Program by the United States. FDA on animal health medications namely liver, kidneys, muscles and fat. Tissue remains the presence of the distribution pattern in sheep, with levels in fat is slightly higher than that found in the liver at all times, and the rest of the remains consist of more polar metabolites. The half-life of the parent drug total tissue residues close agreement in cattle and sheep liver tissue, suggesting equally efficient depletion of the parent drug and its metabolites.

(Núñez *et al*., 2007), A study was carried out to validate an accurate and reliable analytical method for detecting ivermectin (IVM) tissue residues in sheep. The study also aimed to determine the depletion patterns of drug concentrations in edible tissues, including the liver, kidney, muscle, and fat. Among the tissue samples from sheep treated with IVM, the highest concentrations were observed in the liver and adipose tissue, while the lowest concentrations were found in the muscle samples.

(Degroodt *et al*., 1994), the study aimed to investigate the presence of ivermectin residues in the liver and meat of sheep. Analytical methods were employed to detect these residues, demonstrating their suitability as a routine analysis technique for ivermectin. The results showed that the residue levels were below the maximum residue limit (MRL), which aligns with findings in previous studies (Hiwa and Khulod, 2024). The primary objective of this study was to rapidly detect the presence of anthelmintic drugs in sheep meat using high-performance liquid chromatography

(HPLC), with the lowest concentration found to be within the acceptable limits compared to the MRL.

4.2. Comparison between pesticides (cypermethrin and ivermectin)

The detection of anti-parasitic residues in sheep meat and edible tissues is crucial for ensuring food safety and protecting consumer health. Recent advancements in analytical techniques have significantly enhanced detection accuracy. Among the most effective methods are high-performance liquid chromatography (HPLC) techniques.

4.2.1. Comparison of pesticides in sheep meat

As presented in Table (4.9), the comparative analysis of the studied areas demonstrates significant variations in waste levels, particularly in the area effect rate column. The data indicates that the presence of residues notably influences the percentage of residual substances in meat samples. Notably, the Tauweraij area exhibited the highest residue concentration, reaching a concerning 3.00, in sharp contrast to the Al-Hussaina area, which recorded a much lower percentage of 0.56.

In terms of comparing the substances themselves, as shown in the pesticides effect rate of the table, cypermethrin emerged as the substance with the highest survival rate at 2.66. This finding confirms persistence of cypermethrin residues in meat. Furthermore, when examining the intersection of regions and substances, the Tauweraij region emerged again with the highest overall concentration of (3.17). These overlapping data underscore the need for targeted interventions in areas with high waste levels, especially in Tauweraij.

Table (4.9): The effect of geographic region and pesticides on the percentage of residues in meat within the sample and the interaction between them.

Area	pesticides		Area effect rate
	Cypermethrin	ivermectin	
Al-Hussaina	0.85± 0.003 e	0.26± 0.002 d	0.56± 0.06 E
AL Hur	1.82± 0.02 c	1.05± 0.009 a	1.43± 0.08 C
Tauweraij	3.17± 0.01 a	0.82± 0.003 b	3.00± 0.49 A
center	1.04± 0.006 d	0.25± 0.009 d	0.64± 0.09 D
Aeen tumer	2.40± 0.03 b	0.75± 0.007 c	2.57± 0.41 b
pesticides effect rate	2.66± 0.25 a	0.63± 0.04 b	
LSD	Area	pesticides	Area * pesticides
	0.0318	0.0201	0.045

Means with common or similar letters do not differ significantly.

4.2.2. Comparison of pesticides in sheep tail fat

It is shown in table (4.10) of the tail fat samples when comparing the studied areas with each other, shown in area effect rate column, that the residue has a significant effect on the percentage of survival of materials on the tail fat. The highest percentage of survival of materials was in the Aeen al-Tumer area, which reached a percentage of (4.31), compared to the Al-Hussaina area, which reached (0.39). As for the materials compared to each other, which are shown in the row in pesticides effect rate, the highest survival rate was in the first substance (cypermethrin), which reached a rate of (2.64). As for the overlap between regions and materials, the Aeen al-Tumer region for the first substance (cypermethrin), which reached (3.78).

* It indicates the dual interference of LSD between the area and the pesticide

Table (4.10): The effect of geographic region and pesticides on the percentage of residues in tail fat within the sample and the interaction between them.

Area	pesticides		Area effect rate
	Cypermethrin	ivermectin	
Al-Hussaina	0.46± 0.003 c	0.31± 0.003 e	0.39± 0.01 e
AL Hur	0.25± 0.001 e	1.87± 0.007 a	1.06± 0.18 d
Tauweraij	0.42± 0.005 d	1.83± 0.01 c	1.12± 0.16 c
center	2.27± 0.01 b	0.58± 0.004 d	2.92± 0.51 b
Aeen tumer	3.78± 0.03 a	1.85± 0.02 b	4.31± 0.56 a
pesticides effect rate	2.64± 0.40 a	1.29± 0.09 b	
LSD	Area	pesticides	Area * pesticides
	0.0304	0.0192	0.043

Means with common or similar letters do not differ significantly.
* It indicates the dual interference of LSD between the area and the pesticide.

4.2.3. Comparison of pesticides in sheep liver

As shown in table (4.11) when comparing the studied areas with each other and shown in area effect rate column, the residue has a significant effect on the survival rate of materials. The highest percentage of material remains was in the Al-Hussaina area, which reached a percentage of (2.75), compared to the Aeen Al-Tumer area, which reached (0.29). As for comparing the substances with each other, which are shown in pesticides effect rate row, the highest percentage of substance residues was for the second substance (ivermectin), which amounted to (1.49). As for comparing the substances with each other, which are shown in the yellow row, the highest percentage of substance residues was for the second substance (ivermectin), which amounted to (1.49). As for the overlap between areas and substances, it was noted that the free zone was for the second substance (ivermectin), which had a residual rate of (3.74).

Table (4.11): The effect of geographic region and pesticides on the percentage of residues in liver within the sample and the interaction between them.

Area	pesticides		Area effect rate
	Cypermethrin	ivermectin	
Al-Hussaina	3.62± 0.04 a	1.87± 0.009 b	2.75± 0.20 a
AL Hur	0.50± 0.005 b	3.74± 0.04 A	2.12± 0.37 b
Tauweraij	0.19± 0.002 d	0.52± 0.003 d	0.35± 0.03 d
center	0.12± 0.002 e	1.13± 0.003 c	0.62± 0.11 c
Aeen tumer	0.36± 0.005 c	0.21± 0.003 e	0.29± 0.01 e
pesticides effect rate	0.96± 0.19 b	1.49± 0.17 a	
LSD	Area	pesticides	Area * pesticides
	0.0404	0.0256	0.0572

Means with common or similar letters do not differ significantly.

