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University of Kerbala
College of Veterinary Medicine
Department of Veterinary Public Health

Detection of Ectoparasiticide in Cattle Products (Meat, Milk and Milk Products) in Karbala Province

Thesis submitted to the Council of the College of Veterinary Medicine/University of Karbala, as a Partial Fulfillment of the Requirement for the Degree of Master in Science of Veterinary Medicine/Veterinary Public Health

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2024 **1446**

بِسْمِ اللَّهِ الرَّحْمَـٰنِ الرَّحِيمِ

فَوَجَدَا عَبْدًا مِّنْ عِبَادِنَآ ءَاتَيْنَـٰهُ رَحْمَةً مِّنْ عِندِنَا
وَعَلَّمْنَـٰهُ مِن لَّدُنَّا عِلْمًا ﴿٦٥﴾ قَالَ لَهُۥ مُوسَىٰ هَلْ
أَتَّبِعُكَ عَلَىٰٓ أَن تُعَلِّمَنِ مِمَّا عُلِّمْتَ رُشْدًا ﴿٦٦﴾

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We certify this thesis entitled **(Detection of Ectoparasiticide in Cattle Products Meat, Milk and Milk Products in Kerbala Province)**carried out by **(Zainab Ali Jabur)** has been prepared under our supervision at the College of Veterinary Medicine/University of Karbala as a partial fulfillment of the requirement for the degree of Master in science of Veterinary Medicine/Veterinary Public Health.

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In view of the available recommendation, I forward this thesis to debate by the examining committee.

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Committee Certification

We, the members of the examining committee, certify that we have read this thesis entitled **(Detection of Ectoparasiticide in Cattle Products Meat, Milk and Milk Products in Kerbala Province)** presented by **(Zainab Ali Jubar)** from the department of (Veterinary Public Health) and we have examined the student in its contents. We have found that it is adequate for the award of the Degree of Master in **Science of Veterinary Medicine/ Veterinary Public Health.**

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Dedication

To the one who is closer to me than my jugular vein, who guards me, protects me, and takes care of me, my God Almighty

To those whom God recommended me to them goodness, mercy, and love, my father and mother

To those whom God supported me with them brothers and sisters

To the light of my eyes and the joy of my heart, my nephew and my niece

To my esteemed teachers

I dedicate my humble scientific work

Zainab Ali Jabur

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Summary

This study was planned to investigate the residue of some pesticides (cypermethrin and ivermectin) in 200 animal food products (cattle meat, milk, cheese and ghee) by High Performance Liquid Chromatography (HPLC), which compared with the maximum residual levels (MRLs) of pesticides topic by the World Health Organization (WHO). The samples were collected from the local market and butcher shop from different districts (Al- Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) of Karbala province.

The current results of cypermethrin revealed a significant increase is at ($P \leq 0.0001$) in cattle meat samples of (Al-Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) for (0.1569; 5.408; 6.409; 5.509 and 5.408**)** ppm, respectively compared with WHO (0.03) ppm. The results of residues of cypermethrin in cow milk showed a high significant increase values specially in (Ain Al-tumar, Al Hur, Twairij, Center and Al-Hassainya) that registered (5.651, 4.778, 0.1569 ,3.073 and 0.1026) ppm respectively compared with WHO (0.015)ppm. All cheese samples collected from different districts of Kerbala province (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) for (0.3271,5.720, 6.127,0.07613 and 4.625) respectively had a significant difference compared with maximum residue level (0.015)ppm of cypermethrin and all ghee samples were purchased from various districts of Kerbala province (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) for (0.06863 , 5.632, 0.1700, 2.788 and 0.0452) respectively had a significant difference compared with maximum residue level (0.2)ppm of cypermethrin. On the same side, the ivermectin residue showed less significant residue level than cypermethrin residues and the result showed that all meat samples were collected from different districts of Kerbala province (Al-Hassainya,

city center, Al-Hur, Twairij and Ain Al-Tamer) for (0.4293,0.1969,0.2425,0.4461 and 0.7081) respectively had a significant difference compared with maximum residue level(0.026)ppm of ivermectin pesticide; the milk samples were collected from different district of Kerbala province (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) recorded (0.6843, 1.065, 0.04360, 0.3634 and 0.4288) respectively had a significant difference compared with maximum residue level (0.01) ppm of ivermectin pesticide.

All cheese samples were collected from different districts of Kerbala province (Al-Hassainya, Center, Twairij, Al-Hur, Ain Al-Tumar) for (0.4262, 0.0075, 0.3336, 0.2453and 0.0052) respectively had a significant difference compared with maximum residue level (0.01)ppm of ivermectin pesticide and all ghee samples were collected from different district of Kerbala province (Al-Hassainya, Center, Twairij, Al-Hur, Ain Al-Tumar) (0.4023, 0.3786 , 0.3595,3.858 and 0.1362) respectively had a significant difference compared with maximum residue level of ivermectin (0.4) pesticide in the (WHO) index for 2022 year.

We conclude the high levels of pesticides (cypermethrin and ivermectin) in some food products of animal origins (meat, milk, cheese, and ghee) that were found in the results of this study are attributed to the use of high dose of pesticides for the purpose of eliminating ticks spread in Iraqi governorates**.**

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List of Abbreviations

No.	Abbreviations	Full Name
1.	PTDs	Pyrethroids
2.	CYP	Cypermethrin
3.	IVM	Ivermectin
4.	MRL	Maximum Residue Level
5.	Bti	Bacillus Thuringiensis
6.	PIPs	Protective Instruments Plants
7.	LD_{50}	Lethal Dose 50
8.	GABA	Gamma-Amino butyric acid
9.	T	Tremor-type syndrome
10.	LPO	Lipid peroxidation
11.	ROS	Reaction Oxygen Species
12.	Ca^{++}	Ionized Calcium
13.	DNA	Deoxyribose Nucleic Acid
14.	ACHE	Acetylcholine esterase enzyme
15.	ACH	Acetylcholine
16.	LOP	Lowest Level of Detected

17.	GluCls	Glutamate Gated Chloride
18.	Cis	Indicates that functional groups substituents are on the same side of same plane
19.	TBEV	Tick -Borne Encephalitis Virus
20.	CCHF	Crimea and congo hemorrhagic fever virus
21.	HPLC	High-performance liquid chromatography
22.	tR	Retention Time
23.	ACN	Acetonitrile
24.	TCA	Trichloroacetic Acid
25.	NMT	N-Methylimidazole
26.	WHO	World Health Organization
27.	ANOVA	Analysis of Variance
28.	Arc GIs	Earth Client Geographic Information System
	ODs	Octadacylsilyl Group
29.	UV detector	Ultra violate detector
30.	S.D	Standard Deviation
31.	PPM	Part Per million
32.	B. Cells (NF-κB)	The nuclear factor kappa B
33.	HIV-1	Human Immunodeficiency Virus-1
34.	IMP α /BL	Importin
35.	DENV	Dengue virus
36.	SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
37.	H2B1a	highly-active broad-spectrum antiparasitic
38.	LC	Liquid chromatography
39.	ARFD	acute reference dose
40.	PPDB	Pesticide Properties Database
41.	CAC	Chemometrics in Analytical chemistry
42.	Trans	Transverse
43.	LOD	Limit of determination
44.	HIV-1	Human Immunodeficiency Virus-1
45.	TCA	Trichloroacetic Acid

46.	CPM	
47.	LOQ	Lowest Concentration
48.	DLM	Deltamethrin

Chapter One
Introduction

1.Chapter One:

1.1. Introduction:

The use of pesticides to increase production is very important, but pesticide residues remaining in animal products have serious consequences for human health (Raheem & Niamah, 2021). Pesticides have emerged as a global threat to human health, especially in developing countries (Fazal *et al*., 2022).The use of veterinary drugs in food-producing animals has recently become a public health problem. These drugs are widely used in the prevention and treatment of parasitic diseases in farm animals (Baz *et al*., 2014).

Controlling livestock parasites are important from a production point of view, as they cause significant economic losses due to reduced production (Müller *et al*., 2020). Pyrethroids are the most common of the commercial pesticides and represent almost a quarter of the pesticide market these materials are widely used in agriculture, residential areas (indoor and outdoor), public health, disinfection of stored products and veterinary medicine (Wren *et al*., 2021).

Cypermethrin (CYP) is a man-made toxic chemical compound that belongs to the environmentally polluting pyrethroid insecticides(Aioub *et al*., 2021). However, there are reports that pyrethroids can also accumulate in fat of mammalian body. Some of the more serious toxic effects in humans include respiratory, brain, and gastrointestinal symptoms, as well as skin and eyes (Hudson *et al*., 2014).

Ivermectins are semi-synthetic macrocyclic lactones derived from avermectin, a chemical produced by *Streptomyces avermitilis* during fermentation. Ivermectins are commonly used to treat diseases caused by endo- and ectoparasites in various species, including cattle (Gupta *et al.*, 2020). Ivermectin (IVM) is one of the most widely used antiparasitic drugs worldwide and has become the drug of choice for anthelmintic and acaricide treatment in production beef cattle. Drugs used in production animals require a withdrawal period after treatment to avoid residual concentrations above a specified maximum residue level (MRL) (Brossi *et al*., 2024).

It is estimated that 5.2 billion pounds of pesticides are used worldwide each year. The use of pesticides to control pests has become a common practice worldwide. Its use is not limited to agricultural lands, but is also used in homes as sprays, poisons and powders against cockroaches, mosquitoes, rats, insects, ticks and other pests (Kaur *et al*., 2019). Although the largest number of pesticides are used in developed countries, their use has increased significantly in developing countries in recent decades (Damalas & Koutroubas, 2016).

To combat pesticide resistance in target pests and diseases, farmers have generally increased the amount, concentration and frequency of pesticide application (Schreinemachers & Tipraqsa, 2012). This continuous increase in dependence on pesticides has led to dramatic consequences for the environment and human health, including chronic human diseases such as Parkinson's disease, Alzheimer's disease or cancer (Rani *et al*., 2021) ;and the contamination of food sources, land and water and the destruction of beneficial pollinators and natural enemies of pests (Stehle & Schulz, 2015).

The effects of pesticides on human health and non-target organisms have been a source of global concern for more than four decades and are the basis of most legislation aimed at controlling or prohibiting the use of specific products. Despite the high environmental, health and financial costs, farmers continue to use pesticides, often in inappropriate doses. In general, most research on pesticide abuse in developing countries has been done at the farmer level, assuming that pesticide abuse is largely their responsibility. Examples include studies of large-scale extension programs related to safety precautions (Mengistie *et al*., 2017).

To ensure that pesticide residues do not end up in food or feed at levels that pose an unacceptable risk for human consumption, maximum residue limits (MRL) are established by the European Commission. MRLs are legal upper limits for the concentration of pesticide residues in or on food or feed. They are in place for a wide range of food products of plant and animal origin, and generally apply to the product once it is placed on the market. The MRLs are not only established as toxicological thresholds, they are derived from a complete evaluation of the properties of the active substance and the behavior of the residues (Kowalska *et al*., 2020).

The analysis of multicomponent samples is a difficult and delicate problem for chemical analysts. High performance liquid chromatography (HPLC) is recognized as a good analytical technique to address this problem (Amr & Sarah, 2020).

Most of used pesticides are toxic and have caused a number environmental concerns, especially regarding risks to human and animal health. Thus , these potentially toxic chemicals have proven to be a major threat" A health." One Health is a collective approach deal with humans, animals and environmental (A. Kumar *et al*., 2019). Maximum residue limits were set by national governments and the international government due to the serious negative impacts of using pesticides heavily in agricultural activities around the world. Maximum residue limitations are influenced by environmental and agricultural circumstances, which differ from one country to another(Abdulrahman *et al*., 2023)

Aims of study:

1-Finding the percentage of anti-parasitic residues in meat, milk and milk products.

2-Assessing the fitness of local meat, milk and milk product for human consumption.

3-Raising awareness of the importance of using the correct and safe doses of anti-parasites and commitment with withdrawal period to avoiding the risks associated with the precipitation of these substances in food products of animal origin.

Chapter Two

Review of the Related Literature

2. Review of the Related Literatures:

2.1. Pesticides:

The word "pesticides" is a complex word that encompasses all compounds that are applied to destroy or regulate pests; this includes insecticides (insects), herbicides (weeds) and fungicides (fungi). One of the primary sponsors of the green revolution was finding ways to improve and use safe pesticides to control the wide range of herbal and insect pests, which affect negatively the quantity and quality of world food production (Hassaan & El Nemr, 2020).

Pesticides have many effects on the environment and humans. Due to the wide variety of active ingredients and different mechanisms of action, a general classification of the different classes of insecticides cannot be determined (Garraway, 2020) . However, the unwise use of pesticides has led to spread of their residues on land in soil, water, crops, seasonal weeds and animal feed. Thus, a portion of the pesticide can be stored in the tissues of animals when they are fed feed contaminated with these pesticides (Gullick *et al*., 2016).

On the other hand, indiscriminate usage of these chemicals has led to several environmental implications and caused adverse effects on human health (Sharma *et al*., 2020). Insecticides work by attracting, and then killing insects. Pests can generally be defined as plants or animals that threaten our food, health and/or comfort. Pesticide use has increased by percent in recent years (Kaur *et al*., 2019).

2.1.2. Classification of Pesticides:

The word "pesticides" is a broad term that includes insecticides, herbicides, fungicides, and others rodenticides that can be used to kill some specific pests (Abubakar *et al*., 2020).

2.1.2.1. Natural (bio-pesticides):

Pesticides are chemical or biological substances that are designed to destroy insects or prevent damage caused by pests. According to their source, pesticides can be classified as chemical pesticides and biopesticides. The key benefits of using pesticides is unique. While pesticides are often very broad and target a large group of organisms, they only work on target birds and related organisms. Pesticides are generally harmful to the environment because they are toxic, break down easily, and are in low demand. Chemical Pesticides cause significant environmental pollution because they are highly toxic not always biodegradable (Yadav & Devi, 2017).

Another important advantage of using biocides is the fact that it is less susceptible to genetic modification in the plant population. This confirms the low chance of pest resistance to insecticides. This is something we rarely see in the case of chemical pesticides. It is further divided into organochlorine, organophosphate, carbamate. Biocides is a group of pesticides that is derived from natural materials such as animals, plants and microorganisms (bacteria, viruses, fungi, and nematodes). They are classified into three groups (Yadav & Devi, 2017).

2.1.2. 1.1.Microbial pesticides:

The active ingredient in microbial pesticides is microorganisms e.g Bacteria, fungi, or protozoa. These pesticides kill insects either through toxins secreted by microbial organisms, or through infection by organisms, two the most common pesticides that fall into this group include bacterial pesticides toxin produced by Bacillus thuringiensis (Bti), live Bacillus bacteria Spherical (B). The mode of action in general is to produce a protein that. It binds to receptors in the larval gut, causing the larvae to starve. These two bacteria poisons are used against mosquito larvae and black fly larvae. Most microbial insecticides are more selective than biochemical pesticides

(Ayilara *et al*., 2023).Unlike synthetic pesticides, microbial pesticides have unique effects, are easily available without expensive chemicals, and are environmentally friendly without any damage (Valicente, 2019).

2.1.2. 1.2.Plant incorporated protectants:

These groups of pesticides are produced by plants naturally. And so is the gene necessary for the production of pesticides is introduced into the plant through Genetic Engineering. Hence, the pesticide produced by this plant and together, the introduced genetic material is defined as a compact plant Protective Instruments (PIPs) (Ali *et al*., 2024).

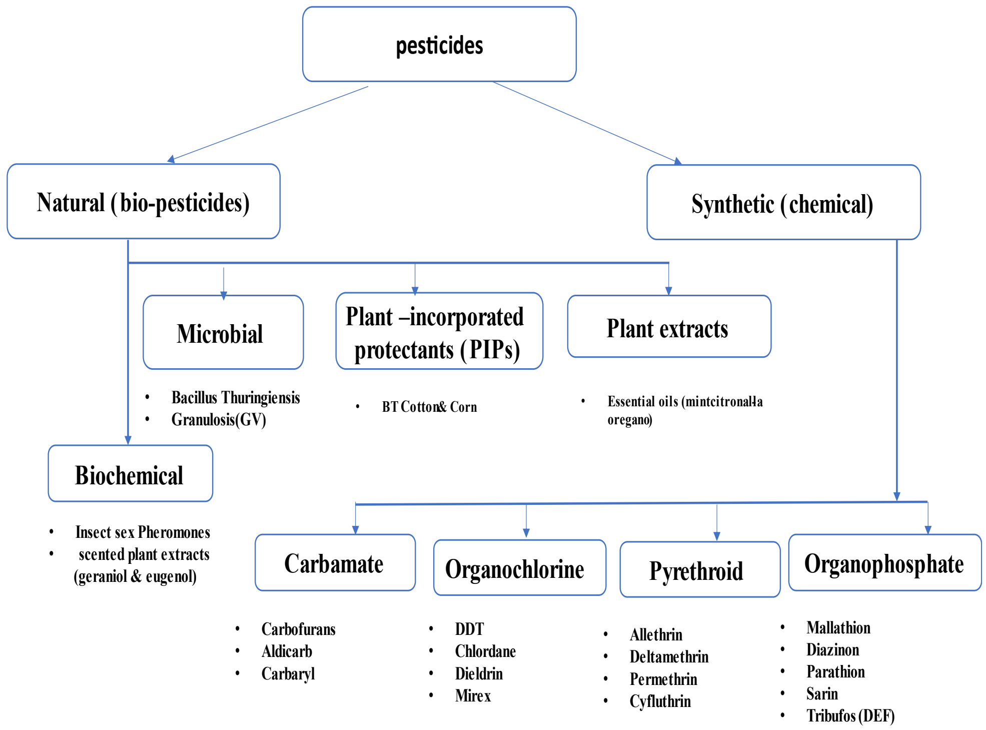
2.1.2.1.3. Biochemical pesticides:

The third category is chemical insecticides, which contain natural ingredients that have a non-toxic effect on insects. Examples of biochemical insecticides are insecticides (work through mating), plant extracts (work to attract insects to traps) (Rohani, 2023).

2.1.2.B. Synthetic Pesticides:

Synthetic pesticides are man-made chemicals, but they do not occur in nature. They are classified into different categories according to needs. Nowadays, there are three most popular proposed pesticide classification method (Książek-Trela & Szpyrka, 2022). Synthetic pesticides are made from chemicals and carriers, such as polymers (Rakhimol *et al*., 2020)

These are specific to different insects, weeds (weeds), algae (algaecides), fungi (fungicides), mites or ticks (miticides/acaricides), bacteria (bactericides), rodenticides (rodenticides), termites (termitecides), insects (insectides). lethals are different from the substances used in combat. forms the basis of the classes molluscs (molluscs) and nematodes(nematicides)(Anakwue, 2019).



Figure(2-1):Classification of Pesticides (Ar & Kaushik, 2022)

2.2. Ticks:

Ticks are an important group of arthropod vectors characterized by a variety of infectious diseases due to their impact on human and animal health as well as economic well-being, especially in Southern Hemisphere countries Their increasing presence in the environment since the beginning of the 20th century is undeniable, due to significant changes in diversity caused by humans. Increased awareness among health professionals and the public are needed to effectively control the disease. Therefore, "a better understanding of tick-borne diseases for better protection" is a simple and effective method that takes into account their presence in the environment and the specific mode of transmission of the virus (long-term blood of hard ticks), and latency transmission of bacteria and viruses. Finally, these ectoparasites are a problem because of possible allergic reactions and other problems that they cause in humans and animals (Boulanger *et al*., 2019).

Many viruses can be transmitted to humans and animals by ticks. Most of these viruses belong to three families Viruses transmitted by hard ticks include bunyaviruses, flaviviruses, and retroviruses (Lani *et al*., 2014). The most important viruses transmitted by ticks infections in humans are: tick-borne encephalitis virus (TBEV), Endemic to Central and Eastern Europe; Crimea and Congo Hemorrhagic fever virus (CCHF)(Cutler *et al*., 2021).

2.3. Crimean and Congo Hemorrhagic fever in Iraq:

Crimea-Congo Hemorrhagic fever virus (CCHF) is a viral infection caused by a virus (CCHFV) belonging to the Bunyaviridae family. Domestic and wild animals are asymptomatic reservoirs of the virus, this puts pastoralists, livestock workers and agricultural workers at high risk in endemic areas where secondary infection through contact with infected blood and other body fluids is possible. Human diseases are characterized by severe symptoms that often result in death. Although CCHFV transmission is known to be limited to Africa, Asia, and Europe, its global prevalence and potential impact in these regions is not well defined (Messina *et al*., 2015).

CCHF was first identified in Iraq in 1979, where the virus was isolated from humans and characterized. Since then, there have been minor outbreaks in 2019, 2021 and 2022. During this period, awareness trainings were organized for managers, veterinarians, doctors and assistants (Al Salihi *et al*., 2024; E *et al*., 2022).

Crimean-Congo hemorrhagic fever (CCHF) is spread by ticks and is classified as a viral infection. CCHF is a fatal disease in Iraq and has been reported sporadically since the first report in 1979. The last epidemic and its fatal consequences in 2021-2023 are important for this research. CCHF is a tick-borne disease that poses a major public health, social and economic problem. The geographical distribution of CCHF is closely related to the distribution of Hyalomma ticks. Therefore, predicting and mapping the spread of disease-associated environmental conditions provides useful information for establishing early warning systems based on preventive measures to reduce the spread of CCHF risk (Khwarahm, 2023).Crimea-Congo Hemorrhagic Disease (CCHF) was sporadically reported in Iraq over the past four decades until 2022, when outbreaks affected many provinces. Two individuals were seen in the Al-Hassainya region of Karbala province (June and July 2022). Inspection of livestock, fields and warehouses to reduce human CCHF (Al-Adhadh, 2024).

At 1980 seroprevalence study showed that CCHFV was first detected in animals from three biological regions in Iraq; 57.6% of sheep, 49.64% of goats, 29.28% of cattle, 58.73% of horses and 23.23% of camels were found. It is serologically positive (Tantawi *et al*., 1981). The annual number of confirmed CCHF cases ranged from zero to six between 1998 and 2009. In 2010, there were 11 and 28 suspected cases. Death toll in Iraq reached to the accounts for 36% of confirmed cases (Majeed *et al*., 2012). Incidental cases of CCHF outbreaks in Iraq include many anecdotal reports; for example, (i) two confirmed deaths in 1979 (one doctor, one nurse), (ii) two confirmed cases in 1992 (doctors), and (iii) one confirmed case in 1996 (physician) (Ibrahim *et al.*, 2014).

2.4. Pyrethrin:

Pyrethrin is a potent biopesticide, a natural mixture of six compounds (pyrethrin I and II, cinerin I and II, jasmolin I and II) produced by the Dalmatian pyrethrum plant (*Tanacetum cinerariifolium*). It is a species that is widespread on the eastern coast of the Adriatic, but is cultivated all over the world. Many of the unknown natural insecticides contain high-quality pyrethrins, which are very effective against a wide range of insects and have little harm to human health and the environment. In recent decades, pyrethrins have been largely replaced by their synthetic counterparts, pyrethroids. However, due to their harmful effects on various species and ecosystems, the use of pyrethrins is again considered old.(Jeran *et al*., 2021).

Pyrethrins are a class of terpene derivatives with high insecticidal activity and are mainly produced in the heads of the important vegetable plant *Tanacetum Cinerariifolium* (Zeng *et al*., 2022).The term pyrethrum refers to the dried and powdered flower heads of the white daisy-like flowering plant belonging to the genus Chrysanthemum. The insecticidal properties of Pyrethrum were identified in the mid-19th century when an American named Jumticoff discovered that was used by many tribes of the Caucasus to control lice. Pyrethrum, also known as Persian pyrethrum or Persian dust, was first cultivated in the Caucasus region, which extends to northern Persia (Sastry *et al.*, 2001).

2.4.1. Pyrethroid pesticides:

Pyrethroids are insecticides derived from *pyrethrum chrysanthemum* flower extract, known as pyrethrin, found in Kenya. It acts on the central nervous system, causing changes in the function of sodium cation channels in the membrane of nerve cells, resulting in an increase in the opening time of sodium channels. Sodium cation current extends across the membrane in both larvae and insects(Perry *et al*., 2013).

Due to the high demand for pesticides, most of the Pyrethroid pesticides (Figure2-2) contain compounds with low toxicity to birds and mammals; very toxic to arthropods as very low doses such as are required to kill insects; Very toxic to fish when used in water; and is particularly useful against greedy insects in the morning. Although many pyrethroid insecticides are absorbed by insecticides when dry residue is walked on, they are not effective in penetrating soil to control soil insects because they bind strongly to soil and organic matter. It also does not dissolve well in water (Saari *et al*., 2018)

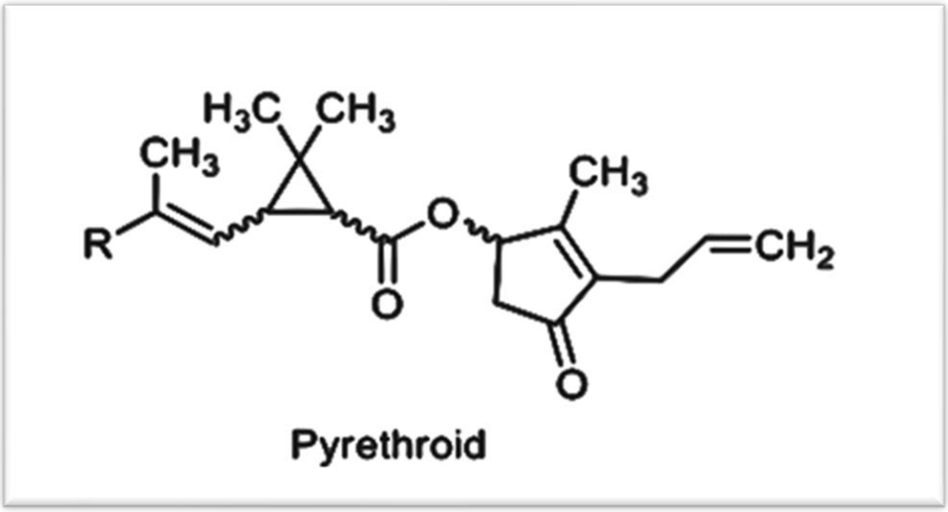


Fig (2.2): chemical structure of pyrethroids

Pyrethroids are used as active ingredients in many commercial products, including pet shampoos, human skin treatments, pet sprays, mosquito repellents, and of course insecticides for businesses, homes, and farms (Neima & Hassan, 2020).

2.4.2. Toxicity of Pyrethroids:

Synthetic pyrethroids have been used since the 1940s. They are derived from pyrethrins, which are natural substances found in the extract of the flower heads of *Chrysanthemum cineraria folium*, while the natural toxicity of pyrethroids is high, their LD50 values range from 0.5 mg/kg to 250 µg/kg, especially for class II. type 2 compounds are generally considered safe for humans (Bradberry *et al*., 2005). However, there are reports that pyrethroid compounds may not be as safe as previously reported (Ramchandra *et al*., 2019).

Pyrethroids are lipophilic compounds and are widely distributed throughout the body in the liver, stomach, intestine, fatty tissue, nervous system, and kidneys(Bradberry *et al*., 2005). Despite their lipophilic nature, no bioaccumulation has been observed after ingestion in mammals. They are generally not objectionable. In humans, it passes through the skin and is rapidly metabolized in the dark by ester cleavage, hydroxylation, and conjugation. However, data indicate that pyrethroids appear in the urine for less than 14 days (Miyamoto *et al*., 1995).

Synthetic pyrethroids are now made by combining piperonyl butoxide (S. Kumar *et al*., 2004) or organophosphorus compounds. Piperonyl butoxide is an active ingredient that reduces pesticide resistance and prevents the breakdown of pyrethroids. Organophosphorus compounds may increase the toxicity of pyrethroids by inhibiting their degradation by carboxylesterase (Martin *et al*., 2003).

Swallowing a pyrethroid causes a sore throat, nausea, vomiting, and abdominal pain within minutes. There may be mouth ulceration, increased secretions, and/or dysphagia. Systemic effects occur 4-8 hours after exposure. Dizziness, headache, and fatigue are common, and heart palpitations, chest tightness, and blurred vision are less common. Coma and convulsions are the main life-threatening features. Most patients recover within 6 days, although there were seven deaths out of 573 cases in one series and one out of 48 in another series (Bradberry *et al*., 2005). Pyrethroids are lipid-soluble, so their contact with the skin, digestive system and respiratory tract penetration the body (Chrustek *et al*., 2018). The degree of penetration is influenced by the stability of the barrier. It has been shown that a 15-second contact of deltamethrin on rabbit skin is insufficient to induce a depolarization reaction caused by the influx of sodium ions into cells (Hughes & Edwards, 2010)

Pyrethroids are divided into two groups, based on the pharmacological symptoms that appear in vertebrates after their administration (Bradberry et al., 2005). Some pyrethroids, including permethrin, cause symptoms known as "tremor-type syndrome" (T), characterized by generalized tremors, restlessness, aggressive behavior and ataxia. Type II pyrethroids, represented by deltamethrin and cypermethrin, are associated with choreoathetosis-mucosal disease, which is salivary and muscle dysfunction (Hedges *et al*., 2019).

In the fact that it has been proven that the level of pyrethroids in the body, as well as the severity of symptoms of poisoning, depends on the type of diet. Higher concentrations of pyrethroid metabolites in urine have been reported in people following a vegetarian diet. However, it should be emphasized that despite the proven negative effect of pyrethroids on human health, at the current stage of knowledge, it is not possible to propose a safer measure of personal protection against insects (Chrustek *et al*., 2018).

Pyrethroids are synthetic pesticides commonly used in agriculture and appear to replace organophosphates and carbamates. According to in *vitro* studies and human data, pyrethroids are considered neurotoxic (Clark & Symington, 2007). In laboratory studies, some pyrethroids such as (permethrin, cypermethrin, and deltamethrin) have been shown to cause genotoxic changes (DNA damage) (Sun *et al*., 2007). Some pyrethroids (cyfluthrin, permethrin, and cypermethrin) have been documented to exhibit endocrine-disrupting properties, such as antiandrogenic activity (Zhang *et al*., 2008).

2.4.3. Pyrethroid's Mode of Action:

The mechanism of toxicity of pyrethroids is complex, as it affects many channels and proteins. Pyrethroids mainly affect sodium and chloride channel The tissues of interest (nerves and muscles) are therefore important targets of pyrethroid toxicity (Ramchandra *et al*., 2019).

Pyrethroids have been found to alter the cap characteristics of sodium-sensitive channels, delaying their closure. This result in a sustained sodium current (called a sodium "tail current") which, if large and/or long enough, lowers the level of the action potential and causes re-firing. The magnitude of the current depends on the strength of the pyrethroid and the number of sodium channels involved (Ray *et al*.,2000).

Type II pyrethroids have been found to be long-lasting. At high levels of pyrethroids, the sodium tail can increase to the extent that it blocks action potential formation, resulting in " conduction block". This effect on the sodium channel may be responsible for the clinical symptoms of paresthesia and proarrhythmogenic potential (Bhaskar *et al*., 2010).

Pyrethroids also reduce chloride channels in the brain, nerves,muscles, and salivary glands (Forshaw *et al*., 2000). This can lead to increased salivation and myotonia. Type II pyrethroids act on voltage-gated chloride channels, and this effect may play an important role in the toxicity properties of type II. At high concentrations, pyrethroids may also act on gamma-aminobutyric acid (GABA) chloride channels, which may be responsible attacks seen in type II toxicity (Bradberry *et al*., 2005).

2.4.4. Cypermethrin:

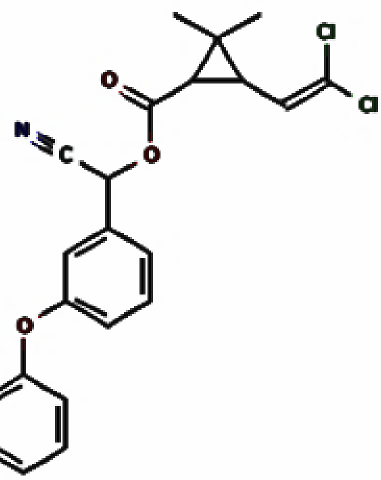
2.4.4.1. Identification of Cypermethrin:

Cypermethrin (CYP) is a man-made toxic chemical compound that belongs to the environmentally polluting pyrethroid insecticides (Aioub *et al*., 2021). Cypermethrin is a broad-spectrum Type II pyrethroid insecticide because it contains a cyano group in its structure (Ujihara, 2019).Chemically, synthetic pyrethroids are esters of chrysanthemum acid and alcohols. The center may be asymmetric present in acid ions and/or alcohol. Synthetic pyrethroids have a complex chemical structure and can contain two eight optical isomers (Mehta & Mehta, 2022).

2.4.4.2. Chemical structure of Cypermethrin:

Cypermethrin was synthesized (Figure-2-3) by (Elliott *et al*., 1987) in by the esterification of hydroxy(3-phenoxyphenyl) acetonitrile, **1a** with 3-

(2,2-dichloroethenyl)-2,2dimethylcyclopropanecarboxylic acid,**2**. Chemically Cypermethrin is[cyano-(3-phenoxyphenyl) methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane 1carboxylate,**3a.** The technical products commonly available contain more than 90% cypermethrin and the ratio of *cis*- to *trans*-isomers varies from 50:50 to 40:60 (Mehta & Mehta, 2022a).



(Figure-2-3): Chemical structure of cypermethrin (Elliott *et al.*, 1987)

2.4.4.3. Uses of Cypermethrin:

Cypermethrin is a pyrethroid used to control ectoparasites that infect cattle, sheep, chickens and other livestock. The use of the plant in the control of marine diseases in farmed fish is being investigated. However, cypermethrin is toxic to aquatic organisms and it is important to prevent contamination of surface water. Cypermethrin can be used orally or topically (paddling, dipping, spraying, pouring) (Organization, 1989).

Cypermethrin is a type II synthetic pyrethroid and is a widely used household, veterinary and agricultural insecticide due to its high pressure on cotton, fruits and vegetables and poultry products (lal hit, baygon). The bed nets in West Africa were treated with cypermethrin to prevent malaria (Guessan *et al.*, 2014).

Cypermethrin neurotoxin rapidly destroys insects by attacking the sytemanervosum (Lessenger, 1992), stopping the activity of motor nerve fibers and the supra-esophageal tendon sheath, and having a strong effect on respiratory fibers, causing paralysis and disrupting the brain's communication with the brain(Ghosh *et al.*, 2006). It caused the death of insects. Additionally, since this pesticide is generally used to control insects around leaves, its toxicity may not affect groundwater or plants (Farmer *et al.*, 1995).

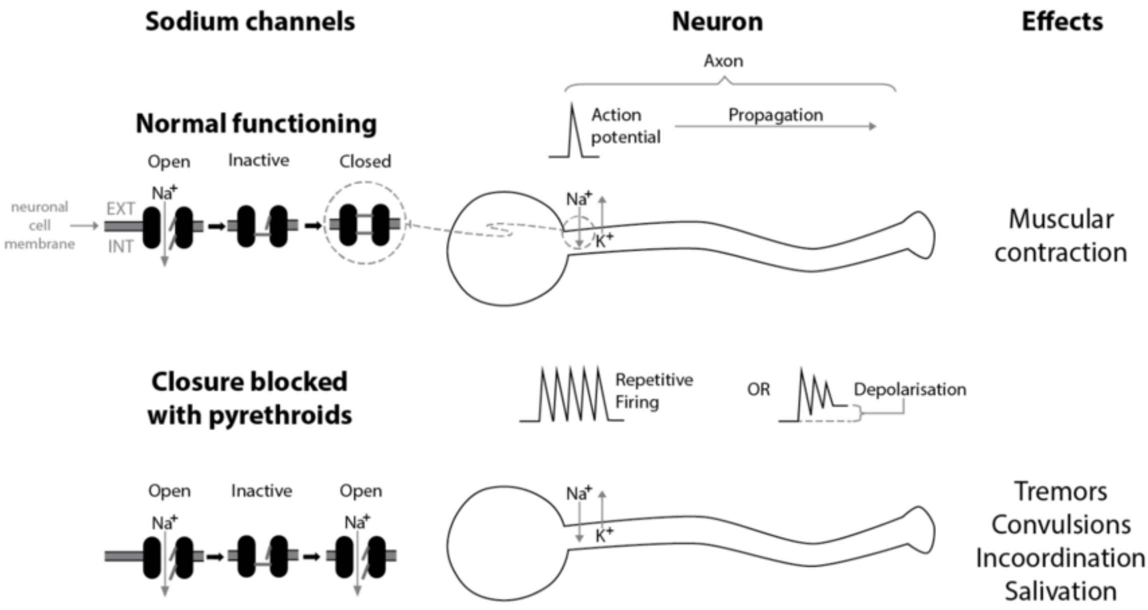
Technical Cypermethrin varies from a viscous, yellow liquid to particles of crystal at ambient temperature. In a study demonstrating the use of cypermethrin to control tsetse flies, dead and dying insects were found within a few hours, demonstrating the immediate effect of cypermethrin (Bequaert, 1956). Intensive CIS-cypermethrin treatment reduced the infection rate by % (almost 100%) after one day of treatment (Changizi, 2014).

2.4.4.6. Mode of Action of Cypermethrin:

Cypermethrin has a similar mode of action to organochlorine compounds. They act on the membrane of nerve cells and prevent closure Ion gating of the sodium channel during repolarization. This severely disrupts the transmission of nerve impulses, which causes spontaneous depolarization of the membranes or frequent discharge. In low concentrations insects and others arthropods suffer from hyperactivity. In high concentrations they become paralyzed and die. Sensory and nervous cells are particularly sensitive (Lawrence & Casida, 1982).

Cypermethrin leads to form cyanohydrin, and thus decompose to cyanide and aldehydes, which ultimately leads to the formation of ROS (Wielgomas & Krechniak, 2007). ROS stimulate lipid peroxidation (LPO) and enhance the level of calcium (Ca++), which leads to cytotoxicity and genotoxicity in exposed organisms (Ullah *et al*., 2018).

The harmful effects of pyrethroids are due to their neurotoxic effect,which leads to inhibition of AChE leading to its retention Ach in the synaptic gaps of neurons. It also reacts with sodium channels and stimulates depolarization of neurons (Idris *et al*., 2012).



Fig(2-4):mode of action of cypermethrin(Idris *et al*., 2012)

Pyrethroids act on voltage-gated sodium channels .The binding of the pyrethroid molecule to the α part of the channel stabilizes its opening and prevents its closure (Soderlund, 2012).

As a result, the entry of sodium ions into neurons and a permanent depolarization occur. In addition, pyrethroids reduce the enzymatic activity of acetylcholinesterase (EC 3.1.1.7), changing the active site of the substrate. They also change the activity of the cytochrome p450 system in brain and liver neurons. Pyrethroids kill more insects than mammals and birds due to the sodium channel effect in the insect's sensory system at low temperatures (Hedges *et al*., 2019).

2.4.5. Residue of Cypermethrin:

2.4.5.1 Residue of Cypermethrin in Cattle Meat:

Meat is an excellent source of highly nutritious proteins and fats such as essential amino acids, as well as rich minerals such as iron. Therefore, meat must be produced from animal feed without harmful chemical residues (Lehotay *et al.*, 2005).

Pesticide residues are widely used in developing countries to control animal pests or farmers in many African countries. Pesticides are used to control plant pests that are transmitted to humans through the food chain. In addition, pesticides are used to control ectoparasites, so pesticides bioaccumulate in animal food residues , such as meat, milk and the fish(Qiu *et al*., 2005).

The residues of pyrethroid pesticides represented by cypermethrin, deltamethrin, esfenvalerate and permethrin were not detectable in winter, while in the muscles of cattle and in the fat of food animals until summer, while Lambda-cyhalothrin, bifenthrin, cyfluthrin and meothrin were found in most of the tested samples. from different species to the winter and summer seasons, most of these results showed higher average levels than the residue limits (Zhou *et al*., 2019).

There are seven types of pyrethroids (bifenthrin, lambda-cyhalothrin, deltamethrin, cyfluthrin, fen valerate, cypermethrin, and permethrin). Widespread use of pyrethroids in crop protection and livestock can result in the transfer of residues into animal milk, tissues, honey, and eggs. Veterinary applications of this type of pesticide include sprays, ear drops, drops, and sprays. According to their chemistry, physical properties and toxic profile importance, pyrethroids should be controlled in animal feed in order to control feed quality and prevent potential risks to human health (Niewiadowska *et al*., 2010).

2.4.5.2. Residue of Cypermethrin in cow Milk:

Pyrethroids are synthetic insecticides. It is widely used to treat lactating mothers' Dairy cows, especially against ticks and flies, Lice and skin(Sassine *et al*., 2004). Pesticide residues are an organic environmental pollutant mostly with lipophilic properties which easily dissolve in milk(Bissacot, Vassilieff, 1997).

Cypermethrin is a Class II pyrethroid. A moderately dangerous insecticide according to the World Health Organization (WHO). Because it may be dangerous Human health, maximum residue levels (MRL) in milk has been stated different organizations, e.g.0.2 mg/ kg by the European Union (Tait, 2009).While The European Commission has reducing the MRLs to the limit of determination (LOD) on several products, and to 0.005 mg/kg (below the standard LOD of 0.01) on oranges, pears, melons, and potatoes. (The LOD is the lowest level that can be detected using the most modern and reliable analytical methods)(Countries, 2024).

Pesticides can cross the skin barrier, enter the bloodstream, and enter milk(Braun *et al*., 1985). Residues of pyrethroids applied to cattle were found in milk 28 days after exposure to the animals (mainly absorbed through the skin). These observed levels were several times higher than the limits accepted by Codex Alimentarius(Bissacot and Vassilieff, 1997).

On the other hand, the widespread use of pyrethroids (PTDs) in the control of livestock pests also leads to contamination of animal meat, as their lipophilic nature facilitates their rapid spread. Administration through legal or accidental contaminated feed suggests that milk remains due to bioaccumulation mediated by the dissolution of these substances (Kayitsinga *et al*., 2017).

2.4.5.3. Residue Of Cypermethrin in Cheese and Ghee:

Pesticide residues remaining in various forms in foods after harvest cannot be controlled by the consumer and affect human health (Bajwa & Sandhu, 2014).

Milk and dairy products are sometimes contaminated with pesticides and food residues from their oils. Therefore, residues may be found in a more concentrated form (fat) in dairy products (butter, cheese, ghee, malai) than in the resulting milk (Li *et al*., 1970). The most organic pesticides have high affinity for oils which makes their removal extremely difficult from different oils and fats. The fat soluble insecticides, like organochlorines, after reaching a body of water tend to adhere to suspended organics which are consumed by small invertebrates that dwell upon them (Kannan *et al*., 1997).

2.5. Macrocyclic Lactones:

Macrocyclic lactones (avermectins and milbemycins) are products or chemicals from streptomyces soil bacteria. Commercially available avermectins are ivermectin, abamectin, doramectin, eprinomectin, and selamectin. The commercially available milbemycins are milbemycin oxime and moxidectin (Wang *et al*., 2017).

2.5.1. Avermectins and Ivermectin:

Avermectins are a new class of macrocyclic lactones derived from the *mycelium* of *Streptomyces avermitilis* (a common soil pathogen). These compounds are said to have insecticidal, acaricidal and nematocidal properties(Reddy, 2013).

Avermectins were discovered in 1967 from fermentation plates of actinomycete cultures found at the Kitasato Institute in Japan, after intense research to find natural products with anthelmintic effects (Bai & Ogbourne, 2016).

Avermectins are a group of drugs that occur in nature as products of the yeast Streptomyces avermitilis, actinomycetes isolated from soil. Eight different compounds including ivermectin, abamectin, doramectin, eprinomectin, moxidectin and selamectin were isolated and divided into four classes (A1a, A2a, B1a and B2a) and four subclasses (A1b, A2b, B1b and B2b) (El-Saber Batiha *et al*., 2020).

Avermectins are often used as insecticides to treat insects and parasitic worms, causing anthelmintic in insects. They are also anti-cancer, anti-diabetic, anti-viral and anti-fungal and are used to treat many metabolic diseases (Zaidi & Dehgani-Mobaraki, 2022).

Avermectin usually works by blocking the transmission of electrical impulses in the muscles and spinal cord, increasing the effect of glutamate on internal chloride channels. Avermectin has adverse effects or reactions, especially when given inappropriately, including respiratory depression, hypotension, and coma(El-Saber Batiha *et al*., 2020).