* It indicates the dual interference of LSD between the area and the pesticide

4.2.4. Comparison of pesticides in sheep kidney

As shown in table (4.12), when comparing the studied areas with each other and shown in Area effect rate column, the residue has a significant effect on the percentage of substances remaining on the kidneys. The highest percentage of residues of substances was in the city center area, which reached a percentage of (3.89), compared to the AL Hur area, this amounted to (0.27). As for to compare the materials with each other shown in the row pesticides effect rate, the highest percentage of residues was for the first substance (cypermethrin), which reached a rate of (1.99). As for the overlap between areas and materials, it was the city center area for the first substance (cypermethrin), which reached (4.35), and the Al-Husseiniyah area for the second substance (ivermectin and cypermethrin), which amounted to (3.51) and (3.97).

Pesticides have been used in veterinary medicine interest since the 18th century, when tick-borne diseases became widespread the use of insecticides (internal and external parasiticides) in veterinary medicines is a good thing. It has been documented, and its use has been shown to be beneficial to health for animals and the economy (Akre, 2016). Their heavy use of pesticides has led to their emergence they are widely distributed and transmitted to animals. These pollution method can lead to bioaccumulation of pesticides in food products of animal origin, ultimately leading to the transfer of pesticides to humans via the food chain (Castillo *et al*., 2012).

Table (4.12): The effect of geographic region and pesticides on the percentage of residues in kidney within the sample and the interaction between them.

Area	pesticides		Area effect rate
	Cypermethrin	ivermectin	
Al-Hussaina	3.97± 0.06 b	3.51± 0.01 a	3.74± 0.06 b
AL Hur	0.17± 0.008 d	0.37± 0.002 e	0.27± 0.02 e
Tauweraij	0.18± 0.004 d	0.82± 0.003 d	0.50± 0.07 d
center	4.35± 0.009 A	2.44± 0.01 b	3.89± 0.33 a
Aeen tumer	0.28± 0.001 c	1.68± 0.008 c	0.98± 0.16 c
pesticides effect rate	1.99± 0.31 a	1.76± 0.16 b	
LSD	Area	pesticides	Area * pesticides
	0.0473	0.0299	0.0669

Means with common or similar letters do not differ significantly.

* It indicates the dual interference of LSD between the area and the pesticide

Widespread use of these antiparasitic drugs may pose a potential risk to the consumer if the waste enters the food chain. Therefore, monitoring their residues in meat and other animal products used for human consumption is an important task. Maximum residue limits (MRLs) have been set for a number of avermectins in edible tissues (muscle, liver, kidneys, fat) with the aim of reducing the risks to human health associated with their consumption. To do monitoring confirmatory analytical programs and methods are needed (Seelanan *et al*. 2006).

A comparative study of pesticide pollution was conducted in Karbala Governorate, which included comparison between different geographical regions, including cities and agricultural areas. Levels of contamination with different pesticides were also analyzed, with a focus on the percentage of overlap between types of pesticides. The results showed that there are high levels of pesticide pollution in all areas studied, whether urban or agricultural, which indicates that there is a major problem in environmental pollution with pesticides in the governorate. The results of the analysis of pesticide residues in sheep meat and its various parts showed a marked variation in contamination levels between tissues. Residues of the pesticides cypermethrin and ivermectin were measured in multiple parts of sheep including meat, kidneys, fat, and liver. Sheep meat, kidneys and fat were shown to have higher levels of cypermethrin pesticide residues in these tissues compared to ivermectin.

This suggests that cypermethrin accumulates more in meat, kidneys and fat, which may be a result of its chemical properties and behavior in the body. The results indicate that the levels of cypermethrin residues in the sheep liver were significant, but it should be noted that the percentage of ivermectin residues in the liver was significantly higher compared to other tissues. The liver is the main organ responsible for metabolism and detoxification in the body, which makes it a suitable environment for the accumulation of ivermectin residues. This accumulation may be attributable to the nature of ivermectin as a commonly used veterinary drug, where it interacts differently with the body's tissues.

Analyzes indicate that the liver not only stores these residues, but also shows a greater ability to absorb them than other tissues. In contrast, cypermethrin levels show greater accumulation in meat, kidney, and tail fat, which may reflect differences in how tissues handle these pesticides. Overall, these differences in accumulation could reflect variation in tissue properties in terms of the ability to store or transform pesticides, warranting further research to understand the precise mechanisms that influence these phenomena. Understanding these differences will help improve control strategies and reduce health risks associated with pesticides in animal products.

4.3. Comparison between samples based on the percentage of residues

4.3.1. Ivermectin residues in sheep meat and edible parts

Table as shown in (4.13) analysis of samples of sheep meat and edible products found close to high levels of recommended natural residues, but when studying the remains of ivermectin in the Karbala areas. Al-Hussaina area showed that both liver and kidneys had high concentrations compared to meat and fat, while both liver, fat and meat had high levels in the AL Hur area. The percentages of residues in fat are of high concentration compared to the rest of the samples. As for the whole city center and Ain al-Tumr, the percentages of concentration of residues in each of the liver, kidneys, and meat are high. Therefore, when comparing samples in relation to regions, there is a difference in the percentages obtained. For this reason, tests are conducted on products of animal origin to detect these substances in them.

Table (4.13): Concentrations of ivermectin residues in both sheep meat and edible parts.

ivermectin				
Area	Meat	Fat tail	Liver	Kidney
Al-Hussaina	0.26± 0.002 d	0.31± 0.002 d	1.87± 0.009 b	3.51± 0.01 a
AL Hur	1.05± 0.009 b	1.87± 0.007 a	3.74± 0.04 a	0.37± 0.002 e
Tauweraij	0.82± 0.003 c	1.83± 0.01 b	0.52± 0.003 d	0.83± 0.003 d
center	0.25± 0.009 e	0.58± 0.004 c	1.13± 0.003 c	2.44± 0.01 b
Aeen tumer	2.40± 0.007 a	1.85± 0.02 ab	0.21± 0.003 e	1.68± 0.008 c
LSD	0.0163	0.0366	0.0567	0.0333

Means with common or similar letters do not differ significantly.