Ivermectin (IVM) is an antiparasitic agent widely used in animal husbandry (Peluso *et al*., 2024). The IVM market remains very strong in livestock, particularly for the control of intestinal worms, although it is also licensed for the control of bovine lungworm and various ectoparasites (Burgess *et al.*, 2012).

After oral and parenteral administration, it has strong activity against many parasitic nematodes. IVM is not active against flukes or tapeworms, but it is active against many arthropods, including lice, mites, and some ticks (Mealey *et al*., 2001). Ivermectin and pentobarbital, which open chlorine channels, eliminate these effects and may be useful in treating these effects. However, more research is needed to better understand this effect on the chloride channel (Soderlund, 2012).

2.5.2. Discovery and Synthesis of Ivermectin:

In 1970, biologist Satoshi Ōmura collected soil samples from trees near a golf course in Kawana, on the southeast coast of Honshu., Japan (Van Voorhis *et al*., 2015). Ōmura isolated and grew a Gram-positive culture; sample NRRL 8165 – a then-unknown Streptomyces species; This was sent to William Campbell at Merck (along with 50 other Streptomyces species considered unusual in morphology or culture) to determine the evaluation effects of antiparasitic agents (Burg *et al*., 1979).

Strain NRRL 8165 showed potent activity against Nematospiroides dubious (currently Heligomosoides polygyrus) infection in mice, and the active compounds were purified, yielding a family of macrocyclic lactones. These compounds are often called avermectins (by the bacterium Streptomyces avermitilis), reflecting the 'weather dissipating' nature of the worms that produce (Burg *et al*., 1979).

Subscripts 'A' and 'B' indicate the presence of methoxy or hydroxyl at the C5 position, while subscripts 1 and 2 indicate double bonds between C22 and C23 or hydrogen atoms at C22, C23 - with a hydroxyl group as before. Type "a" contains secbutyl C25, while type "b" contains isopropyl. These subtle differences in chemical composition have been found to have significant functional consequences; Although previous tests found that four avermectins showed some activity against intestinal nematodes in sheep, series 'B' avermectins showed the highest activity (Blair & Campbell, 1979). In addition, avermectin B1 is more active than B2 when administered orally, while avermectin B2 is more active than B1 when administered parenterally (Campbell *et al*., 1983).

Based on this, the development of commercial anthelmintics focused on the "B" system and the chemical structure of the C22 and C23 positions. IVM is a derivative of avermectin B1 containing ~80% 22,23-

dihydroavermectin B1a and ~20% 22,23-dihydroavermectin B1b (Figure2-5) (Campbell, 2012).

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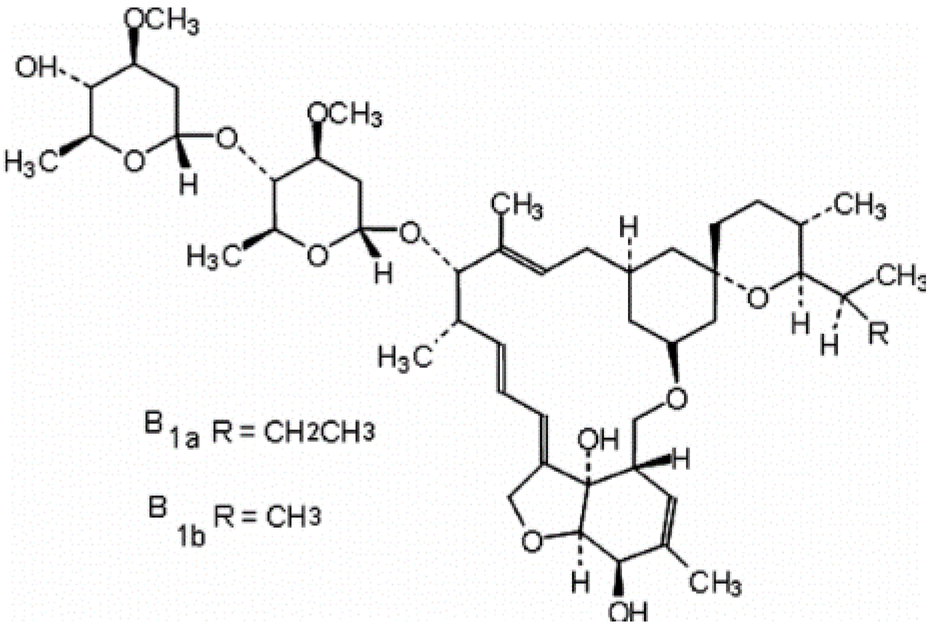

Fig (2.5): chemical structure of ivermectin (Fernandez *et al.*, 2009)

2.5.3. Ivermectin Mode of Action:

Although the effectiveness of IVM in the treatment of many parasitic infections is well known, its method is not well established. At the nanomolar level, IVM affects nematode motility, feeding, and reproduction and acts on ligand-gated chloride channels, particularly those gated by glutamate (Yates *et al.*, 2003). Glutamate-gated chloride channels (GluCls) do not exist in vertebrates and are therefore thought to provide a protective margin for IVM. However, at micromolar concentrations, IVM can interact with ligand-gated channels found in the spinal cord, including GABA, glycine, histamine, and nicotinic acetylcholine (Wolstenholme & Rogers, 2005).

2.5.4. Uses of Ivermectin:

Ivermectin is the 22,23-dihydro derivative of avermectin B1, a macrocyclic lactone produced by the actinomycete Streptomyces avermitilis. It is active at very low doses against various species of nematodes and arthropod parasites, apparently due to its neurotransmission activity and aminobutyric acid. It is currently used commercially in many countries for the treatment and control of parasites in cattle, horses and sheep, and is expected to be used in pigs and dogs. It is not yet known whether ivermectin will be effective in human treatment, as research on the use of the drug in humans is still in its early stages (Tang *et al*., 2021).

Ivermectin (IVM) has been used in veterinary practice to control multiple parasitic infections over the past two decades. (Batiha *et al*., 2019).Ivermectin is suspected to affect the gamma-aminobutyric acid (GABA) binding neurotransmitter in infected arthropods (Campbell, 1981)**.**

Ivermectin is an endectocide against many parasites. Although it is a macrocyclic lactone, its antibacterial activity is not well known, probably due to the micromolar concentration at the tissue level that should have a therapeutic effect (Ashraf *et al*., 2018)**.**

The small molecule macrocyclic lactone ivermectin, approved by the US Food and Drug Administration for the treatment of parasitic infections, has received renewed attention in the past eight years because of its clearly exciting potential as an antiviral. It was identified in a high-throughput chemical screen to inhibit recognition of the human immunodeficiency virus-1 (HIV-1) (Jans & Wagstaff, 2020).

A few antiparasitic are used for stimulation Immunity in various human diseases, including leprosy, Hodgkin's disease and rheumatoid arthritis Adjuvant treatment of colorectal cancer. in animals, Simultaneous use of vaccines and immunomodulator Antiparasitic medications may be helpful. There have been some studies. The properties of ivermectin as immunomodulators have been shown The catalyst is associated with the modified function of T Lymphocytes in particular, T helper lymphocytes in malesCD-1 mice (Blakley & Rousseaux, 1991). Reports from in vitro studies have suggested that ivermectin can interfere with the attachment of SARS-CoV-2 spike protein to the human cell membrane, inhibition of host transport proteins that the virus uses to evade the antiviral response, and some studies suggest anti-inflammatory properties (Kinobe & Owens, 2021).

2.5.5. Toxicity of Ivermectin:

The administration of ivermectin in food-producing animals poses a risk related to the presence of drug residues in meat, milk and other products (Escribano *et al*., 2012). Remnants of the ivermectin marker H2B1a, which is the main component of the original compound, were found. Tissue distribution of residues and ratios of total marker to total residue were generally similar in most species, and the highest concentrations of ivermectin residues were found in fat and liver with high levels also detected in injection site muscles. Ivermectin is not approved for use in dairy animals for human consumption. However, since it has been present in milk and dairy products for a long time, its excessive use should be taken into consideration for human safety (Escribano *et al*., 2012).

The metabolism of IVM by human cytochrome P450 enzymes, interactions with drug transporters, and the potential to cause drug–drug/drug–chemical interactions and toxicity which might occur in a wide range of its clinical applications (Rendic, 2021).

Ivermectin residues are responsible for many health ailments Risks: It may cause a mild Mazzotti reaction, including fever, itching, Arthralgia, myalgia, postural hypotension, edema, lymphadenopathy. Hence the gastrointestinal symptoms, sore throat, cough, and headache; it becomes necessary for waste to be strictly regulated by food safety point of view (Koesukwiwat *et al*., 2007).

2.5.6. Residues of ivermectin

2.5.6.1. Residues of ivermectin in cattle meat:

Ivermectin in cows, sheep and rats, and fat and liver tissues were found to contain the highest concentrations of residue, while muscle tissue contains the lowest concentrations. Tissue redistribution is not affected by the direction of administration (Riviere & Papich, 2013).

Different levels of ivermectin residue were detected in bovine skin samples collected from slaughterhouses however the degree of damage caused by ectoparasites exceeds the damage caused by ivermectin. Ivermectin residues can be present in animal products such as milk and meat (Baz *et al*., 2014).

Ivermectin is lipophilic and residues may be found in the meat of treated animals, especially animals with a high fat content. The absorption period of ivermectin is long in mammals (Fernandez *et al*., 2009)**.** The main residue of beef food is the un-metabolized medicine. After administration, most residues were found in the liver, followed by fat, kidney, and muscle (Celis-Giraldo *et al*., 2020)**.**

2.5.6.2. Residues of ivermectin in milk and milk products:

Ivermectin is not authorized for use in animals that produce milk for human consumption. However, its off-label use should be considered in relation to human safety, due to its long persistence in milk and milk-derived products (Escribano *et al*., 2012). The IVM molecule has high liposolubility, this fact is important because their excretion could be by milk (Campillo *et al*., 2013) **.**

However, the concentration of IVM in milk is very low and does not have a significant effect on consumers(Campillo *et al*., 2013). Its use in food-producing animals may cause accumulation of its residues into the animal tissues that find their way into the food products, such as milk, and poses a health hazard to the consumer(Zakaria *et al*., 2019)

2.6. High-Performance Liquid Chromatography (HPLC):

High-performance liquid chromatography (HPLC) was developed in the late 1960s and early 1970s. Today, it is widely used for separation and purification purposes in a wide variety of fields, including the chemical, biological, environmental, polymer and food industries. HPLC has become the method of choice for the analysis of many substances up to over the last decade. The main advantage of GC is that the analysis does not need to be volatile, so macromolecules are suitable for HPLC analysis(Bhardwaj *et al*., 2015). HPLC is performed by injecting small amounts of liquid flow (called mobile phase) through a column filled with stationary phase. The differences in composition are due to the different degrees of retention of each element in the column. The amount of fraction up to remaining in the column is determined by the partition between the liquid mobile phase and the stationary phase(Sahu *et al*., 2018). In HPLC, this distribution is affected by compared to the dissolved/stationary phase and the dissolved/mobile phase. So, unlike the GC, a change to the mobile unit can have a significant impact on your differential. Because the units have different engines, they display column at different times; i.e. tR, they have a different retention value time. Holding time and injection time and detection. For this reason, HPLC is most commonly used when performing objective analysis, where one has a good idea of the compounds present in the mixture and thus measurements can be used to determine retention times(Gerber *et al*., 2004).

2.6.1. Principle of HPLC:

HPLC is a separation technique that involves: injecting a small liquid sample into a tube filled with particles (3 to 5 microns) (diameter, called stationary phase), where the sample components are moved along the tube (column) filled with water (mobile phase) by high pressure given by the pump. These parts are separated between the packing columns and packing parts containing different chemicals and/or physical interactions between the molecules. These parts are separated from this tube (column) by the outlet, a detector that measures their number(Patil, 2017). The outlet of this detector is called “HPLC”. In principle, LC and HPLC work in the same way, but in terms of speed, efficiency, sensitivity and ease of use, is much better than HPLC. Although HPLC holds most of the credit on the analytical side, the first simple liquid chromatography still has the necessary recommendations for preparative activities(Bergh & Breytenbach, 1987).

Chapter Three

Methodology

3. Chapter Three: Methodology

3.1. Materials:

3.1.1. Equipment and Instruments

Table 3.1: Equipment and Instruments

No.	Equipment and Instruments	Company and Country
1	Hand Blender Set	Silver crest (Germany)
2	Centrifuge	HETTICH(Germany)
3	Deep freezer	LG (Korea)
4	High Performance Liquid Chromatography (HPLC)	Shimadzu (Japan)
5	Homogenizer	Silver crest (Germany)
6	Magnetic stirrer	Lab tech (Korea)
7	Capsule Magnetic Stirrer Beads	LARK (India)
8	Micropipette size(100-1000µg)	Dragon lab (China)
9	Water bath	Memmert (Germany)
10	Poly propylene Centrifuge tube	IWAKI (Japan)
11	Fume Hood	Lab tech (Korea)
12	Containers	China
13	Sensitive Balance	Denver (Germany)
14	Glass Beaker	China
15	Cellulose Acetate Syringe Filter	CHM(Spain)

16	Disposable Syringe	Golden gate (Iraq)
17	Insulin syringe	Golden gate (Iraq)
18	Glass pipette	Achema (Germany)
19	Glass conical flask	Achema (Germany)
20	Amber glass bottle	Satya (India)

3.1.2. Chemicals

Table 3.2: Chemicals used and their manufacturer company

No.	Chemicals	Manufacturer company
1	Cypermethrin standard	Mobedco (Jorden)
2	Ivermectin standard	KEPRO(Holland)
3	Methanol HPLC grade	LAB ALLEY (Austin, Texas)
4	Deionized water	SIDDHIC LABs (india)
5	Acetonitrile	LOBA Cheme (India)
6	n-methyimidazole	Fluka (Switzerland)
7	Trichloroacetic acid AR	SDFCL(Mumbai)

3.2. Study design:

Five administrative areas of the city of Karbala were surveyed (Al-Hassainya, City Center, Al-Hur, Twairij and Ain Al-Tamer), and twenty samples were taken from each area, and five samples were entered from each. Beef, milk, cheese, ghee) at October 2023 to January 2024, samples are collected 24 hours before the extraction process and stored in a cold place at 4 degrees Celsius.

Two hundred samples were randomly collected from the local market and butcher's shop, 50 samples of fresh cattle meat, 50 samples of cow's milk, 50 samples of cheese and 50 samples of ghee from different supermarkets in Karbala Province. Determination of residual levels of antiparasitic drugs are (cypermethrin and ivermectin) in fresh cattle meat, milk, cheese and ghee.

The aim of this study was also to determine the level of residual antiparasitic drugs (cypermethrin and ivermectin) using HPLC in order to determine the degree of contamination of this food of animal origin with residues of harmful chemical pesticides.

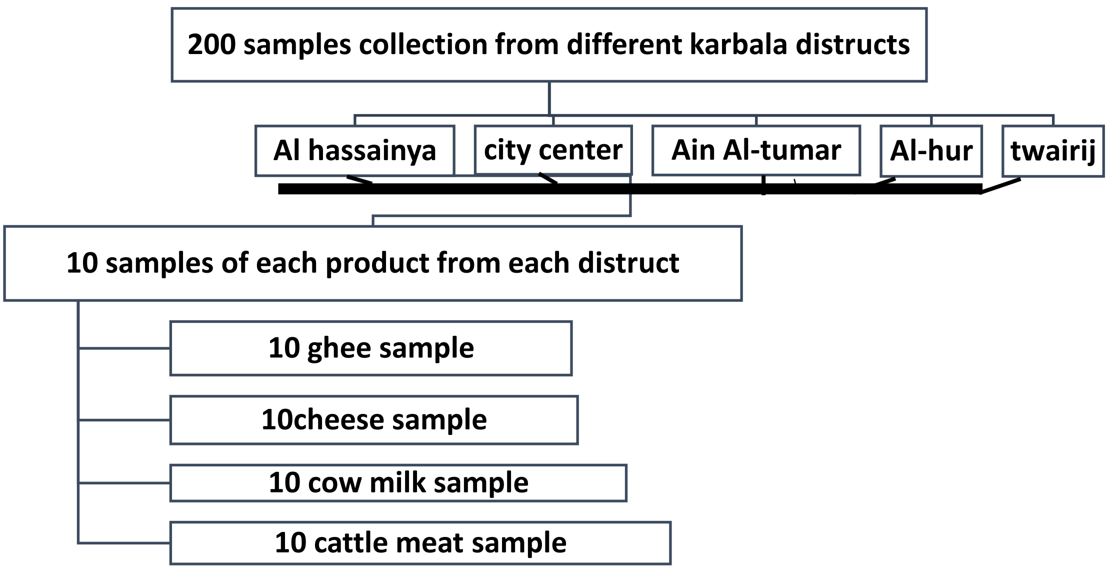

Figure 3.1: General experimental design for the current study

3.3. Methods:

3.3.1. Samples collection:

Every sample was gathered into a sterile, clean polyethylene plastic bag, and each bag had a label with the product's name and any other pertinent information for additional identification. During the transit to the laboratory of the Veterinary Public Health Department at the College of Veterinary Medicine at Karbala University, the samples were preserved and refrigerated in a portable ice-cooled box.

To determine the degree of ectoparasiticides contamination, each sample was applied. Cattle meat, cow milk, cheese, and ghee were tested for ectoparasiticides (cypermethrin and ivermectin) contamination using High-Performance Liquid Chromatography (HPLC) analysis.

3.3.2. Preparation of Solvents:

3.3.2.1. Preparation of Solvent A:

Acetonitrile with n-methylimidazole is widely used as a solvent in various chemical reactions, especially in organic synthesis. So, the solvent is prepared by mixing 5ml of n-methylimidazole (NMI) with 5ml of acetonitrile (ACN).

3.3.2.1. Preparation of Solvent B:

Acetonitrile (ACN) with Trichloroacetic acid (TCA) is widely used as a solvent in various chemical reactions, especially in organic synthesis. So, the solvent is prepared by mixing 10ml of 0.1 % Trichloroacetic acid (TCA) with 5ml of acetonitrile (ACN)

3.4. Sample Preparation:

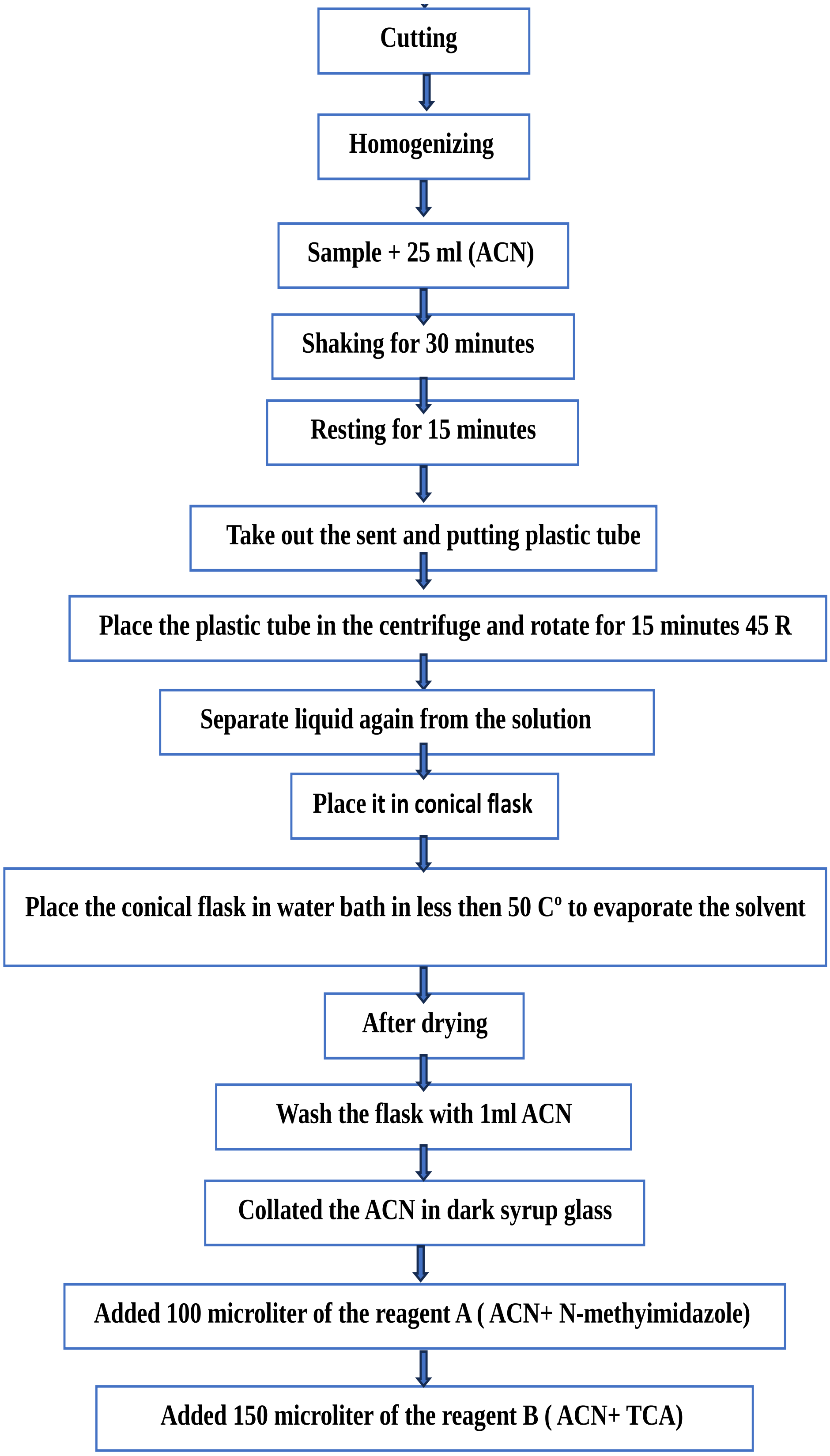

Figure 3.2: The samples preparation and extraction (liquid-liquid(L/L) extraction)

3.4.1. Extraction:

All samples were performed in the Nutrition and Public Health Laboratory at the College of Veterinary Medicine/ Karbala University. Samples were taken from fresh cattle meat, milk, cheese and ghee from local Iraqi market in Kerbela province from different district (Al-Hassainya, Al-Hur, Ain Al-Tamur, Center of Kerbela and Twuriji).

The extraction method used in this study was a modification of the method for multiresidue analysis as outlined by (Lentza-Rizos *et al.*, 2001). In their paper, acetonitrile was used to extract the pyrethroid insecticides from the oil and they stated that 25 mL acetonitrile was needed for optimum extraction of pyrethroids from the oil matrix. In the present study, we used the same solvent for the extraction of cypermethrin, and found that 50 mL acetonitrile was optimal for a complete extraction of the residues from 5 g of each sample (cattle meat, cow's milk, cheese and ghee).

In this study, 5 grams were collected separately from each sample. Then, the samples (beef and cheese) were crushed and homogenized, after which 25 ml of acetonitrile was added. For cow's milk and ghee samples, 25 ml was added directly to the sample after weighing 5 grams using a sensitive balance, then the sample was shaken with a magnetic stirrer for 30 minutes, after which the sample was left to stand for 15 minutes, then the liquid was removed and the extract was centrifuged to remove the solids at 45,000 rpm for 15 minutes, the extraction was repeated on the same sample with the same steps as the previous steps. After collecting the liquid from the centrifuge, it is placed in an Erlenmeyer flask and placed in a fume hood in a water bath with a temperature below 50 ° C until the liquid is completely dry. After that, the sediment remaining after drying the flask is washed with 1 ml of acetonitrile, then collected in an opaque glass vial and 100 μm of solvent is added. A, then 150 μm solvent B.

The samples were sent to the laboratory of the Materials Research Department at the Ministry of Science and Technology in Baghdad for the purpose of examining the samples using a high-performance liquid chromatography device, and then the samples were filtered using a special filter with a size of 25mm to be Ready to read with (HPLC High-Performance Liquid chromatography – Japan) (Almashhadany, 2020).

3.5. Preparation of standard curves:

Stock standards solution of (CYP) and (IVM) were made by 1ml of (CPM) or (IVM) in 0.1L of methanol to get a concentration 100 ppm (100 ml/L). Then diluted to (10,5,2.3,3.3…. etc.) ppm. Calibration solutions were prepared in the Laboratory of the Materials Department of the Iraqi Ministry of Science and Technology in Baghdad.

3.6. Analysis of antiparasitic drug in samples:

The samples were collected and prepared for quantitative analysis using HPLC figure (3-3):

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 reverse phase, 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 was 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

standard solutions (3.3,5,2.5 µL) that were produced weekly(de Lima *et al*., 2016; Shaheed & Dhahir, 2020).

4. 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).

- The chromatographic column (cypermithrin): 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).
- 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).

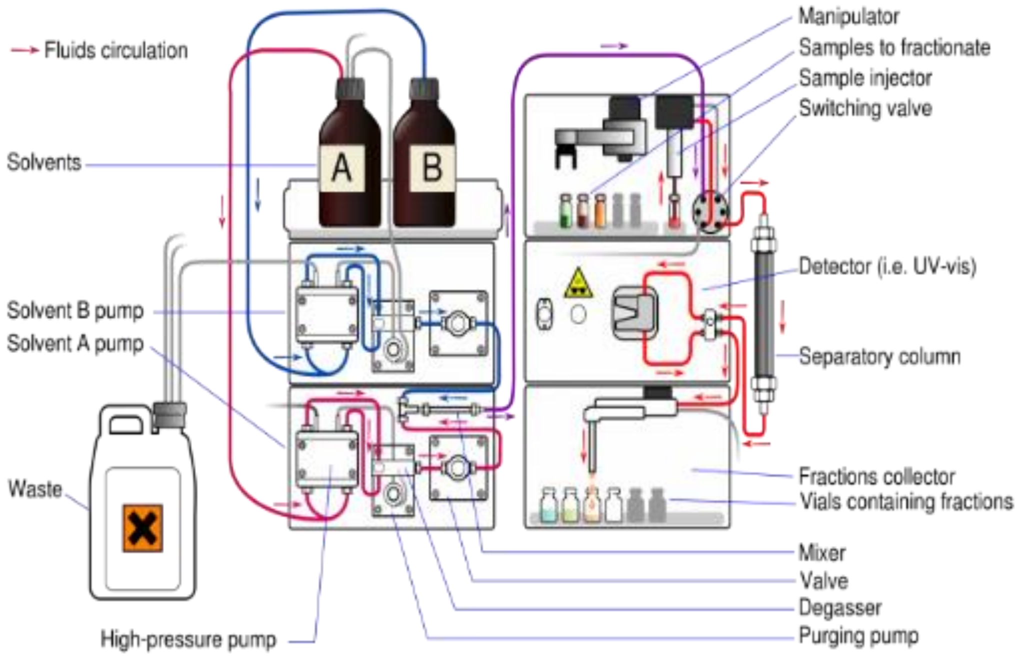

Fig (3-3): HPLC Function Chart (Megahed *et al*., 2020)

3.7. Statistical Method:

The Padgraph Prism program was used to create graphs, find the average and standard deviation, and compare them with the ratios approved by the World Health Organization (WHO) for the year 2023 for cypermethrin and the year 2022 for ivermectin. One Way ANOVA and T. Test were used(Mavrevski *et al*., 2018). the charts prepared using the program (ArGIs10.8) designed to make geographical charts(Liu *et al*., 2023).

Chapter Four

Results and Discussion

4.Chapter Four: Results and Discussion

4.1. Residue of Cypermethrin in cattle Meat:

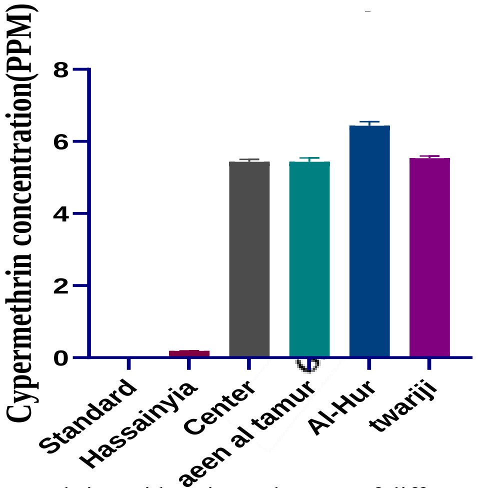

Figure (4-1): Cypermethrin residues in cattle meat of different district for Kerbala province.

Table (4-1): Cypermethrin residues concentrations for cattle meat samples PPM (Means±SD).

Groups	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.03	0.1569 ±0.0018 *	5.408 ±0.0682 **	5.509 ±0.0554 ***	6.409 ±0.113 ****	5.408 ±0.1038 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (1), ** Appendix (2), *** Appendix (3), **** Appendix (4) and***** Appendix (5).

The current study showed that all meat samples collected from different district of Kerbala province (Al-Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) had a significant difference at p≤0.0001 (0.1569; 5.408; 6.409; 5.509; 5.408) ppm respectively compared with maximum residue level of cypermethrin pesticide in the world health organization (WHO) index for 2023 year.

On the same side , it was found that Al-Hur district (6.409 ±0.113) had highly significant from (Al-Hassainya, center of Kerbala, Twairij and Ain Al-Tumar) as shown in figure (4-1) and table (4-1).

4.2. Residue of Cypermethrin in Milk:

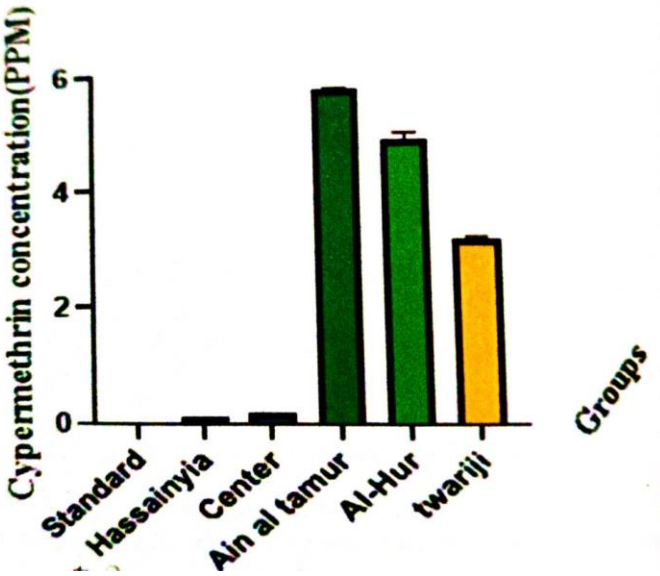

Fig. (4-2): Cypermethrin residue in cow 'milk of different district for Kerbala Province

Table (4-2): Cypermethrin residue concentrations in cow 'milk samples PPM(Means±SD.).

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.015	0.1026 ±0.0005 *	0.1569 ±0.0018 **	3.073 ±0.0480 ***	4.778 ±0.1256 ****	5.651 ±0.0217 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (6), ** Appendix (7), *** Appendix (8), **** Appendix (9) and***** Appendix (10).

The residues of cypermethrin in cow milk showed in the table (4-2) and figure (4-2) during the study of experiment. The results of the study showed highly significant at $p \leq 0.0001$ specially in (Ain Al-tumar, Al Hur , Twairij, Center and Al-Hassainya) where registered(5.651, 4.778, 3.073, 0.1569 and 0.1026) ppm respectively compared with world health organization(WHO).

4.3. Residue of Cypermethrin in cheese:

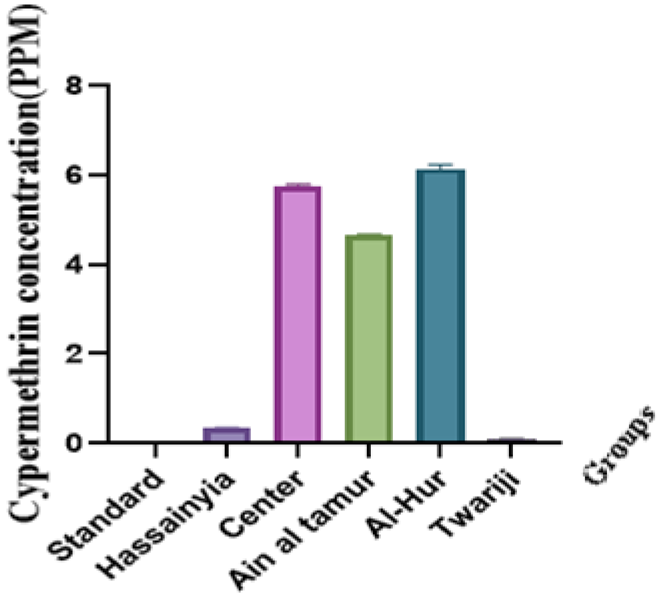

Fig. (4-3): Cypermethrin residue in cheese of different district for Kerbala province

Table (4-3): Cypermethrin residue concentrations in cheese samples PPM(Means±SD.).

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.015	0.3271 ±0.0024 *	5.720 ±0.0637 **	0.07613 ±0.0033 ***	6.127 ±0.097 ****	4.625 ±0.037 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (11), ** Appendix (12), *** Appendix (13), **** Appendix (14) and***** Appendix (15).

The current study showed that all cheese samples collected from different district of Kerbala province (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) (0.3271, 5.720, 6.127. 0.07613 and 4.625) had a significant at p≤0.0001 difference compared with maximum residue level of cypermethrin pesticide in the World Health Organization (WHO) index for 2023 year.

On the other hand, it was found that Al-Hur district had highly significant than (Al-Hassainya, center of Kerbala, Twairij and Ain Al-Tumar) as shown in figure (4-3) and table (4-3).

4.3. Residue of Cypermethrin in ghee:

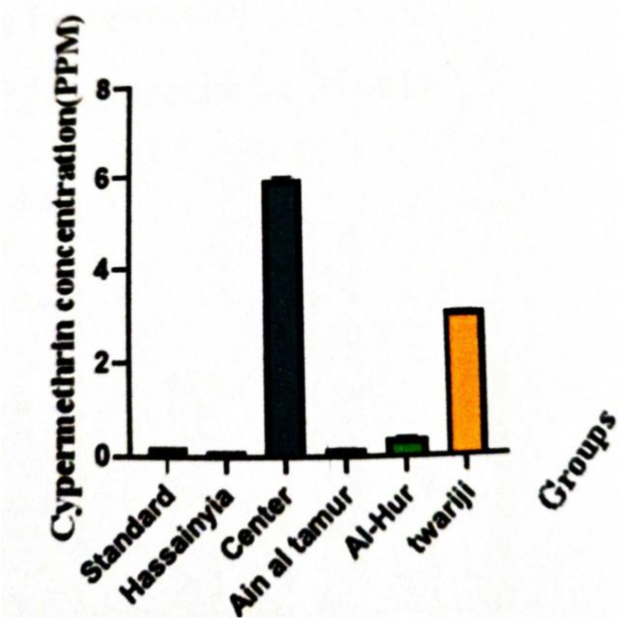

Fig. (4-4): Cypermethrin residue in ghee of different district for Kerbala Province

Table (4-4): Cypermethrin residue concentrations in ghee samples PPM(Means±SD.).

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.2	0.06863 ±0.0016 *	5.632 ±0.0669 **	2.788 ±0.0081 ***	0.1700 ±0.0291 ****	0.0452 ±0.003 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (16), ** Appendix (17), *** Appendix (18), **** Appendix (19) and***** Appendix (20).

The current study showed that all ghee samples collected from different district of Kerbala province (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) (0.06863, 5.632, 0.1700, 2.788, 0.0452) had a significant difference at $p \le 0.0001$ compared with maximum residue level of cypermethrin pesticide in the World Health Organization (WHO) index for 2023 year.

On the other hand, it was found that center of Kerbala district had highly significant from (Al-Hassainya, Al-Hur, Twairij and Ain Al-Tumar) as shown in figure (4-4) and table (4-4).

Table (4-5): Cypermethrin residue concentrations in different food product in (cattle meat, milk, cheese, ghee) samples that collected from various region of Karbala province ppm (Means±SD)

Group	Cypermethrin			
Sample / Regain	Meat	Milk	Cheese	Ghee
WHO	0.03	0.015	0.015	0.2
Al-Hassainya	0.1569 ±0.0018 C	0.1026 ±0.0005 D	0.3271 ±0.0024 D	0.06863 ±0.0016 D
Center	5.408 ±0.0682 B	0.1569 ±0.0018 D	5.720 ±0.0637 B	5.632 ±0.0669 A
Twairij	5.509 ±0.0554 B	3.073 ±0.0480 C	0.07613 ±0.0033 D	2.788 ±0.0081 B
Al-Hur	6.409 ±0.1137 A	4.778 ±0.1256 B	6.127 ±0.0974 A	0.1700 ±0.0291 C
Ain Al-Tumar	5.408 ±0.1038 B	5.651 ±0.0217 A	4.625 ±0.0373 C	0.04525- ±0.0031 D

Different capital letters denoted significant ($p \leq 0.0001$) differences in cypermethrin residue concentration of different districts for Karbala province for same type of sample.

A recent study conducted on cypermethrin residues at the holy city of Karbala showed highly significant differences compared to normal values used by the world health organization (WHO).

Meanwhile, horizontal cattle meat samples showed Al-Hur (6.409) significant difference compared to another region (center of Karbala (5.408), Twairij (5.509) and Ain Al Tumar (5.408). It was also found in the

Al-Hassainya (0.1569) lowest occurrence of cypermethrin residues from another region.

The current study also showed a similarity in the percentage of cypermethrin residue in the Twairij district (5.509) and Ain Al-Tumar district (5.408).

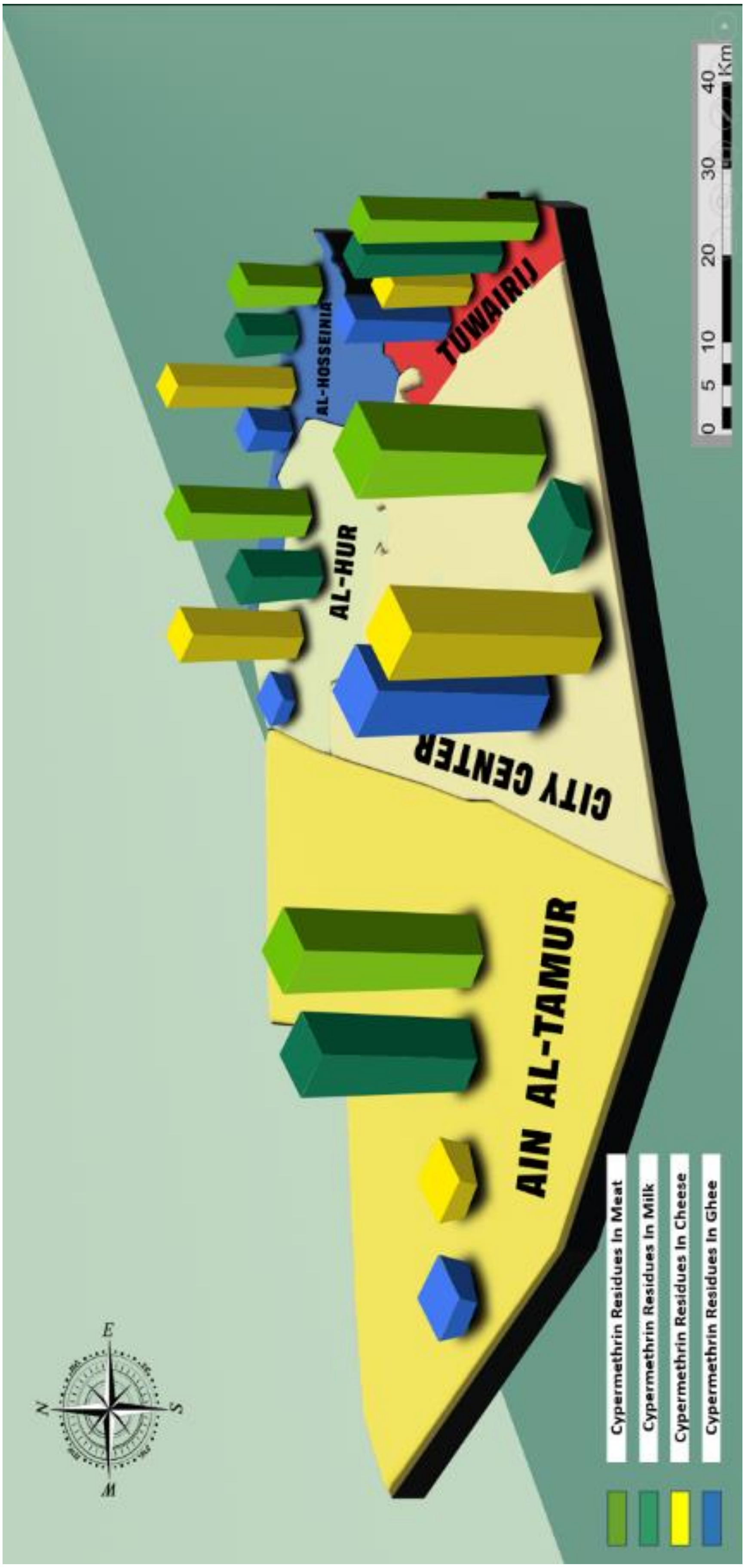

Figure (4-5): A map of the Iraqi Karbala Governorate showing the rates of cypermethrin contamination for some food products of animal origin (livestock meat, milk, cheese, and ghee). Notice how the charts prepared using the program (ArGIs10.8) designed to make geographical charts indicate high rates of contamination, as was explained in the charts and tables previously.

The current result agreed with that found by Jabbar, (2022); Sarmiento-Santos *et al.*, (2024) whom detect high level of cypermethrin residue in cattle muscle after three day of spraying of cattle and confirmed the existence of strong correlation between the amount of pesticides and fat content in cattle meat.

Rothwell *et al.*, (2001) was detected cypermethrin residue in milk sample after two days of treatment of animal in half of collected samples. A maximum residue of 0.025 mg was noted 1 day after treatment in the Jersey study. The spots disappeared in three of the five treated cows' milk one day after treatment and in two of the five samples after days 2 and 3. Approximately half of the remaining in the Jersey group had detectable residues, but were below the LOQ.

FA *et al*., (2019) found Cypermethrin was not detected in cattle samples during winter season, while in summer they were detected in muscle of cattle with a mean value. Cypermethrin was detected in fats samples with a of the same animals.

Jardim *et al*., (2018) pointed to the acute reference level for drug (ARFD) for the compounds with the highest reference doses (ARFD) values were from 21 ng kg-1 bw day-1 for CYP. If these values were below to those recommended by Pesticide Properties Database (PPDB 2017), they present no risk of acute toxicity at this concentration level.

Amr and Sarah (2020) in Egypt found in 2020 that the most abundant pyrethroid residue was cypermethrin in milk samples collected from the cities of Alexandria and Al-Behera, are very high. The level reported in milk samples collected by Matrouh whole milk. When comparing the average contamination level of all milk examined in the three selected provinces. The contamination rate was found in 18.66 and 21.33% of milk samples collected in Alexandria and Al-Behera. This corresponds to a percentage of

44% of the contamination samples reported in Matrouh. Although not all Cypermethrin-contaminated milk exceeded the MRL (100 ppb) according to CAC, the contamination frequency of contaminated samples was significantly different. In the same, the contamination values ± (standard deviation) and mean (Q1-Q3) for the
samples contaminated during the values in ElBehera were very high for Alexandria contaminated milk. Finally, the values of Matrouh-infected samples were higher than the other samples.

Furlani *et al.*, (2015) mentioned even though samples do not remain hazardous concentrations, the presence of low levels of residues of veterinary drugs may be linked to extra-label use or noncompliance withdrawal periods. Additionally, the products evaluated are highly diluted and somewhere in the production chain these levels may have been high. Concerning substances having other approved uses, as pesticides for instance, the real source of milk contamination should be studied in order to improve the dairy management and prevent residues in milk. The results also suggested that the good veterinary practices are not being followed by all producers, since residues of unauthorized drugs have been detected. The illegal use, extra-label use, noncompliance withdrawal periods or the environmental contamination support the need for investigation and continuous monitoring programs to protect human health.

Imperiale *et al.*, (2004) explain during cheese production, the highest residual concentrations of ML were measured in curd and were fold higher in sheep and buffalo cheeses than in the corresponding milks.