4.3.2. Comparison of cypermethrin residues in both sheep meat and edible parts

The results of the analysis indicate a sufficient percentage of the pesticide cypermethrin in the studied groups from the different regions without a clear difference. Simple levels of the substances were found in samples of meat from the Tauweraij area, tail fat from the Aeen tumer area, and kidneys from the city center area. This indicates that the high concentration of cypermarine pesticide is present in all these areas in similar proportions, which indicates the beginning in these areas. Therefore, it could indicate that there is a clear spread of cypermethrin in these areas, with high levels of contamination in all of them.

As it is the case in the rest of the samples bearing the same significant letters, this indicates the similarity between the obtained pollution rates. In on the data in the indicated table (4-14).the assessment of the equal spread of the pesticide in the studied areas. Based on the data in the indicated table (4-14).

Table (4.14) Comparison of cypermethrin residues (PPM) in both sheep meat and edible parts, (Mea±S.E).

Cypermethrin				
Area	Meat	Fat tail	Liver	Kidney
Al-Hussaina	0.85± 0.003 e	0.46± 0.003 c	2.62± 0.0002 a	2.97± 0.0004 ab
AL Hur	1.82± 0.02 c	0.25± 0.001 e	0.50± 0.0002 b	0.17± 0.009 d
Tauweraij	3.17± 0.01 a	0.42± 0.005 d	0.19± 0.04 d	0.18± 0.06 e
center	1.04± 0.006 d	2.27± 0.01 b	0.12± 0.005 e	3.35± 0.008 a
Aeen tumer	2.40± 0.03 b	3.78± 0.005 a	0.36± 0.005 c	0.28± 0.001 c
LSD	0.0624	0.0496	0.0592	0.0899

Means with common or similar letters do not differ significantly.

Regarding the results of ivermectin and cypermethrin, from the comparison of the results of the samples, it is found that the percentages are close, regardless of the regions, there are samples with a high percentage of residues to compare with ivermectin, where he found it through the distribution and metabolism of ivermectin were good documented by (Chiu *et al.*, 1990) with higher residues in the liver and fat and lowest in the muscles. In the liver, ivermectin constitutes the majority (48-60%) of the radiolabeled drug.

(RIvERA *et al*., 2011) also concluded through a study to detect the presence of ivermectin residues in general. 234 liver samples were taken over a period of one month, and ivermectin residues were found to exceed the maximum residue level (MRL). Due to its highly lipophilic nature, ivermectin is widely distributed with a wide volume of distribution (Vd) in all types.

Ivermectin tends to accumulate in fatty tissue, causing it to act as a drug depot. The highest levels of ivermectin are found in the liver and fat, as the liver is the main organ responsible for metabolizing many drugs, including ivermectin. Ivermectin is converted to more excretable forms, resulting in its accumulation in the liver, and kidneys play an important role in filtering chemicals from the blood. After ivermectin is metabolized in the liver, the kidneys can excrete the residue, but some amount may remain in the kidneys for a while (Rohrer and Evans, 1990).

As for cypermethrin, it was found that (regardless of the region) all samples contained cypermethrin residues in proportions exceeding the rate recommended by the World Health Organization, in contrast to the percentages obtained previously (Sartarelli *et al*., 2012). Analytical methods have been applied and validated to measure cypermethrin residues in beef and fat. The samples studied had measurement limits lower than the maximum residue limits (MRL). Presence high level of cypermethrin can be attributed to the regular use of this pesticide by almost all farmers In Iraq to combat ticks, flies, fleas, lice, mites and animal dips on pyrethroid baths or sprayed directly on fields of small animals, or treating seasonal crops, due to its quick effect and low cost toxicity compared to other pesticides (Lainsbury, 2018).

Cypermethrin residues in sheep kidneys have been primarily studied in relation to their distribution and metabolic effects. Research indicates that cypermethrin, a synthetic pyrethroid, can accumulate in various internal organs, including the kidneys, after exposure. It is worth noting that one study found that after cypermethrin administration, the order of tissue residue levels was muscle, spleen, kidney, and liver, with significant levels of residue appearing in the kidney (Oriňák, 1993). Furthermore, exposure to cypermethrin has been linked to changes in drug-metabolizing enzymes in sheep, suggesting potential effects on kidney function due to metabolic changes, the presence of cypermethrin residues raises concerns about food safety and animal health, especially in livestock used for dairy and meat production (Sheweita *et al*., 2012).

Extraction methods used to quantify these residues have shown high recovery rates, indicating the reliability of the results. In the liver, high levels of cypermethrin residues have been consistently found, suggesting its role in detoxification and metabolism. Kidneys, the large accumulation of wastes indicates the involvement of the kidneys in excretory processes, and muscle residues are also present, raising concerns about the safety of meat consumption (Chaparro *et al*., 2014). The presence of cypermethrin residues in edible tissues necessitates monitoring to ensure compliance with safety standards, as residues can pose health risks to consumers (Dahamna *et al*., 2011).

Chapter Five

Conclusions and Recommendations

5. Conclusions and Recommendations

5.1. Conclusions

According to the results obtained from the current study, the following, conclusions can be drawn:

1. The rate of contamination with antiparasitic agents (cypermethrin, ivermectin) reached 90% in all samples.
2. The all regions were highly polluted with pesticides, but according to the different samples.
3. The tail fat samples exhibited elevated levels of cypermethrin, while kidney samples showed high concentrations of ivermectin.
4. A comparison between cypermethrin and ivermectin revealed that cypermethrin concentrations were notably higher in the samples.

5.2. Recommendations

From the conclusions, the following can be recommended:

1. Use the high-performance liquid chromatography (HPLC) method to separate and detect residual substances in samples in order to ensure the health of consumers.
2. Conducting research to detect residues of other medications used to eliminate parasites, such as (Mebendazole, Albendazole and Praziquantel).
3. Awareness must be promoted among farmers and breeders about the correct use of pesticides and veterinary chemicals in accordance with international standards for food and environmental safety.
4. Research and development in the field of biological and chemical analysis should be enhanced to examine and analyze chemical residues in foods and animal tissues, which will help in developing more accurate and effective methods for detecting these substances.
5. To detect the negative effects of residual medications used on the individual and internal organs and studying impact of these residues on the consumer.
6. Use HPLC to detected cypermethrin residue in row milk and dairy products

Chapter Six

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Appendixes

Appendix I:

Extraction process materials

Appendix II:

Sample extraction process

Appendix III:

Extraction results.