Ranjbar and Movassaghghazani, (2023) found in Iran out of the 50, 38, 42, and 100% samples contained cypermethrin over the maximum residue limit set by the EU, respectively. Traditional butter had Cypermethrin residue concentration.

the presence of cypermethrin in cream cheese higher than the level that establish by the Codex establishes the MRL for milk fat, an appropriate parameter for comparing residues detected in cream cheese, a product rich in dairy fat (FNd *et al*., 2024).

Smaldone *et al*., (2013) said that other drugs belonging to the same pyrethroid insecticides group of DLM, such as α-cypermethrin, showed a different fate. found α-cypermethrin in buffalo milk levels were above the maximum residual limit established for bovine milk.

Muhammad *et al.*, (2012) conducted a study in Faisalabad where cow milk was collected from different parts of Faisalabad, Pakistan, and vital microbes were extracted by analysis of insect remains and residues were identified using HPLC. The results of tests showed that a total of 40% of the samples contained pesticides. The analysis of pesticide residues was calculated based on the provisional acceptable daily intake of and the analysis of pesticides. The daily intake of pesticide residues, including cyhalothrin, chlorpyrifos, and cypermethrin, was 3, 11, 2.5, times higher than cow's milk in this study. The results showed that pesticide residues found in milk may cause health problems in the population of the area.

4.5. Residue of Ivermectin:

4.5. Residue of Ivermectin in cattle Meat:

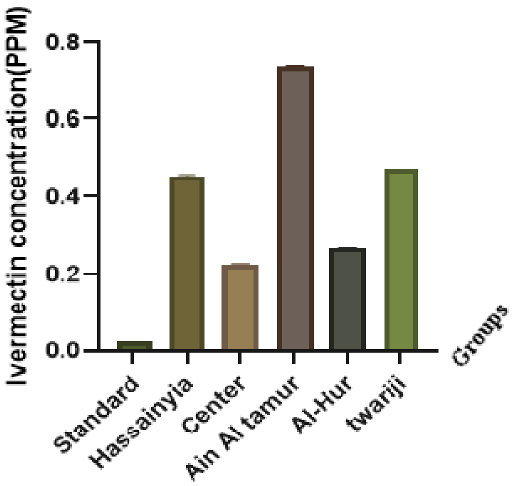

Fig. (4-6): Ivermectin Residue in Meat of Different District for Kerbala Province

Table (4-6): Ivermectin Concentrations in Meat Samples Ppm (Mean±S.D.).

Groups	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.026	0.4293 ±0.002 *	0.1969 ±0.0019 **	0.4461 ±0.0011 ***	0.2425 ±0.0007 ****	0.7081 ±0.0018 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (21), ** Appendix (22), *** Appendix (23), **** Appendix (24) and***** Appendix (25).

The current study showed that all meat samples collected from different districts (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) (0.4293, 0.1969, 0.2425, 0.4461 and 0.7081) had a significant difference at p≤0.0001 compared with maximum residue level of ivermectin pesticide as (WHO) index for 2022 year. On the other hand, it was found that Ain Al-Tumar district (0.7081) had highly value than (Al-Hassainya, Al-Hur, center of Kerbala and Twairij) (0.4293, 0.2425, 0.1969, 0.4461) as shown in figure (4-6) and table (4-6).

4.6. Residue of Ivermectin in Milk:

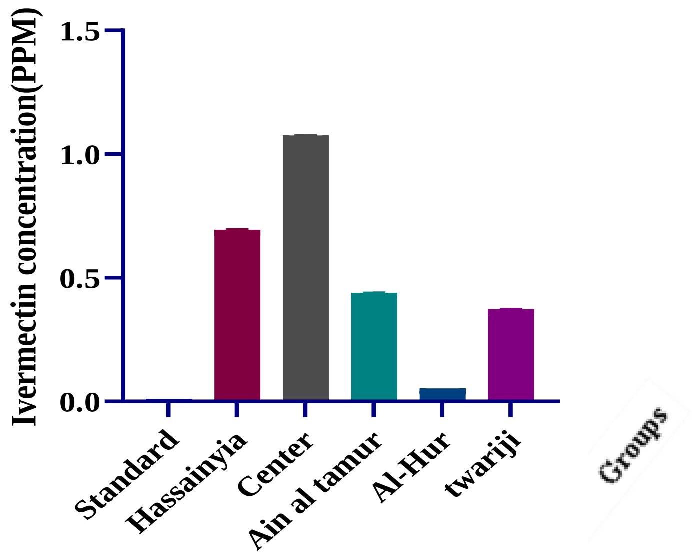

Fig. (4-7): Ivermectin residue in cow' milk of different district for Kerbala Province

Table (4-7): Ivermectin residue concentrations in milk samples ppm (Means±SD.).

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.01	0.6843 ±0.0029 *	1.065 ±0.0013 **	0.3634 ±0.0015 ***	0.04360 ±0.0001 ****	0.4288 ±0.0013 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (26), ** Appendix (27), *** Appendix (28), **** Appendix (29) and***** Appendix (30).

The current study showed that all milk samples collected from different districts (Al-Hassainya, city center, Al-Hur, Twairij and Ain Al-Tamer) (0.6843, 1.065, 0.04360, 0.3634 and 0.4288) had a significant difference at $p \leq 0.0001$ compared with maximum residue level of ivermectin pesticide in (WHO) index for 2022 year.

On the other hand, it was found that center of Kerbala (1.065) district had highly significant than (Al-Hassainya, Al-Hur, Twairij and Ain Al-Tumar) (0.6843, 0.04360, 0.3634 and 0.4288) as shown in figure (4-7) and table (4-7).

4.7. Residue of ivermectin in cheese:

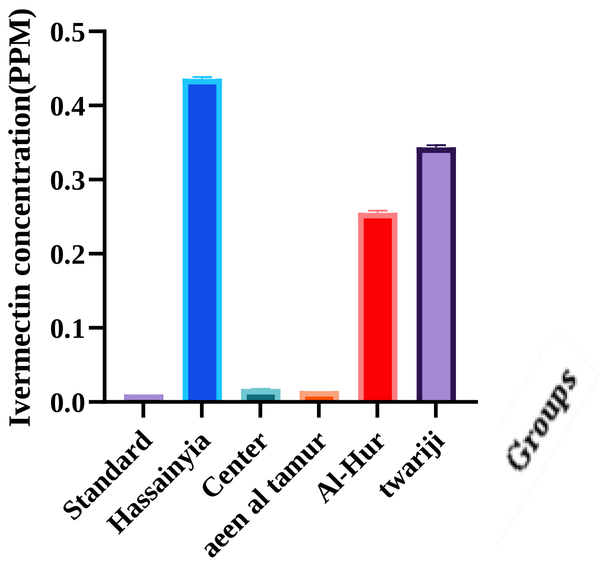

Fig. (4-8): Ivermectin residue in cheese of different district for Kerbala province

Table (4-8): Ivermectin residue concentrations in cheese samples ppm (Means±SD.)

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.01	0.4262 ±0.0022 *	0.0075 ±0.0002 **	0.3336 ±0.0028 ***	0.2453 ±0.0026 ****	0.0052 ±0.0004 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (31), ** Appendix (32), *** Appendix (33), **** Appendix (34) and***** Appendix (35).

The current study showed that all cheese samples collected from different district of Kerbala province (Al-Hassainya, Center, Twairij, Al-Hur, Ain Al-Tumar) (0.4262, 0.0075, 0.3336, 0.2453, 0.0052) had a significant difference at $p\leq0.0001$ compared with maximum residue level of ivermectin pesticide (WHO) index for 2022 year.

On the other hand, it was found that Al-Hassainya district (0.4262) had highly significant than (center of Kerbala, Twairij, Al-Hur and Ain Al-Tumar) (0.0075, 0.3336, 0.2453, 0.0052) as shown in figure (4-8) and table (4-8).

4.7. Residue of Ivermectin in Ghee:

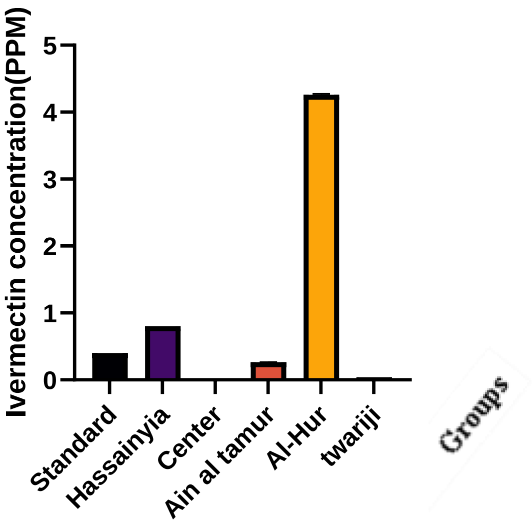

Fig. (4-9): Ivermectin Residue in Ghee in Different District of Kerbala Province

Table (4-9): Ivermectin Concentrations in Ghee Samples ppm (Means±SD.)

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.4	0.4023 ±0.001 *	0.37 0.0001 ± **	0.3595 ± 0.0001 ***	3.858 ±0.0162 ****	0.136 ± 0.001 *****

The stars indicate the original numbers in the table as obtained from the HPLC device and according to the numbers of the following appendices*Appendix (36), ** Appendix (37), *** Appendix (38), **** Appendix (39) and***** Appendix (40).

The current study showed that all ghee samples collected from different district of Kerbala province (Al-Hassainya, Center, Twairij, Al-Hur, Ain Al-Tumar) (0.4023, 0.3786, 0.3595,3.858, 0.1362) respectively had a significant difference at $p \leq 0.0001$ compared with maximum residue level of ivermectin (0.4) pesticide in (WHO) index for 2022 year. On the other hand, it was found that Al-Hur district (3.858) had highly significant from (center of Kerbala, Al-Hassainya, Twairij and Ain Al-Tumar) (0.3786, 0.4023, 0.3595, 0.1362) as shown in figure (4-9) and table (4-9).

Table (4-10): Ivermectin concentrations in different food product in (cattle meat, milk, cheese, ghee) Samples that collected from various region of Karbala province ppm (Means±SD.)

Groups	Ivermectin			
Sample / Regain	Meat	Milk	Cheese	Ghee
WHO	0.026	0.01	0.01	0.4
Al-Hassainya	0.4293 ±0.0020 Bb	0.6843 ±0.0029 Ab	0.4262 ±0.0022 Ba	0.4023 ±0.0013 Cb
Center	0.1969 ±0.0019 Cc	1.065 ±0.0013 Aa	0.0075 ±0.0002 Dd	0.3786 ±0.0001 Bb
Twairij	0.4461 ±0.0011 Ab	0.3634 ±0.0015 Bc	0.3336 ±0.0028 Bb	0.3595 ±0.0001 Bc
Al-Hur	0.2425 ±0.0007 Bc	0.04360 ±0.0001 Dd	0.2453 ±0.0026 Bc	3.858 ±0.0162 Aa
Ain Al-Tumar	0.7081 ±0.0018 Aa	0.4288 0.0013 Bb	0.0052 ±0.0004 Dd	0.1362 ±0.0018 Cd

Different capital letters denoted significant ($p \leq 0.0001$) differences in ivermectin residue concentration of different districts for Karbala province for same type of sample.

The precent showed highly significant differences compared to normal values used by the world health organization (WHO).

Meanwhile, horizontally cattle meat samples showed Ain Al-Tumar (0.7081) significant difference compared to another region (center of Karbala (0.1969), Twairij (0.4461) and Al-Hassainya (0.4293). It was also found in the Center (0.1969) lowest occurrence of ivermectin residues than another regions.

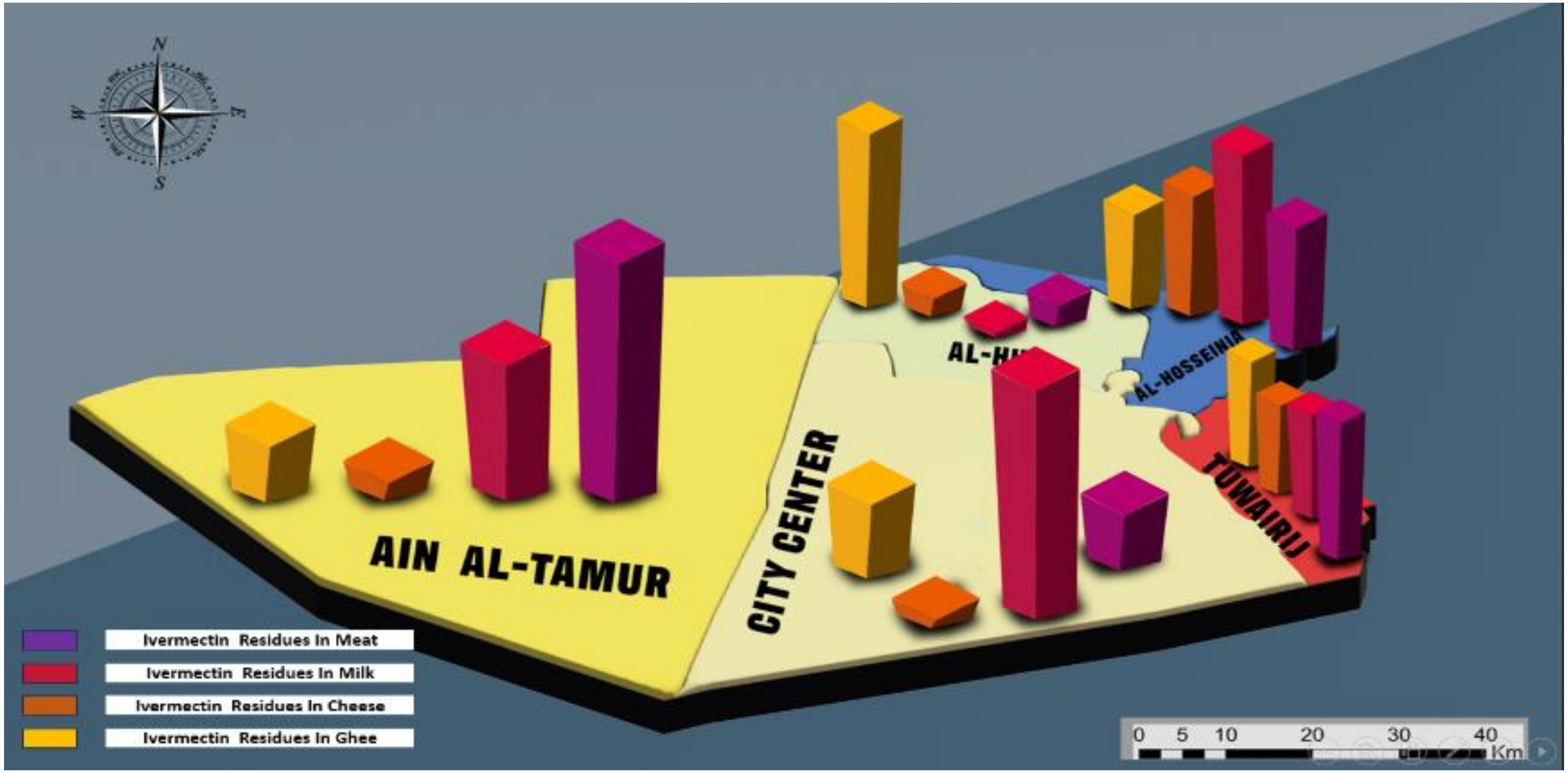

Figure (4-10): A map of the Iraqi Karbala Governorate showing the rates of ivermectin contamination for some food products of animal origin (livestock meat, milk, cheese, and ghee). Notice how the charts prepared using the program (ArGIs10.8) designed to make geographical charts indicate high rates of contamination, as was explained in the charts and tables previously.

Cerkvenik *et al.*, (2004) investigated the destination of ivermectin residues in sheep milk and dairy products (raw milk, raw milk yogurt, pasteurized milk yogurt, cheese, and whey) of animal submitted to a residue depletion test. The highest levels of ivermectin residue were found at 2 days of milking in raw milk, yogurt, and cheese.

Macedo *et al.*, (2015) found a high incidence of the presence of avermectins in butter samples. Butter is not included in the Brazilian National Plan for Control of Residues and Contaminants in Animal Products. As ML residues concentrate in lipophilic compounds, butter and other fatty dairy products should be screened for the presence of ML residues.

Baz *et al.*, (2014)found that only three (7.5%) of 40, meat samples from slaughtered cattle contained ivermectin. The mean value of ivermectin residue at was within the permissible limits. These animals can receive medication and surgery before the waiting period.

Slanina *et al.*, (1989) reported that withdrawal time for ivomec in edible tissues of swine and cattle was 21 and 28 days, respectively. They added that the highest residue levels were found at the injection site.

Macrocyclic lactones (ivermectin and moxidectin) are lipophilic, and the fat in milk promotes drug interactions. Therefore, in the study of ivermectin residues in animals, milk is more important than meat(Flajs *et al.*, 2005). .

Alvinerie *et al.*, (1999)found that ivermectin residue levels reached a maximum concentration in goat milk at 28 days after medication. They estimated that 5% of the ivermectin dose was secreted in the milk.

Cerkvenik *et al.*, (2002)demonstrate that ivermectin residue levels reached a maximum concentration in milk about 13 days after treatment, and residues were still identified 23 days after treatment.

Avermectins, the analytical levels of the milk samples were also below the MRL. However, the results show that the use of ivermectin in dairy cows is significant, as detected/measured residual levels in (46%) of the milk; although it is below the MRL. The presence of doramectin was detected in milks, and the occurrence of abamectin in (1.6%) milks. Abamectin residues in milk will not be reported because it is not intended for use in dairy animals of heading.

Pacheco-Silva *et al.*, (2014) indicate that however, the previous results of the animal control program of the government showed that avermectins, mainly ivermectin, were the main ones reported in total milk, and abamectin residues were reported in 2.80% and 7.25% of milk powder., respectively.

The high levels of pesticides (cypermethrin and ivermectin) in some food products of animal origin (meat, milk, cheese, and ghee) that were found in the results of this study are attributed for the using of this pesticides for purpose of eliminating ticks spread in Iraqi governorates(Obaid *et al.*, 2023) Ticks are vectors for CCHFV(Gargili *et al.*, 2017) A large outbreak of Crimean-Congo hemorrhagic fever (CCHF) has been reported in Iraq and cases have increased without significant control measures(E *et al.*, 2022). Which led to misuse of pesticides and failure to adhere to the pesticide dripping period after injection, dipping, or spraying, which is recommended by the Food and Drug Organization(Zikankuba *et al.*, 2019). This, in turn, led to an increase in pesticide residues (cypermethrin and ivermectin) in food products of animal origin (beef, milk, cheese, and ghee).

Chapter Five
Conclusions and Recommendations

5. Conclusions and Recommendations:

5.1. Conclusion:

1. The results showed high levels of cypermethrin residues in various samples (meat, milk, and milk by-products) in different district of Karbala province.
2. The results showed high levels of cypermethrin residues in various samples (meat, milk, and milk by-products) in different district of Karbala province.

5. 2. Recommendations

From the conclusions, it can recommend the following:

1. Adherence to the prescribed doses mentioned in scientific sources, which are often printed on pesticide containers.
2. Refrain from slaughtering animals during the withdrawal period to ensure that the animal gets rid of the metabolites of these pesticides and their residues.
3. The results showed high levels of cypermethrin residues in various samples (meat, milk, and milk by-products) in different district of Karbala province.
4. The results showed high levels of cypermethrin residues in various samples (meat, milk, and milk by-products) in different district of Karbala province.
5. Conduct a histological study of the liver and kidneys of animal tissues and evaluate the extent of cellular damage resulting from these pesticide residues.
6. Conducting tests at the level of cell DNA and the extent of the ability of these residues to cause changes and mutations in animal cells, which can lead to mutations in human cells.