Appendix IV:

The (HPLC) device used in the experiment.

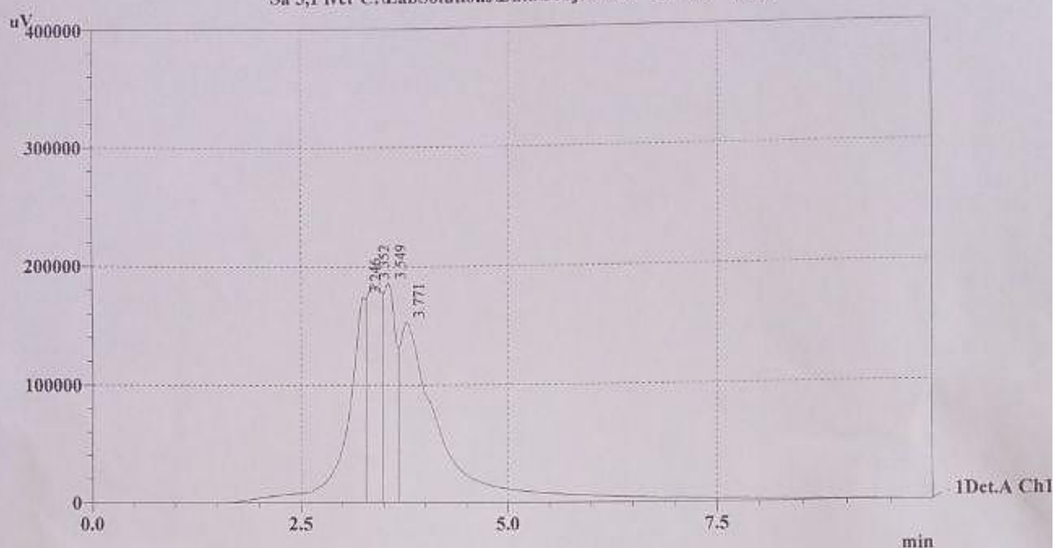
Appendix V:

Sample Information

Acquired by	: Admin
Date Acquired	: 07/03/2024 11:00:51 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa 5,1 iver
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 2
Vial#	: 2
Injection Volume	: 20
Data File	: Sa 5,1 iver 30.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 07/03/2024 11:10:55 ص

Chromatogram

Sa 5,1 iver C:\LabSolutions\Data\Project1\Sa 5,1 iver 30.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.246	2768940	175082	22.200	24.945
2	3.352	2262911	186410	18.143	26.559
3	3.549	1830147	186542	14.673	26.577
4	3.771	5610853	153849	44.985	21.919
Total		12472852	701883	100.000	100.000

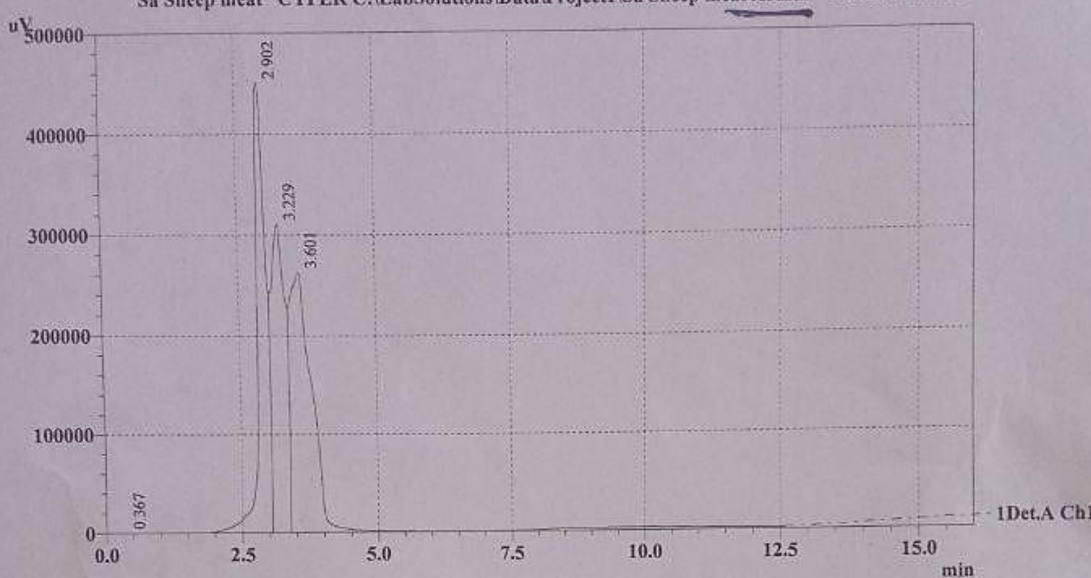
Appendix VI:

Sample Information

Acquired by	: Admin
Date Acquired	: 03/03/2024 11:01:14 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat CYPER
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 5
Injection Volume	: 20
Data File	: Sa Sheep meat Al Hur CYPER 140.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 03/03/2024 11:13:55 ص

Chromatogram

Sa Sheep meat CYPER C:\LabSolutions\Data\Project1\Sa Sheep meat Al Hur CYPER 140.lcd



1 Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.367	1321	76	0.007	0.007
2	2.902	5778849	453034	31.526	43.987
3	3.229	5501679	312844	30.014	30.375
4	3.601	7048828	263975	38.454	25.630
Total		18330678	1029930	100.000	100.000