Chapter Six

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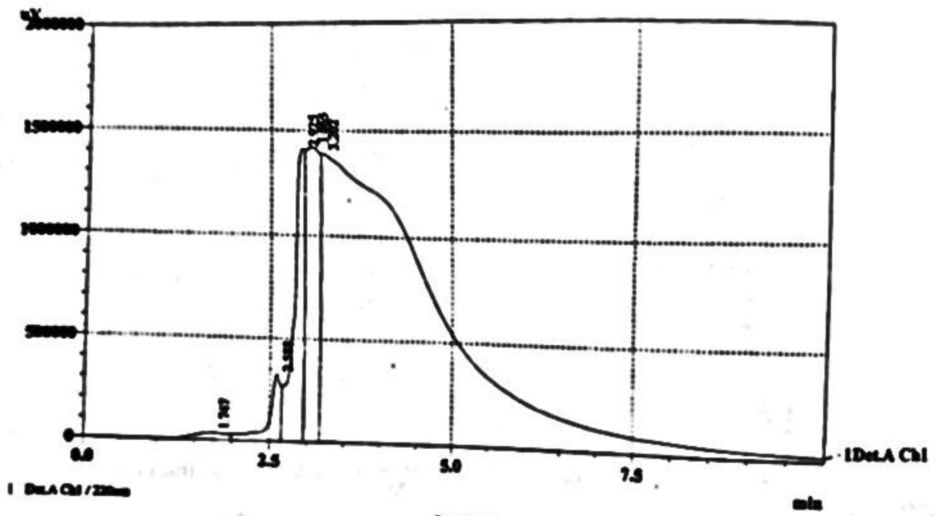
Appendices

Appendix (1): chromatogram from HPLC of detection of cypermethrin in cattle meat sample from Al Hassainya district

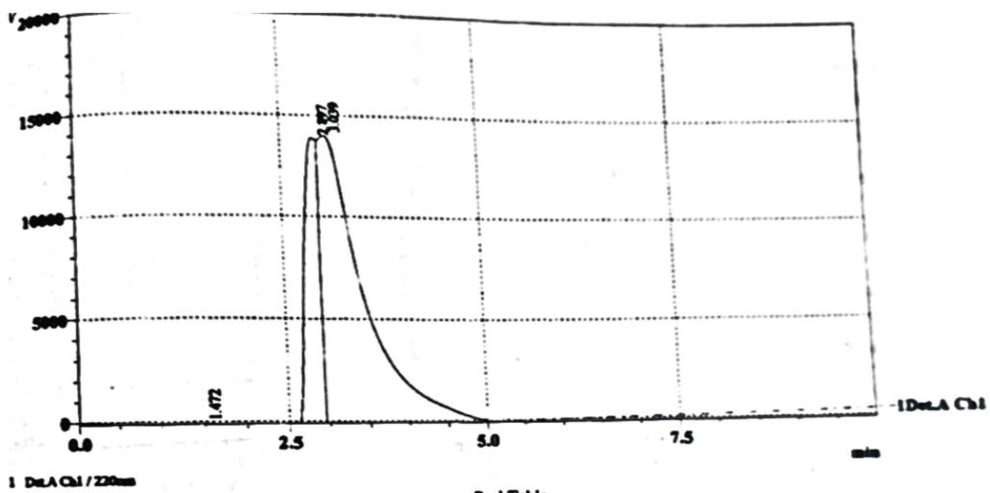


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.747	1075076	34955	0.551	0.763
2	2.588	3665207	322987	1.880	7.054
3	2.925	13882565	1409356	7.119	30.780
4	3.065	18337656	1420561	9.404	31.025
5	3.202	158038810	1390902	81.046	30.377
Total		194999314	4578759	100.000	100.000

Appendix (2): chromatogram from HPLC of detection of cypermethrin in cattle meat sample from city center district



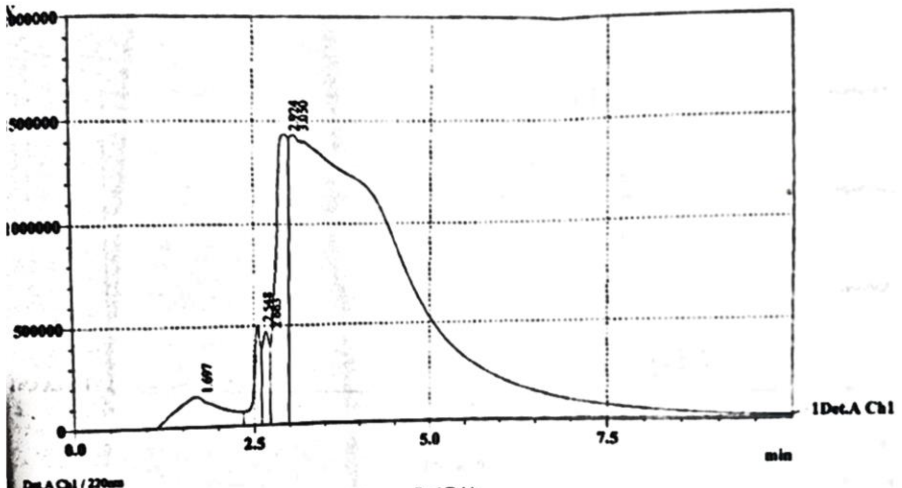
1 Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.472	2784	167	0.354	0.575
2	2.897	183077	14349	23.257	49.436
3	3.039	601315	14509	76.389	49.989
Total		787176	29025	100.000	100.000

Appendix (3): chromatogram from HPLC of detection of cypermethrin in cattle meat sample from Twairij district



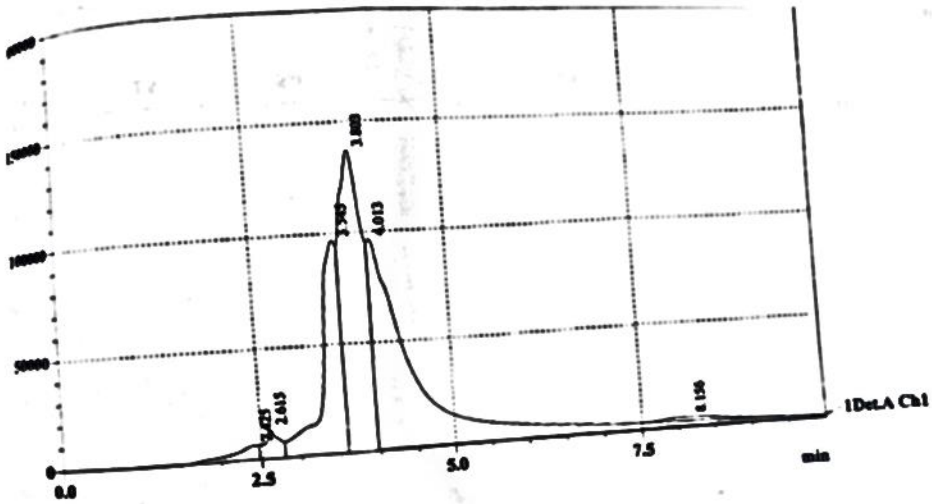
Det.A Ch1 / 220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.697	6790193	152729	3.416	3.828
2	2.548	3551866	500149	1.787	12.534
3	2.663	3058722	470894	1.539	11.801
4	2.924	17604080	1433943	8.857	35.936
5	3.050	167744980	1432553	84.400	35.901
Total		198749840	3990267	100.000	100.000

Appendix (4): chromatogram from HPLC of detection of cypermethrin in cattle meat sample from Al Hur district



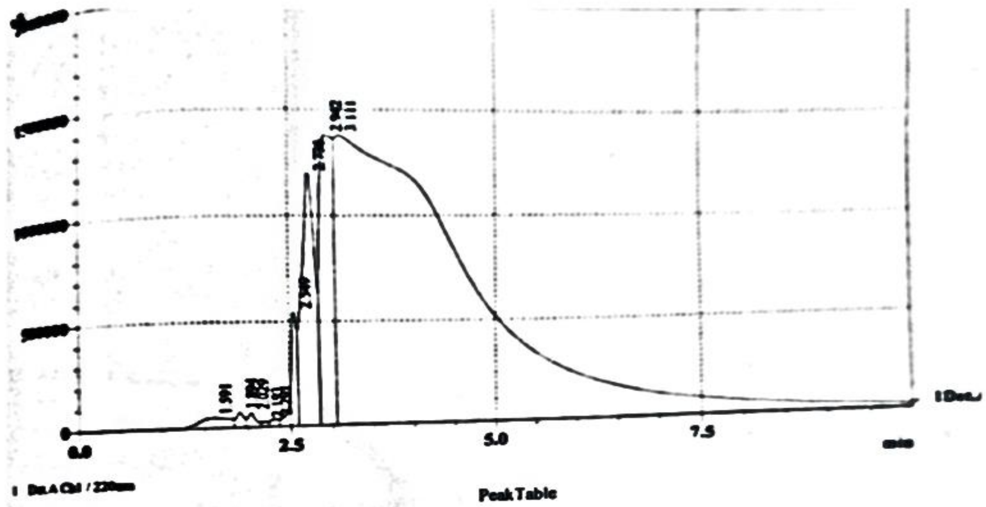
Det.A Ch1 · 245nm

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 (5): chromatogram from HPLC of detection of cypermethrin in cattle meat sample from Ain Al-Tumer district

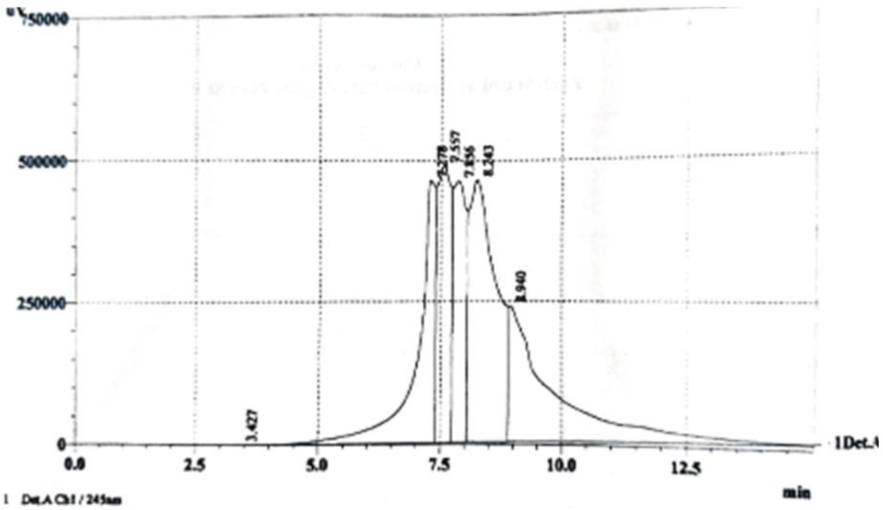


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.591	1223250	49100	0.615	1.033
2	1.894	458113	71793	0.230	1.510
3	2.029	443704	67644	0.223	1.423
4	2.193	137219	26413	0.069	0.556
5	2.291	250689	36099	0.126	0.759
6	2.549	3055224	544636	1.537	11.458
7	2.736	13263755	1198771	6.672	25.221
8	2.942	15495411	1379277	7.794	29.018
9	3.111	164477738	1379406	82.733	29.021
Total		198805103	4753139	100.000	100.000

Appendix (6): chromatogram from HPLC of detection of cypermethrin in milk sample from Al Hassainya district

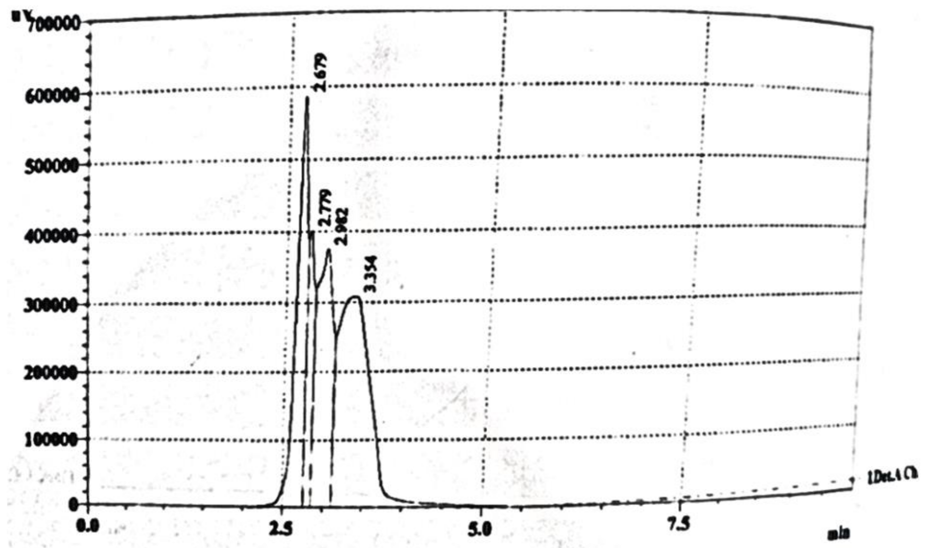


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.427	18757	951	0.030	0.045
2	7.278	11953452	462727	18.949	21.896
3	7.557	9585344	488188	15.195	23.101
4	7.856	8408513	461242	13.329	21.826
5	8.243	17814847	462589	28.240	21.890
6	8.940	15302256	237571	24.257	11.242
Total		63083168	2113269	100.000	100.000

Appendix (7): chromatogram from HPLC of detection of cypermethrin in milk sample from city center district

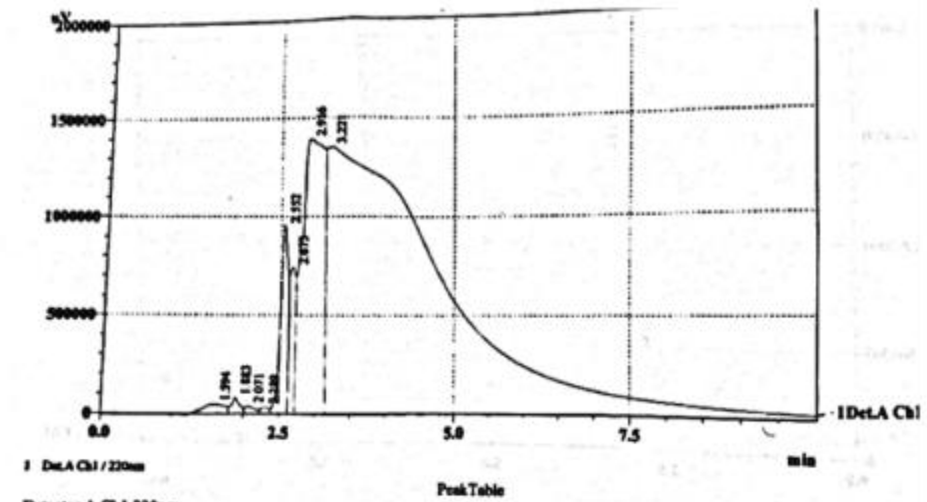


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.679	4861112	590207	22.439	35.091
2	2.779	2194463	403907	10.129	24.015
3	2.982	5077495	377636	23.437	22.453
4	3.354	9531051	310172	43.995	18.441
Total		21664121	1681922	100.000	100.000

Appendix (8): chromatogram from HPLC of detection of cypermethrin in milk sample from Twairij district

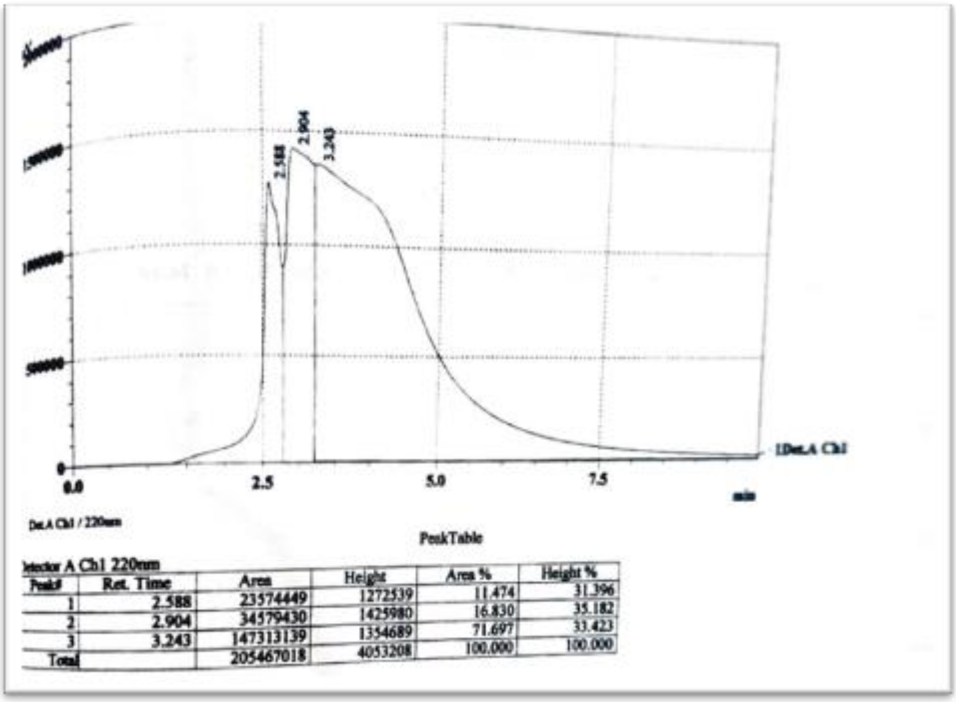


PeakTable

Detector A Ch1 220nm

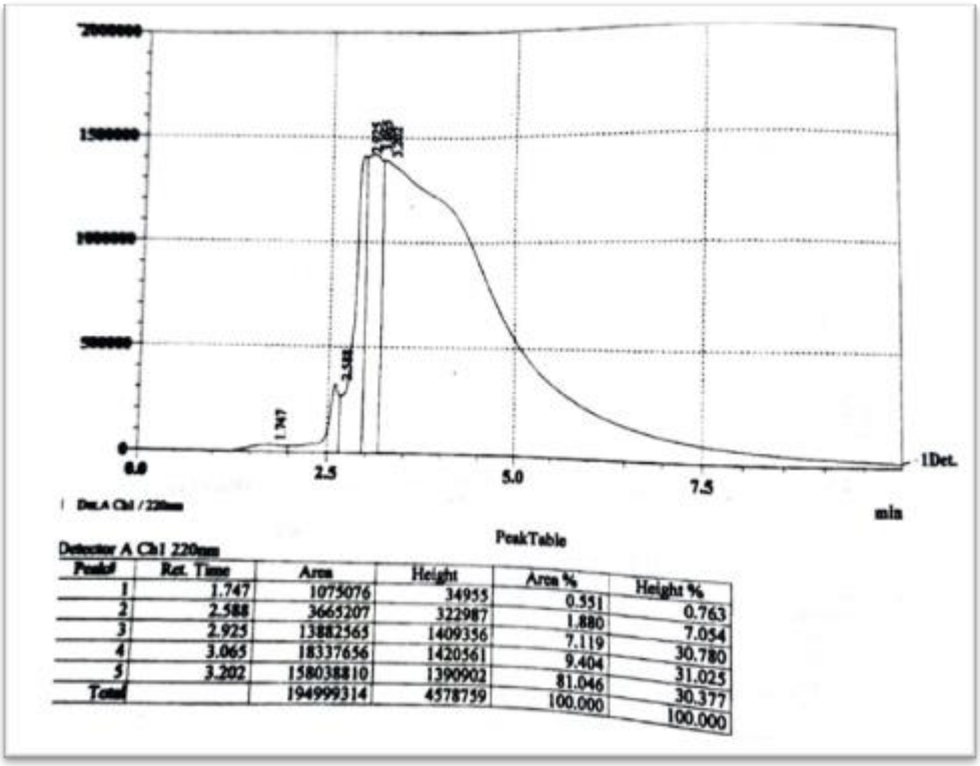
Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.594	1132354	49909	0.542	1.073
2	1.883	706184	82246	0.338	1.768
3	2.071	421284	41447	0.202	0.891
4	2.289	292053	36042	0.140	0.775
5	2.552	6778206	957437	3.247	20.577
6	2.673	4328476	740743	2.073	15.920
7	2.916	31028604	1393453	14.864	29.948
8	3.221	164067480	1351708	78.593	29.050
Total		208754640	4652984	100.000	100.000

Appendix (9): chromatogram from HPLC of detection of cypermethrin in milk sample from Al Hur district



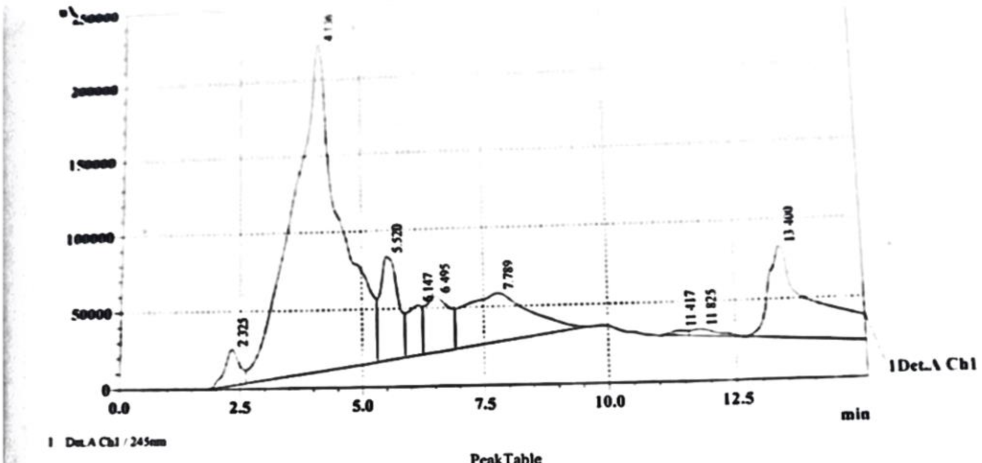
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.588	23574449	1272539	11.474	31.396
2	2.904	34579430	1425980	16.830	35.182
3	3.243	147313139	1354689	71.697	33.423
Total		205467018	4053208	100.000	100.000

Appendix (10): chromatogram from HPLC of detection of cypermethrin in milk sample from Ain Al-Tumer district



Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.747	1075076	34955	0.551	0.763
2	2.588	3665207	322987	1.880	7.054
3	2.925	13882565	1409356	7.119	30.780
4	3.065	18337656	1420561	9.404	31.025
5	3.202	158038810	1390902	81.046	30.377
Total		194999314	4578759	100.000	100.000

Appendix (11): chromatogram from HPLC of detection of cypermethrin in cheese sample from Al Hassainya district

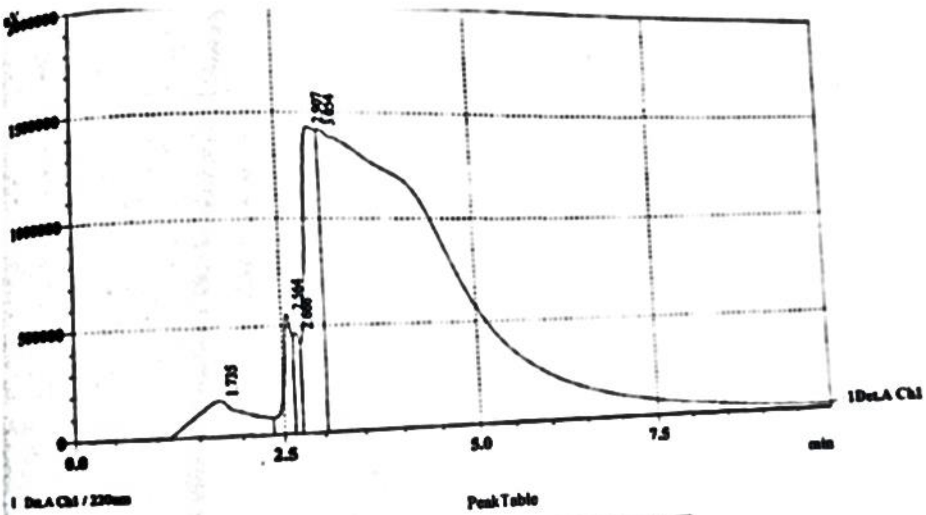


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.325	501057	23366	1.900	5.018
2	4.136	14227462	213221	53.956	45.786
3	5.520	1700332	66325	6.448	14.242
4	6.147	665062	31654	2.522	6.797
5	6.495	1230953	35777	4.668	7.682
6	7.789	2859778	30853	10.845	6.625
7	11.417	73408	3211	0.278	0.689
8	11.825	163384	4396	0.620	0.944
9	13.400	4946987	56891	18.761	12.216
Total		26368422	465693	100.000	100.000

Appendix (12): chromatogram from HPLC of detection of cypermethrin in cheese sample from city center district