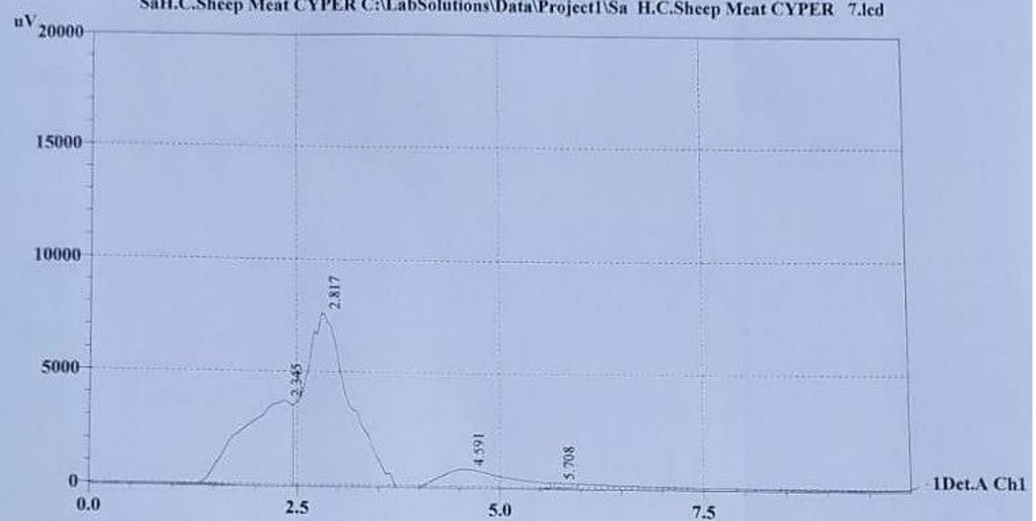
Appendix VII:

Sample Information

Acquired by	: Admin
Date Acquired	: 04/03/2024 09:32:57 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: SaH.C.Sheep Meat CYPER
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 16
Injection Volume	: 20
Data File	: Sa H.C.Sheep Meat CYPER 7.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 04/03/2024 09:43:18 ص

Chromatogram

SaH.C.Sheep Meat CYPER C:\LabSolutions\Data\Project1\Sa H.C.Sheep Meat CYPER 7.lcd



1 Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.345	163220	3892	29.958	29.832
2	2.817	311657	7867	57.202	60.302
3	4.591	66422	1043	12.191	7.996
4	5.708	3535	244	0.649	1.870
Total		544834	13046	100.000	100.000

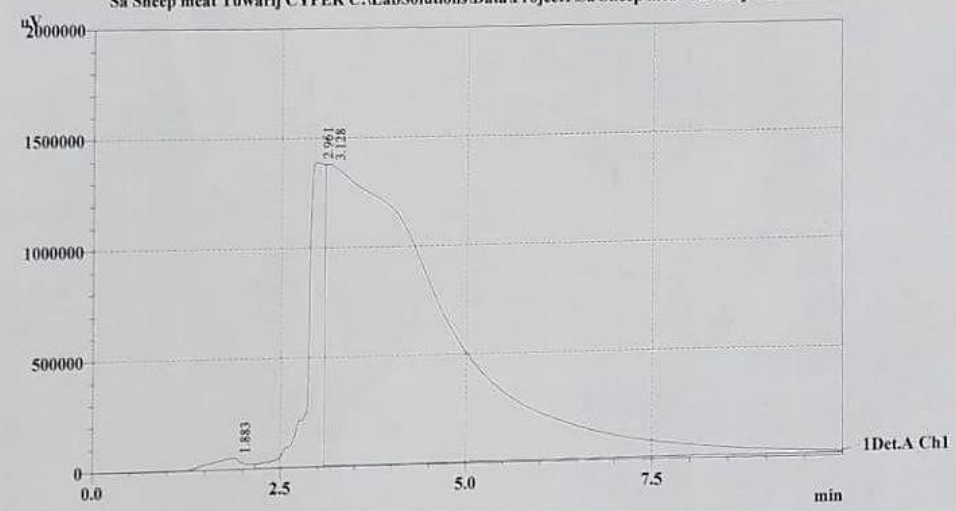
Appendix VIII:

Sample Information

Acquired by	: Admin
Date Acquired	: 03/03/2024 12:43:11
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat Tuwarij CYPER
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 11
Injection Volume	: 20
Data File	: Sa Sheep meat Tuwarij CYPER 2.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 03/03/2024 12:54:02

Chromatogram

Sa Sheep meat Tuwarij CYPER C:\LabSolutions\Data\Project1\Sa Sheep meat Tuwarij CYPER 2.lcd



1 Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.883	1622783	52726	0.905	1.870
2	2.961	21016588	1388280	11.717	49.231
3	3.128	156731599	1378905	87.378	48.899
Total		179370970	2819912	100.000	100.000

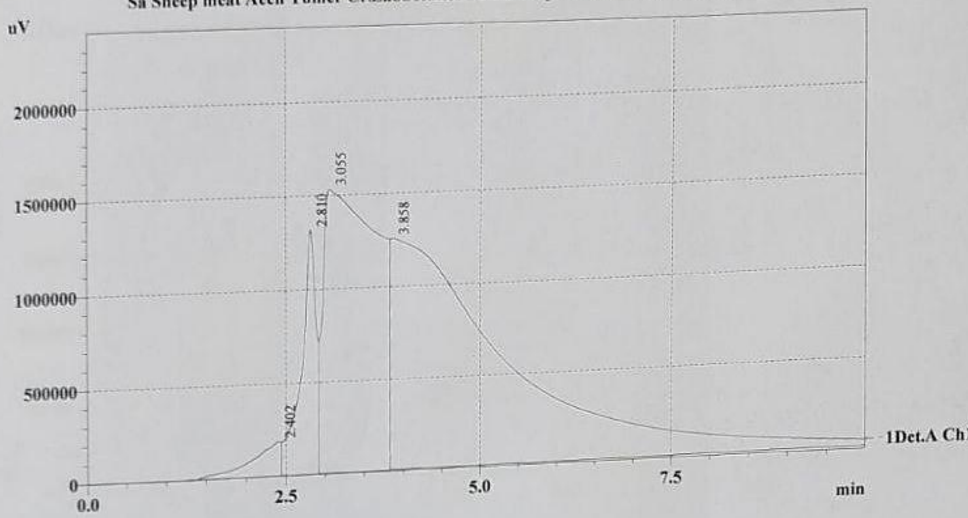
Appendix IX:

Sample Information

Acquired by	: Admin
Date Acquired	: 03/03/2024 10:12:11 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat Aeen Tumer
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 1
Injection Volume	: 20
Data File	: Sa Sheep meat Aeen Tumer 136.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 03/03/2024 10:24:43 ص

Chromatogram

Sa Sheep meat Aeen Tumer C:\LabSolutions\Data\Project1\Sa Sheep meat Aeen Tumer 136.lcd



1 Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.402	4847542	184706	2.080	4.313
2	2.810	17219761	1316440	7.388	30.740
3	3.055	73674045	1529623	31.611	35.718
4	3.858	137323763	1251688	58.921	29.228
Total		233065111	4282457	100.000	100.000