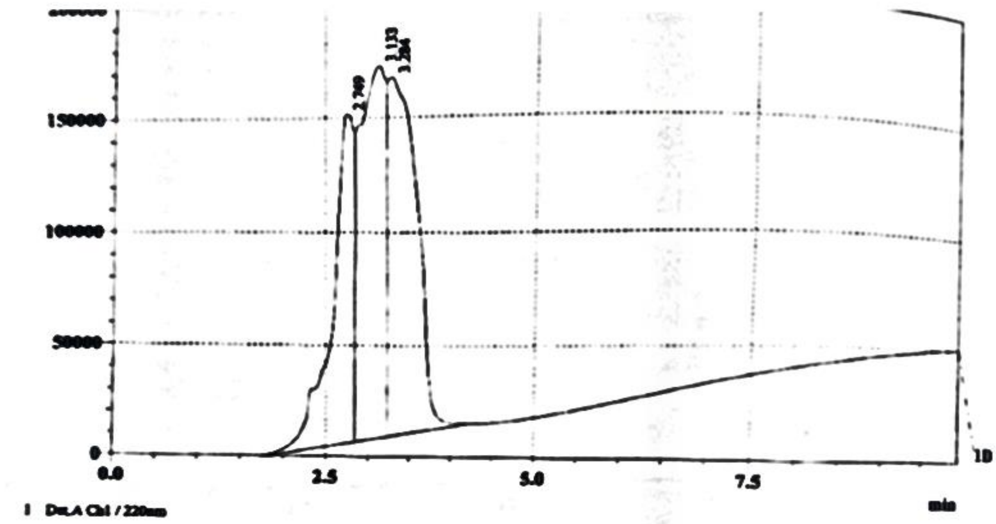


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.735	7315742	164900	3.525	4.086
2	2.564	4456174	555309	2.147	13.759
3	2.666	2485971	466730	1.198	11.564
4	2.907	20431112	1430107	9.845	35.433
5	3.054	172830926	1419017	83.284	35.158
Total		207519926	4036064	100.000	100.000

Appendix (13): chromatogram from HPLC of detection of cypermethrin in cheese sample from Twairij district

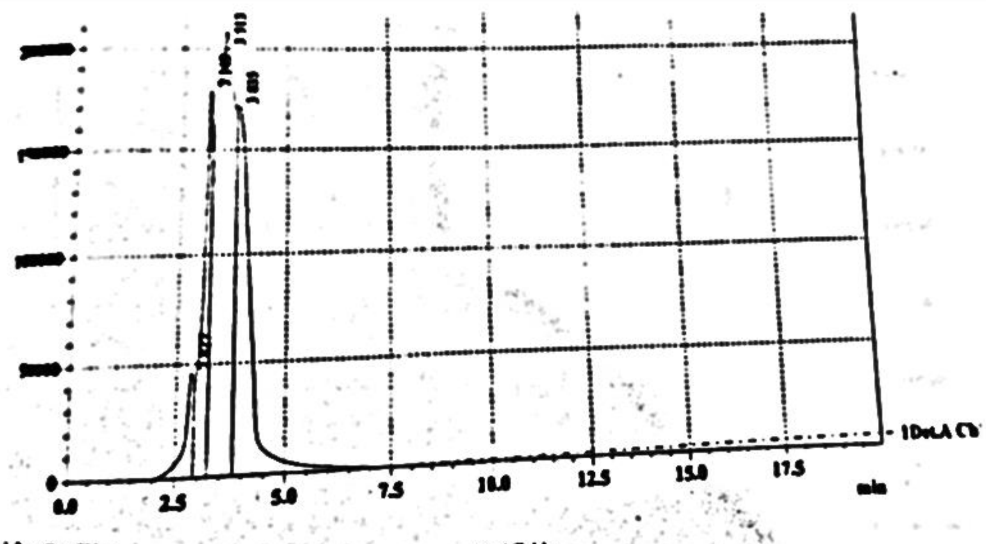


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.749	2630924	145976	26.645	31.202
2	3.133	3509694	163847	35.545	35.022
3	3.284	3733337	158013	37.810	33.775
Total		9873955	467836	100.000	100.000

Appendix (14): chromatogram from HPLC of detection of cypermethrin in cheese sample from Al Hur district

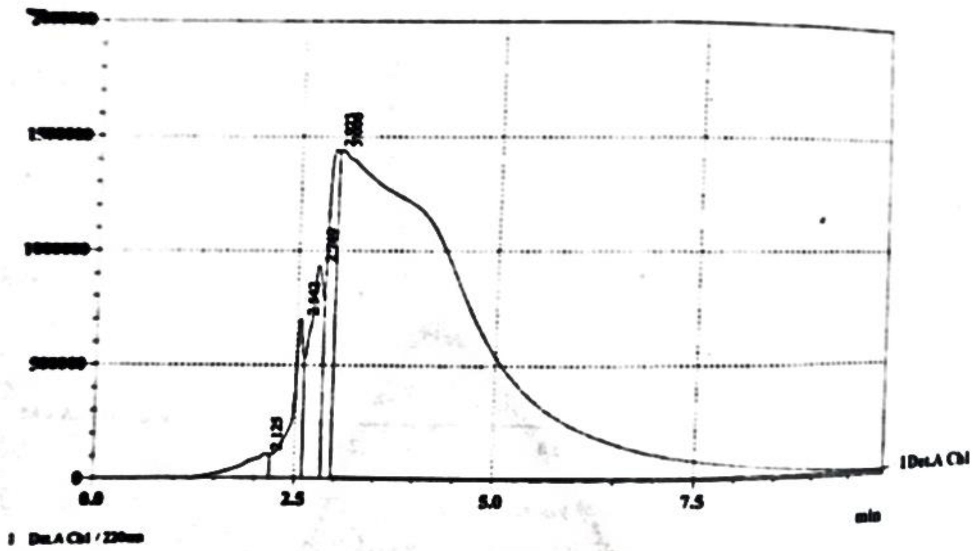


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.827	685266	47328	5.139	7.761
2	3.149	1894089	180567	14.205	29.610
3	3.513	6361132	210025	47.705	34.440
4	3.835	4393845	171907	32.951	28.189
Total		13334332	609827	100.000	100.000

Appendix (15): chromatogram from HPLC of detection of cypermethrin in cheese sample from Ain Al-Tumer district

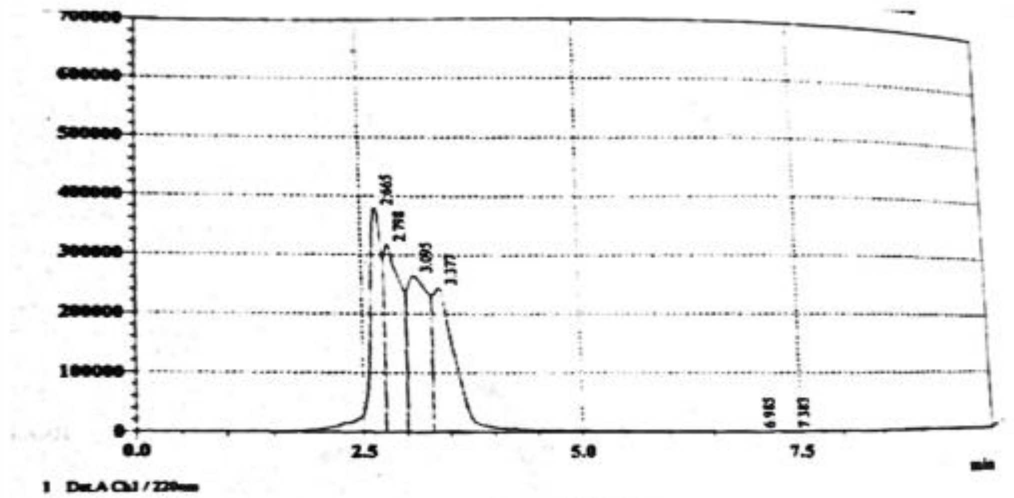


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.125	2611833	107279	1.296	2.320
2	2.542	7042621	702126	3.494	15.187
3	2.749	10732092	934841	5.325	20.220
4	2.923	10631993	1439995	5.275	31.146
5	3.006	170535856	1439075	84.610	31.126
Total		201554395	4623316	100.000	100.000

Appendix (16): chromatogram from HPLC of detection of cypermethrin in ghee sample from Al Hassainya district

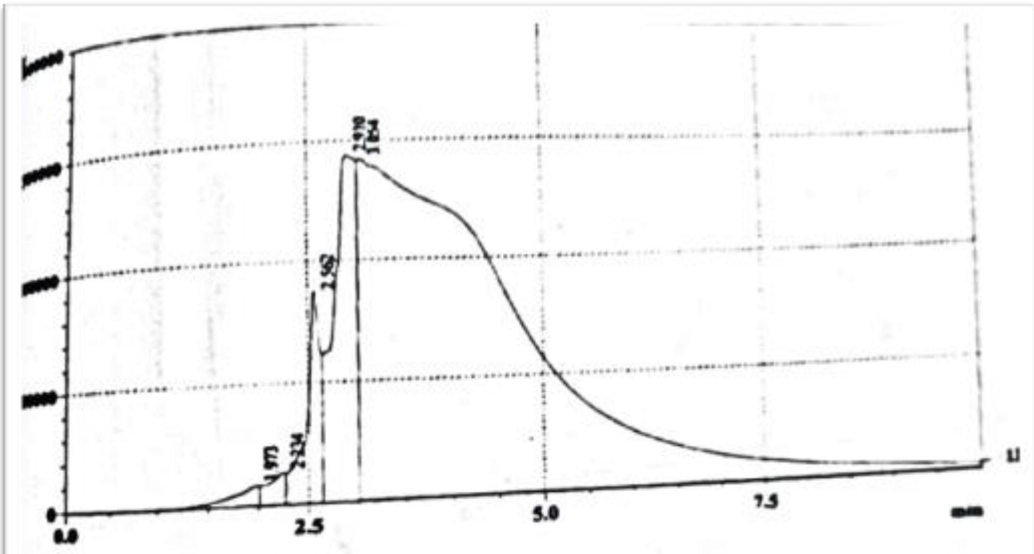


PeakTable

Detector A Ch1 220nm

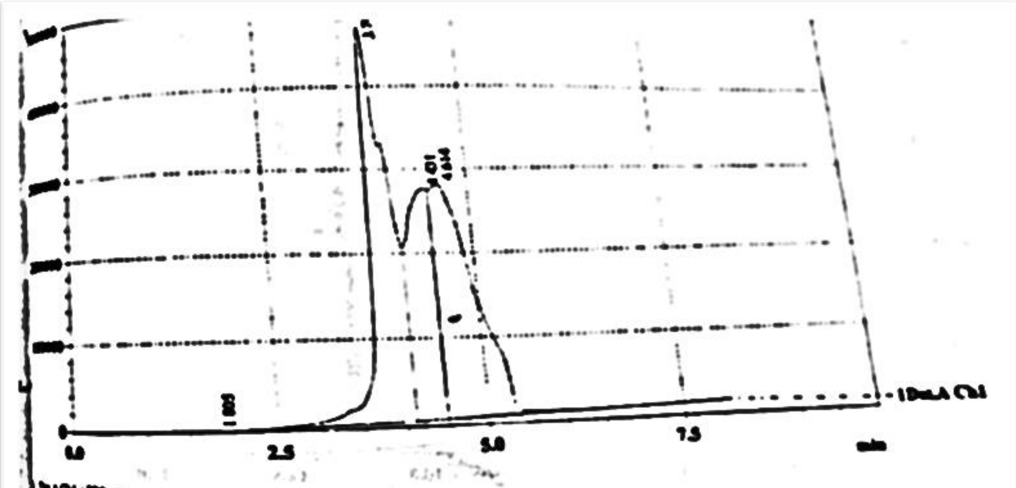
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.665	4022146	379451	23.640	31.435
2	2.798	4176366	317611	24.546	26.312
3	3.095	4373207	265029	25.703	21.956
4	3.377	4418635	244382	25.970	20.245
5	6.985	3535	194	0.021	0.016
6	7.383	1704	114	0.010	0.009
7	10.692	16636	170	0.098	0.014
8	10.908	1946	147	0.011	0.012
Total		17014176	1207098	100.000	100.000

Appendix (17): chromatogram from HPLC of detection of cypermethrin in ghee sample from city center district



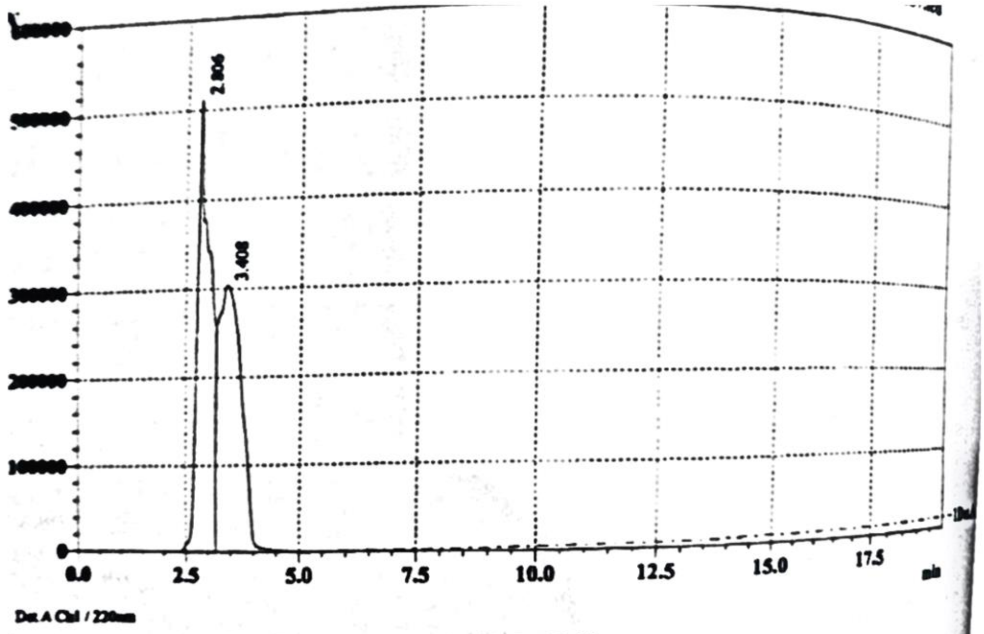
Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.973	1606006	82220	0.759	2.083
2	2.234	1596593	129960	0.755	3.293
3	2.562	8844560	876533	4.180	22.211
4	2.910	23867664	1436725	11.281	36.406
5	3.054	175653089	1420959	83.024	36.006
Total		211567912	3946396	100.000	100.000

Appendix (18): chromatogram from HPLC of detection of cypermethrin in ghee sample from Twairij district



Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.805	25826	619	0.110	0.061
2	3.793	9066958	467684	38.624	46.212
3	4.431	5684309	269212	24.214	26.601
4	4.614	8697984	274518	37.052	27.125
Total		23475076	1012033	100.000	100.000

Appendix (19): chromatogram from HPLC of detection of cypermethrin in ghee sample from Al Hur district

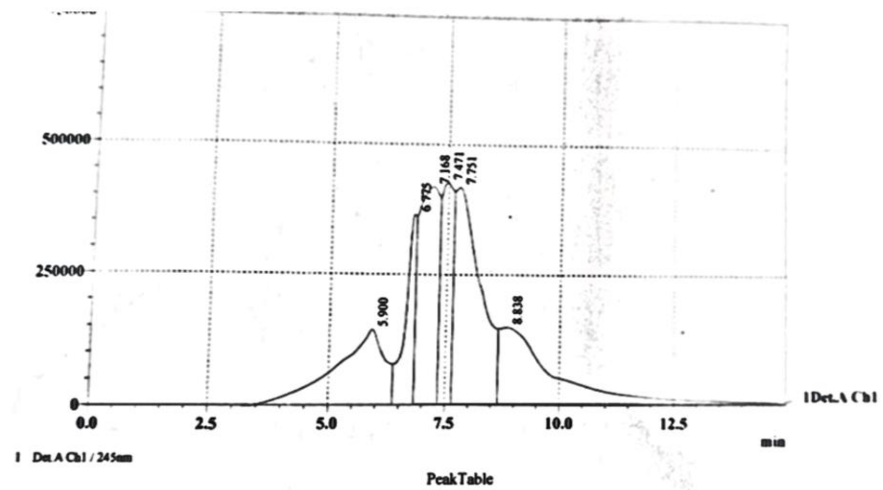


PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.806	10335806	514402	47.824	62.658
2	3.408	11276268	306571	52.176	37.342
Total		21612074	820973	100.000	100.000

Appendix (20): chromatogram from HPLC of detection of cypermethrin in ghee sample from Ain Al-Tumer district

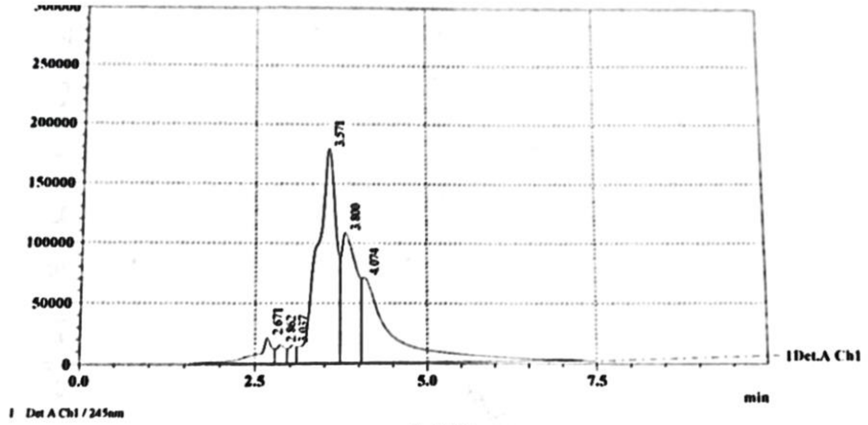


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.900	10387050	142365	16.068	7.437
2	6.775	5187417	364424	8.025	19.037
3	7.168	12414408	417646	19.204	21.817
4	7.471	7493055	425347	11.591	22.219
5	7.751	16560559	417071	25.618	21.787
6	8.838	12601854	147473	19.494	7.704
Total		64644344	1914327	100.000	100.000

Appendix (21): chromatogram from HPLC of detection of ivermectin in cattle meat sample from Al Hassainya district

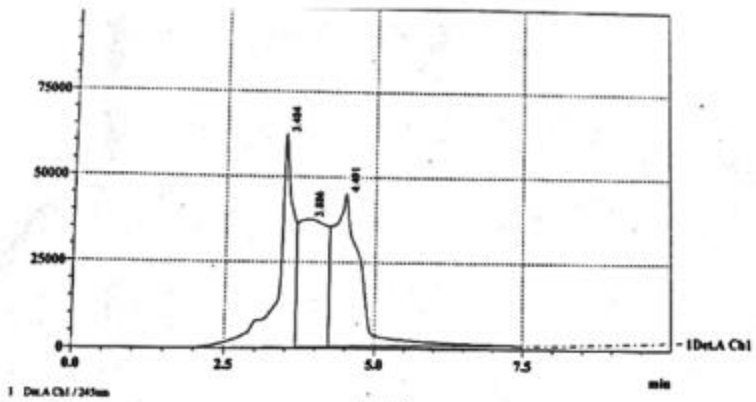


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.671	370222	21350	4.717	5.230
2	2.862	141801	15447	1.807	3.784
3	3.037	110461	15216	1.407	3.727
4	3.571	3378678	178154	43.047	43.639
5	3.800	1667322	107809	21.243	26.408
6	4.074	2180391	70272	27.780	17.213
Total		7848875	408247	100.000	100.000

Appendix (22): chromatogram from HPLC of detection of ivermectin in cattle meat sample from city center district

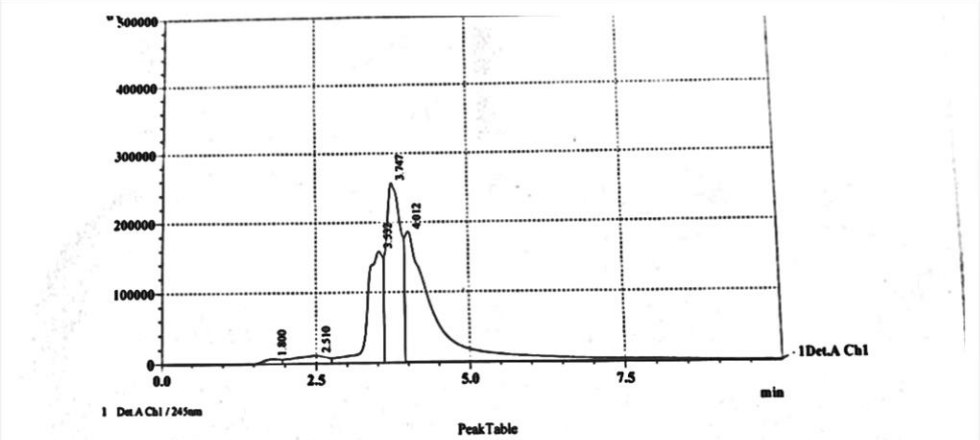


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.484	1098396	62342	29.620	43.230
2	3.886	1206235	37245	32.528	25.827
3	4.491	1403718	44624	37.853	30.944
Total		3708349	144211	100.000	100.000

Appendix (23): chromatogram from HPLC of detection of ivermectin in cattle meat sample from Twairij district

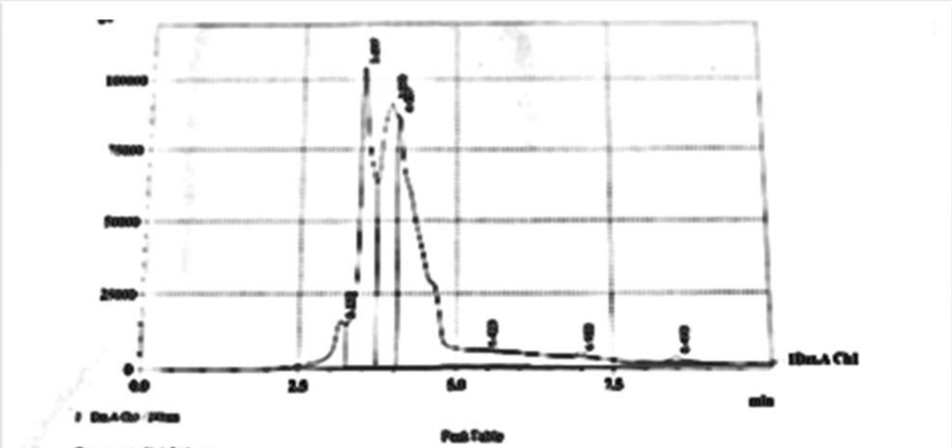


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.800	137436	7198	0.973	1.162
2	2.510	428605	11251	3.034	1.817
3	3.532	2838687	158172	20.094	25.540
4	3.747	4335093	256397	30.686	41.401
5	4.012	6387444	186283	45.214	30.080
Total		14127265	619300	100.000	100.000