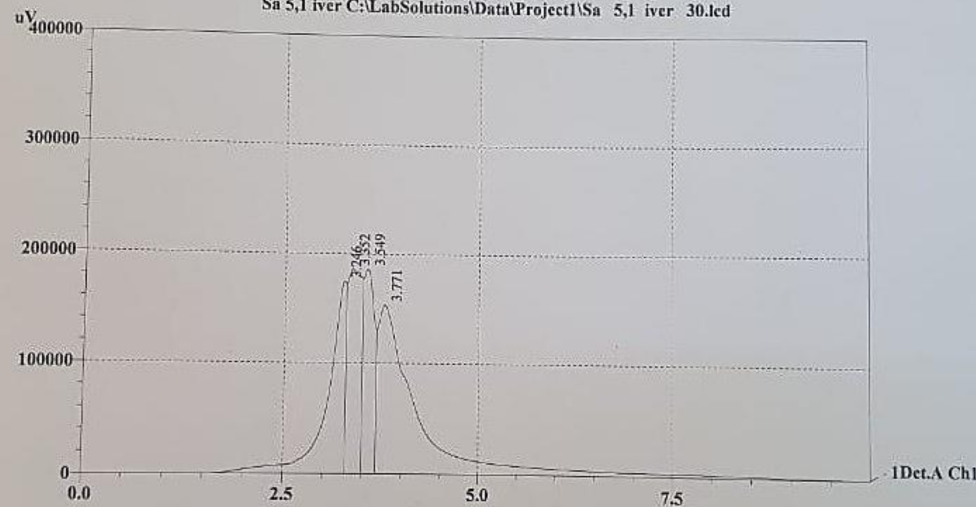
Appendix X:

Sample Information

Acquired by	: Admin
Date Acquired	: 07/03/2024 11:00:51 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa 5,1 iver
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 2
Vial#	: 2
Injection Volume	: 20
Data File	: Sa 5,1 iver 30.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 07/03/2024 11:10:55 ص

Chromatogram

Sa 5,1 iver C:\LabSolutions\Data\Project1\Sa 5,1 iver 30.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.246	2768940	175082	22.200	24.945
2	3.352	2262911	186410	18.143	26.559
3	3.549	1830147	186542	14.673	26.577
4	3.771	5610853	153849	44.985	21.919
Total		12472852	701883	100.000	100.000

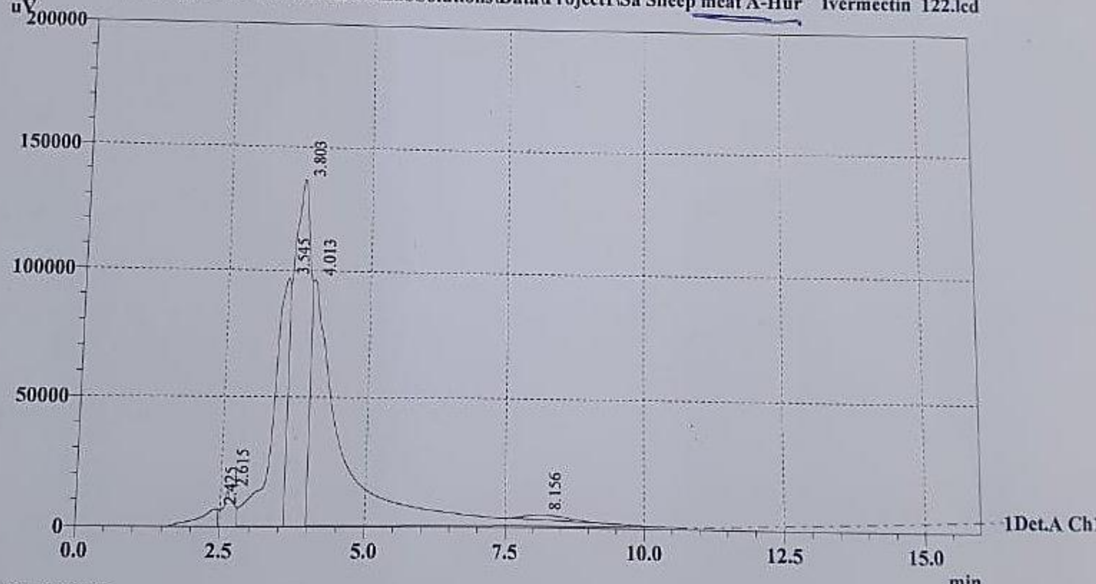
Appendix XI:

Sample Information

Acquired by	: Admin
Date Acquired	: 29/02/2024 11:26:23 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat Ivermectin
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 2
Vial#	: 6
Injection Volume	: 20
Data File	: Sa Sheep meat A-Hur Ivermectin 122.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 29/02/2024 11:37:03 ص

Chromatogram

Sa Sheep meat Ivermectin C:\LabSolutions\Data\Project1\Sa Sheep meat A-Hur Ivermectin 122.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.425	150570	6481	1.733	1.842
2	2.615	169193	13387	1.947	3.805
3	3.545	1666979	96843	19.181	27.527
4	3.803	2678038	136538	30.815	38.810
5	4.013	3906737	96446	44.953	27.414
6	8.156	119106	2117	1.371	0.602
Total		8690623	351813	100.000	100.000

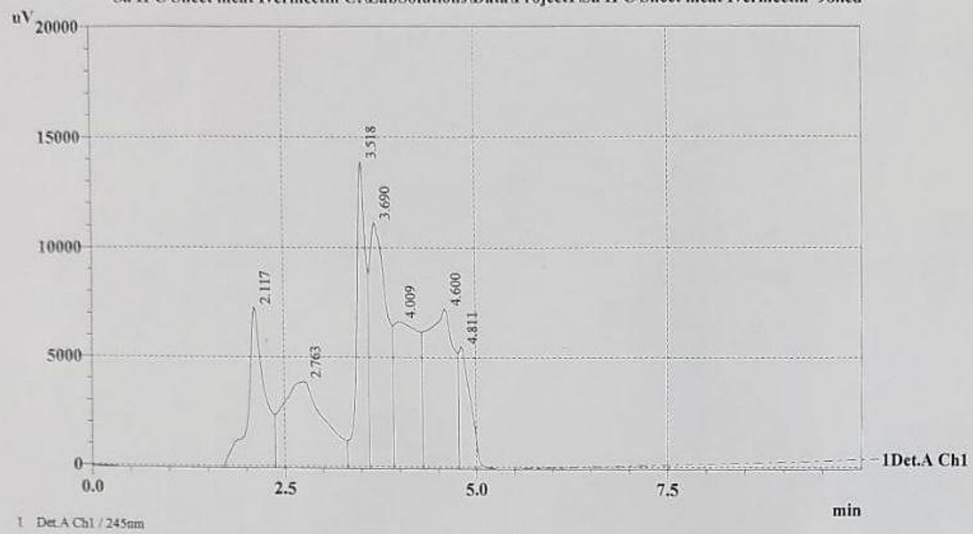
Appendix XII:

Sample Information

Acquired by	: Admin
Date Acquired	: 25/02/2024 11:58:01 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa H C Sheet meat Ivermectin
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 3
Injection Volume	: 20
Data File	: Sa H C Sheet meat Ivermectin 98.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 25/02/2024 12:05:47 م

Chromatogram

Sa H C Sheet meat Ivermectin C:\LabSolutions\Data\Project1\Sa H C Sheet meat Ivermectin 98.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.117	122520	7620	11.802	13.004
2	2.763	170994	4268	16.471	7.283
3	3.518	129429	14318	12.467	24.433
4	3.690	178777	11558	17.221	19.723
5	4.009	155246	7102	14.954	12.120
6	4.600	193482	7712	18.637	13.159
7	4.811	87706	6023	8.448	10.278
Total		1038154	58602	100.000	100.000

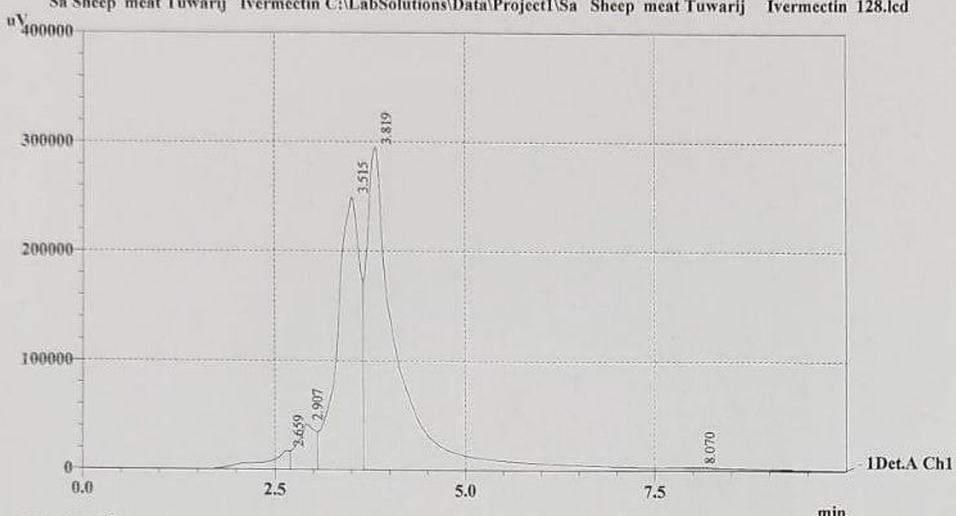
Appendix XIII:

Sample Information

Acquired by	: Admin
Date Acquired	: 29/02/2024 12:55:53 م
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat Tuwarij Ivermectin
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 2
Vial#	: 12
Injection Volume	: 20
Data File	: Sa Sheep meat Tuwarij Ivermectin 128.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 29/02/2024 01:06:30 م

Chromatogram

Sa Sheep meat Tuwarij Ivermectin C:\LabSolutions\Data\Project1\Sa Sheep meat Tuwarij Ivermectin 128.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.659	394112	17490	2.639	2.895
2	2.907	674090	41687	4.513	6.900
3	3.515	5166203	249208	34.590	41.249
4	3.819	8654802	294862	57.947	48.806
5	8.070	46483	902	0.311	0.149
Total		14935691	604149	100.000	100.000

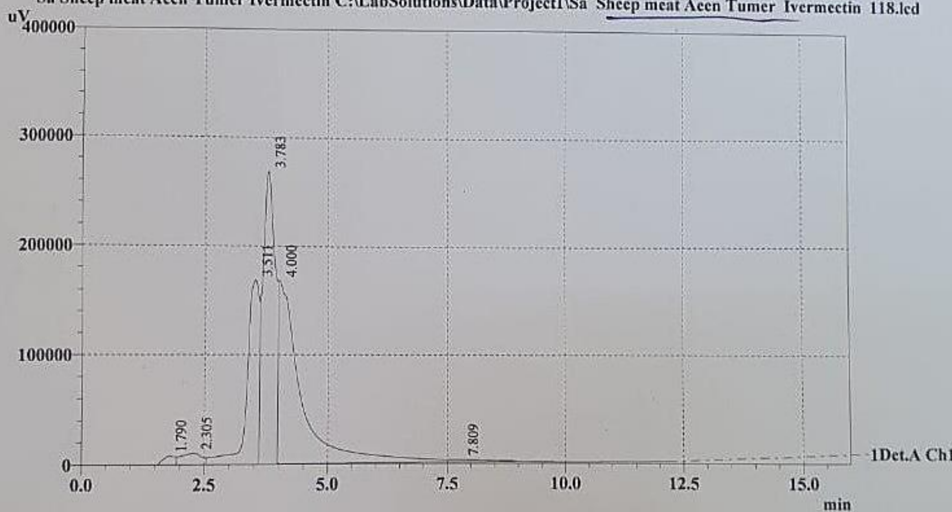
Appendix XIV:

Sample Information

Acquired by	: Admin
Date Acquired	: 29/02/2024 10:20:41 ص
Sample Type	: Unknown
Level#	: 1
Sample Name	: Sa Sheep meat Aeen Tumer Ivermectin
Sample ID	:
ISTD Amount	: [1]=1 [2]=1 [3]=1 [4]=1 [5]=1
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 2
Vial#	: 2
Injection Volume	: 20
Data File	: Sa Sheep meat Aeen Tumer Ivermectin 118.lcd
Method File	: Eman Mohammed.lcm
Original Method	: C:\LabSolutions\Data\Project1\Eman Mohammed.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	:
Processed by	: Admin
Modified Date	: 29/02/2024 10:32:56 ص

Chromatogram

Sa Sheep meat Aeen Tumer Ivermectin C:\LabSolutions\Data\Project1\Sa Sheep meat Aeen Tumer Ivermectin 118.lcd



1 Det.A Ch1 / 245nm

PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.790	154966	8728	1.127	1.393
2	2.305	331484	11069	2.412	1.767
3	3.511	2917253	168854	21.224	26.953
4	3.783	4600959	268965	33.473	42.933
5	4.000	5712951	168276	41.563	26.861
6	7.809	27566	579	0.201	0.092
Total		13745179	626471	100.000	100.000

Appendix XV

Table showing the distance measurements from Al-Hussaina (Starting Point) to other areas in Karbala

Starting area	**End zone**	**Distance**
Al-Hussaina	AL-Hur	17.78 /km
Al-Hussaina	City center	6.21 /km
Al-Hussaina	Tauweraij	15.92 /km
Al-Hussaina	Aeen tumer	63.56 /km

يعد تلوث اللحوم والمنتجات الحيوانية الصالحة للاستهلاك البشري بمخلفات الأدوية البيطرية خطراً محتملاً على صحة المستهلكين. وذلك باستخدام طرق دقيقة للكشف عن أدنى مستويات هذه المخلفات. تم إجراء دراسة البقايا المضادة للطفيليات على 125 عينة (50 عينة من لحم الأغنام و 75 عينة من الأجزاء الصالحة للأكل) تم جمعها من مناطق مختلفة من محلات الجزارة. تهدف هذه الدراسة إلى تحديد متبقيات الأدوية المضادة للطفيليات (الإيفرمكتين والسايبرمثرين) في لحوم الأغنام والأجزاء الصالحة للأكل (الكبد والكلى ودهون الذيل) من الأغنام في خمس مناطق مختلفة في محافظة كربلاء/العراق، باستخدام كروماتوغرافيا سائلة عالية الأداء. تقنية (HPLC). من تشرين الأول 2023 إلى كانون الاول 2024.

وأظهر التحليل أن 96% من اللحوم و95% من الأجزاء الصالحة للأستهلاك البشري كانت ملوثة بما يزيد عن الحدود القصوى للبقايا بمادة السايبرمثرين، أما بقايا الإيفرمكتين فقد كانت 90% في جميع العينات (اللحوم والأجزاء الصالحة للأستهلاك البشري) موجبة لبقايا الايفرمكتين. وقد وجد التحليل الإحصائي للبيانات وجود فروق معنوية (P<0.05) بالنسبة للعوامل المضادة للطفيليات في لحوم الأغنام ومنتجاتها. تأثير المبيدات الحشرية على اللحوم. وكانت نسبة تأثير السايبرمثرين أعلى من نسبة تأثير الإيفرمكتين، إذ بلغت نسبة تأثير السايبرمثرين (2.66) اما الايفرمكتين (0.63).

اما بالنسبة للأجزاء الصالحة للأكل فقد كانت نسبة التلوث بالإيفرمكتين أعلى من السايبرمثرين في الكبد بمعدل (1.49)، وفي الكلى كانت نسبة التلوث بالسايبرمثرين أعلى بمعدل (1.99). كذلك بالنسبة لدهون الذيل كان التلوث بالسايبرمثرين أعلى بمعدل (2.64). وكانت تراكيز بقايا المبيدات بالترتيب التالي: اللحوم > دهن الذيل > الكلى > الكبد. أما بالنسبة لنسبة التأثير على المنطقة فقد جاءت نسبة متبقيات كل من السايبرمثرين والإيفرمكتين في لحوم الأغنام حسب كل منطقة على النحو التالي: الحسينية (0.56)، الحر (1.43)، طويريج (3.00)، المركز (0.09±0.64)، عين التمر (2.57)، كما تبين أن منطقة الطويريج لديها نسبة تلوث أعلى مقارنة بالمناطق الأخرى. أما بالنسبة لدهون الذيل فكانت النسب: الحسينية (0.39)، الحر (1.06)، طويريج (1.12)، المركز (2.92)، عين التمر (4.31)، حيث كانت منطقة عين التمر هي الأعلى في تلوث. وكانت مستويات التلوث في الكبد على النحو التالي: الحسينية (2.75)، الحر

(2.12)، طويريج (0.35)، المركز (0.62)، عين التمر (0.29) وكانت منطقة الحسينية هي الأعلى تلوثاً.أما الكلى فكانت على النحو التالي: الحسينية (3.74)، الحر (0.27)، طويريج (0.50)، المركز (3.89)، عين التمر (0.98). وتبين أن مركز المدينة لديه أعلى نسبة تلوث.

أما بالنسبة لتلوث العينات بالسايبرمثرين فقد كانت نسب التلوث في اللحوم حسب كل منطقة (0.85، 1.82، 5.17، 1.04، 2.40)، أما دهن الذيل فكان كالآتي: (0.46، 0.25، 0.42، 5.27، 6.78). الكبد (3.62، 0.50، 0.19، 0.12 و 0.36) والكلى: (3.97، 0.17، 0.18، 5.35 و 0.28). تلوث العينات بالإيفرمكتين كانت معدلات التلوث في اللحوم حسب كل منطقة (0.26، 1.05، 0.82، 0.25 و 0.75) ودهن الذيل (0.31، 1.87، 1.83، 0.58 و 1.85)، الكبد (1.87، 3.74، 0.52، 1.13). و 0.21). والكلى: (3.51، 0.37، 0.82، 2.44، 1.68).

وبحسب الحدود القصوى للمخلفات المسموح بها من قبل منظمة الصحة العالمية ومنظمة الأغذية والزراعة تبين أن بقايا العوامل المضادة للطفيليات (سايبرمثرين وإيفرمكتين) في لحوم الأغنام ومنتجاتها كانت أعلى من الحدود القصوى للمخلفات. وهذا يدل على الاستخدام الخاطئ للمبيدات الطفيلية أثناء الاستخدام، وكذلك عدم الالتزام بتعليمات الطبيب البيطري.

وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الصحة العامة البيطرية

الكشف عن بقايا المضادات الطفيلية في اللحوم والأجزاء الصالحة للأكل من الأغنام في محافظة كربلاء

رسالة مقدمة إلى مجلس كلية الطب البيطري / جامعة كربلاء لاستكمال جزئي لمتطلبات درجة الماجستير في العلوم في الطب البيطري / الصحة العامة البيطرية

اعداد

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باشراف

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1446 هـ   2024 م