Appendix (24): chromatogram from HPLC of detection of ivermectin in cattle meat sample from Al Hur district

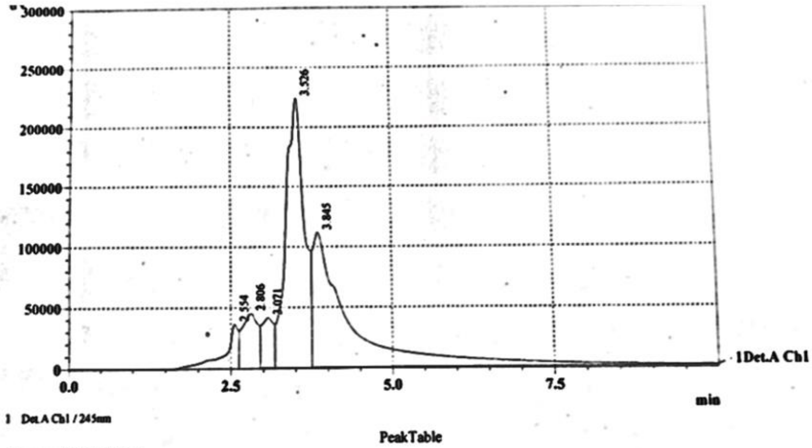


Peak Table

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.150	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	5.425	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	[illegible]	0.644	[illegible]
8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total		[illegible]	[illegible]	100.000	100.000

Appendix (25): chromatogram from HPLC of detection of ivermectin in cattle meat sample from Ain Al-Tumer district

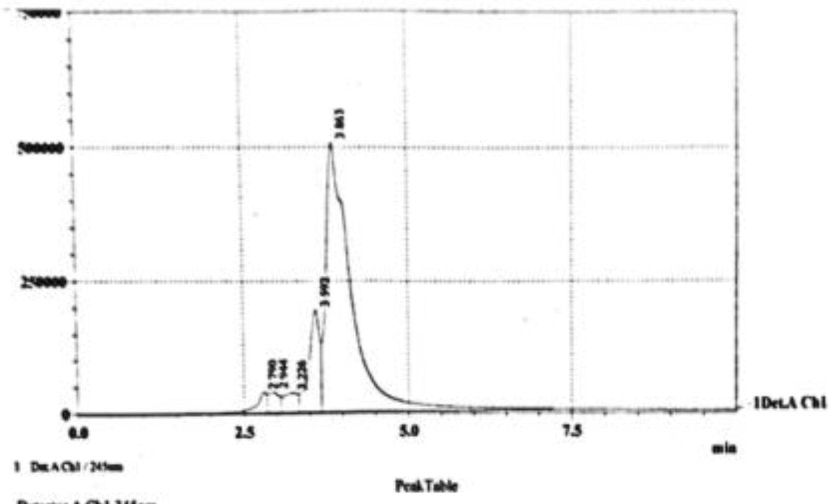


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.554	544123	36058	4.999	7.888
2	2.806	745319	44756	6.847	9.790
3	3.071	513359	41220	4.716	9.017
4	3.526	4412133	223729	40.532	48.941
5	3.845	4670640	111378	42.907	24.364
Total		10885573	457141	100.000	100.000

Appendix (26): chromatogram from HPLC of detection of ivermectin in milk sample from Al Hassainya district

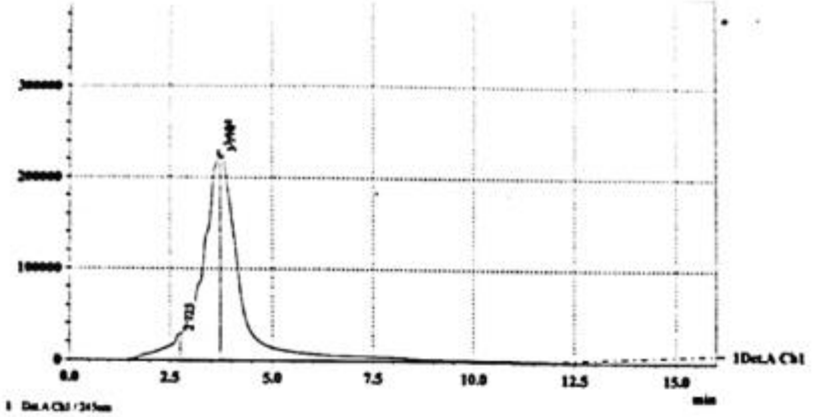


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.790	516826	38755	2.977	4.759
2	2.944	453533	39962	2.612	4.907
3	3.226	560893	36823	3.230	4.522
4	3.592	2249293	193105	12.954	23.713
5	3.863	13581431	505676	78.220	62.096
6	12.183	1065	29	0.006	0.004
Total		17363040	814351	100.000	100.000

Appendix (27): chromatogram from HPLC of detection of ivermectin in milk sample from city center district

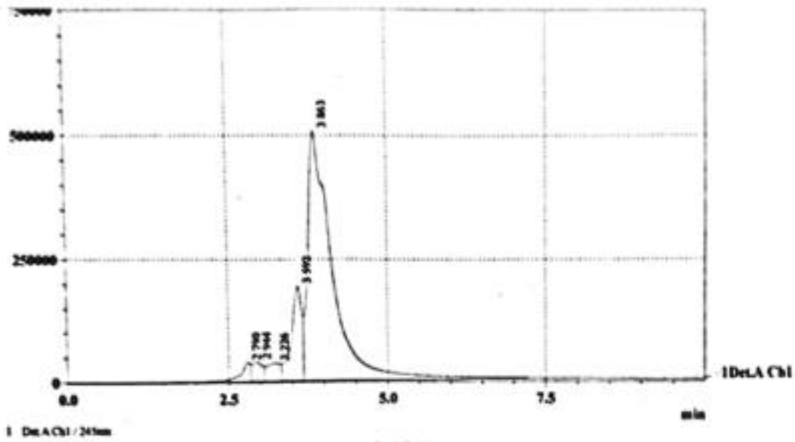


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.725	778009	28735	5.282	5.952
2	3.684	6454242	230130	43.822	47.668
3	3.776	7496193	223916	50.896	46.380
Total		14728444	482781	100.000	100.000

Appendix (28): chromatogram from HPLC of detection of ivermectin in milk sample from Twairij district

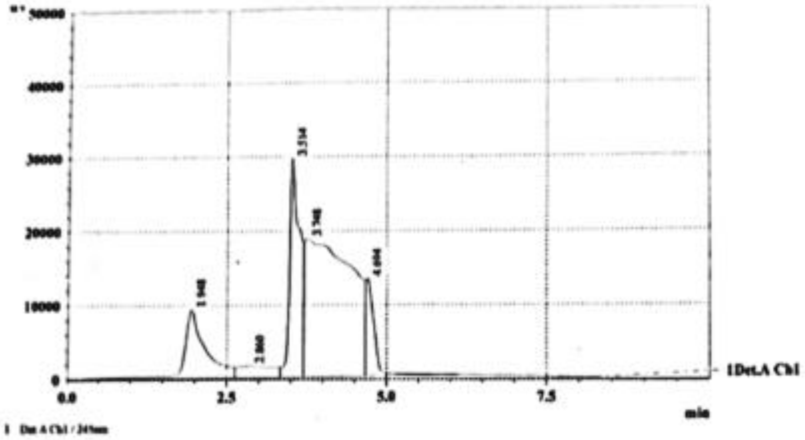


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.790	516826	38755	2.977	4.759
2	2.944	453533	39962	2.612	4.907
3	3.226	560893	36823	3.230	4.522
4	3.592	2249293	193105	12.954	23.713
5	3.863	13581431	505676	78.220	62.096
6	12.183	1065	29	0.006	0.004
Total		17363040	814351	100.000	100.000

Appendix (29): chromatogram from HPLC of detection of ivermectin in milk sample from Al Hur district

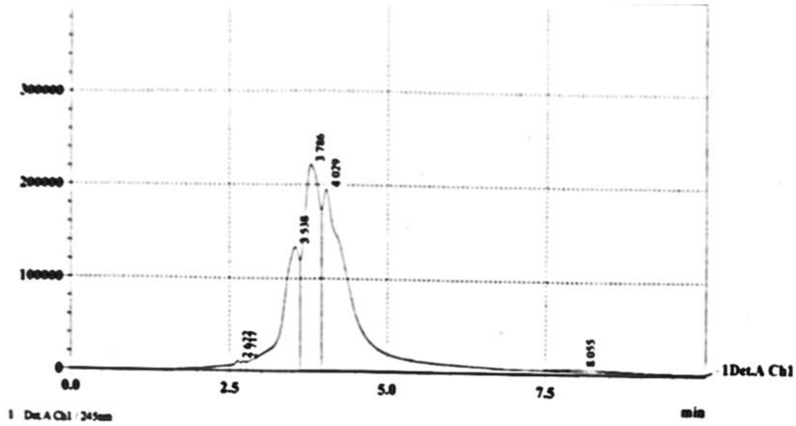


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.948	221317	9268	13.255	12.881
2	2.860	62430	1583	3.739	2.200
3	3.514	322789	29438	19.332	40.915
4	3.748	942771	18568	56.462	25.808
5	4.694	120427	13092	7.212	18.196
Total		1669734	71949	100.000	100.000

Appendix (30): chromatogram from HPLC of detection of ivermectin in milk sample from Ain Al-Tumer district

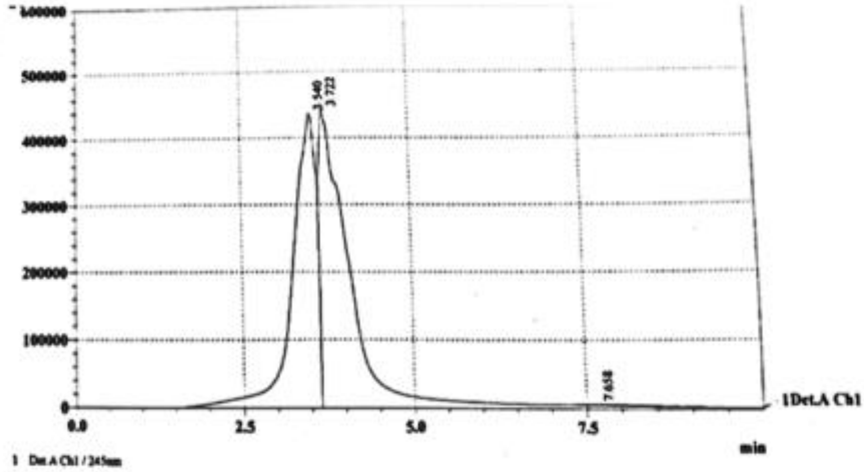


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.622	220025	10222	1.605	1.785
2	2.717	53319	10029	0.389	1.752
3	3.538	2637182	133589	19.242	23.332
4	3.786	3825026	221107	27.909	38.617
5	4.029	6816914	194916	49.738	34.043
6	8.055	153101	2697	1.117	0.471
Total		13705567	572560	100.000	100.000

Appendix (31): chromatogram from HPLC of detection of ivermectin in cheese sample from Al Hassainya district

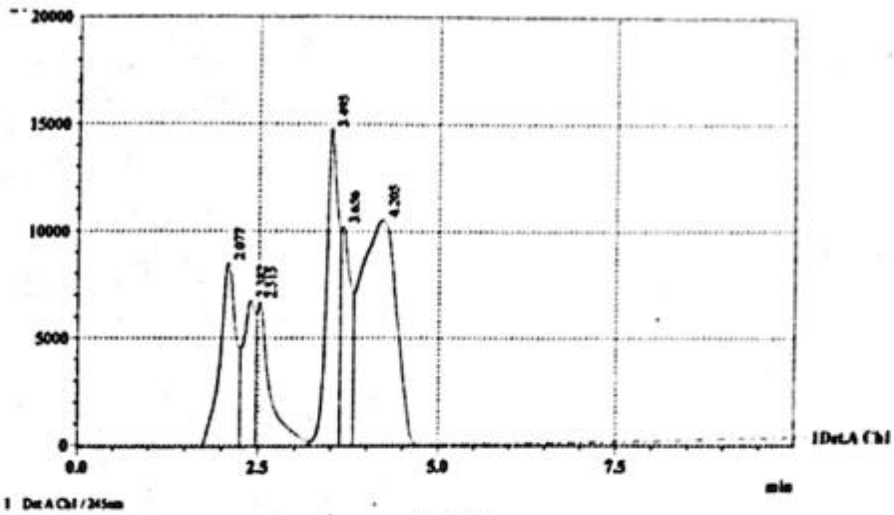


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.540	11059977	436068	43.902	49.572
2	3.722	14019614	442089	55.650	50.256
3	7.658	112819	1509	0.448	0.172
Total		25192410	879665	100.000	100.000

Appendix (32): chromatogram from HPLC of detection of ivermectin in cheese sample from city center district

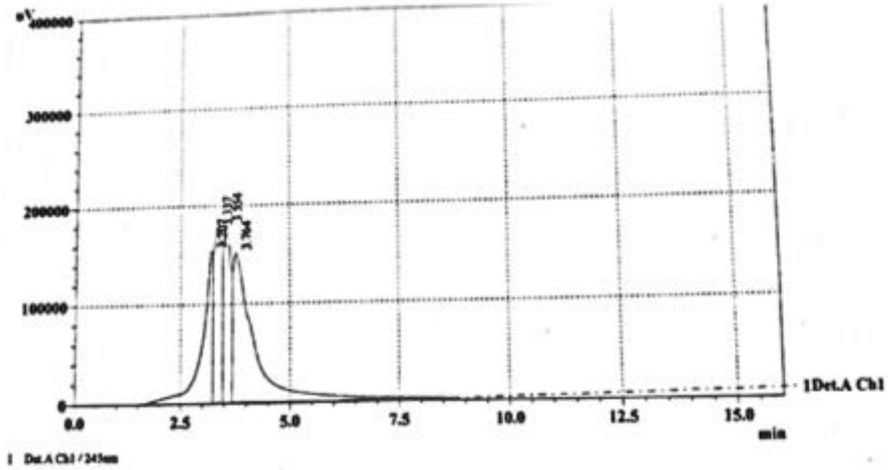


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.077	154052	9333	13.826	14.773
2	2.382	86913	7590	7.801	12.014
3	2.515	122565	7536	11.000	11.928
4	3.495	190300	15687	17.080	24.829
5	3.656	105053	11120	9.429	17.601
6	4.205	435338	11435	39.072	18.099
7	11.792	10421	114	0.935	0.181
8	12.200	2761	117	0.248	0.184
9	12.558	2269	110	0.204	0.174
10	13.050	2856	84	0.256	0.133
11	13.583	1655	53	0.149	0.084
Total		1114183	63180	100.000	100.000

Appendix (33): chromatogram from HPLC of detection of ivermectin in cheese sample from Twairij district

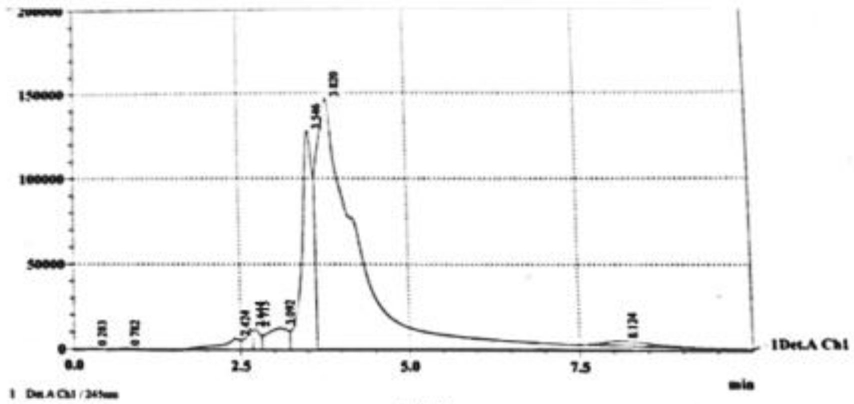


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.207	2632426	153154	21.457	23.178
2	3.337	2235533	176621	18.222	26.730
3	3.554	2096244	180013	17.087	27.243
4	3.764	5304159	150983	43.234	22.850
Total		12268361	660771	100.000	100.000

Appendix (34): chromatogram from HPLC of detection of ivermectin in cheese sample from Al Hur district

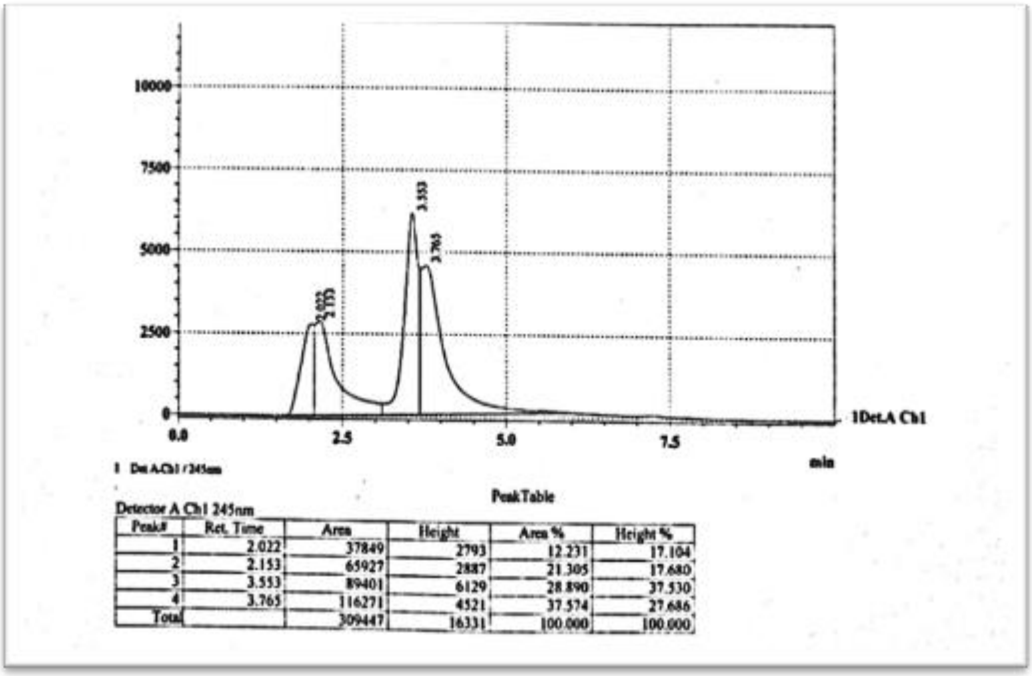


PeakTable

Detector A Ch1 245nm

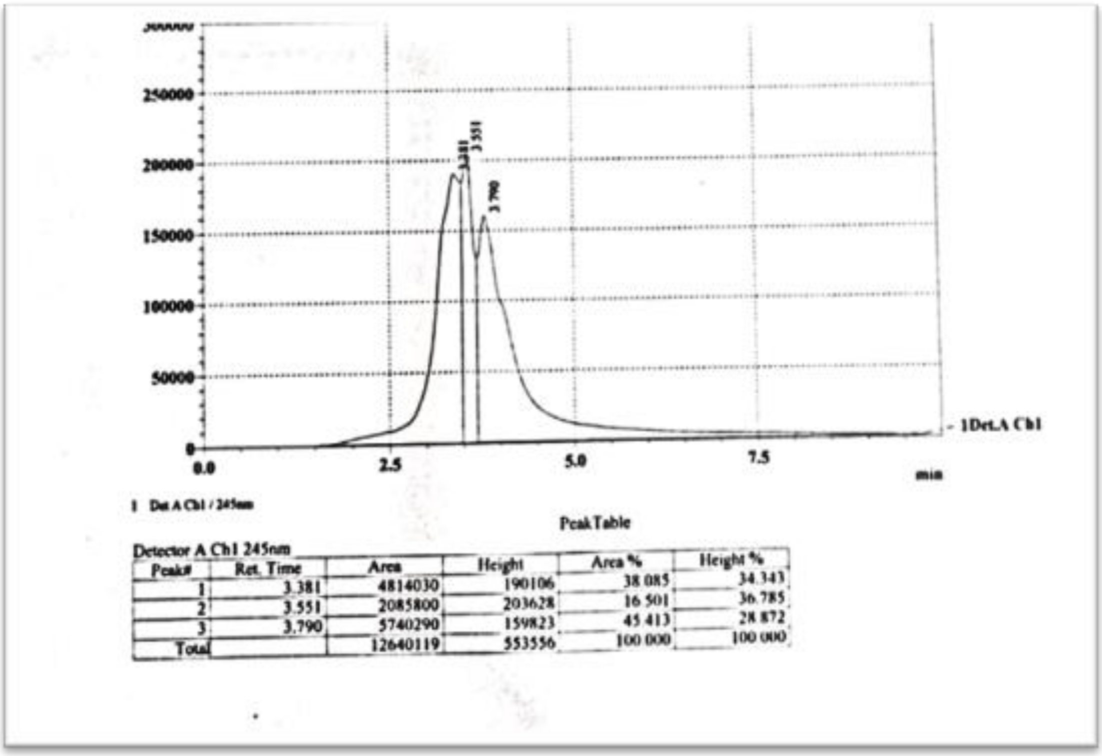
Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.283	6805	405	0.079	0.126
2	0.782	14707	800	0.171	0.249
3	2.424	136207	6724	1.581	2.094
4	2.644	93863	11806	1.089	3.678
5	2.715	82606	12013	0.959	3.742
6	3.092	277512	12638	3.221	3.937
7	3.546	1541417	127580	17.889	39.740
8	3.820	6327578	146254	73.435	45.558
9	8.124	135867	2812	1.577	0.876
Total		8616563	321032	100.000	100.000

Appendix (35): chromatogram from HPLC of detection of ivermectin in cheese sample from Ain Al-Tumer district



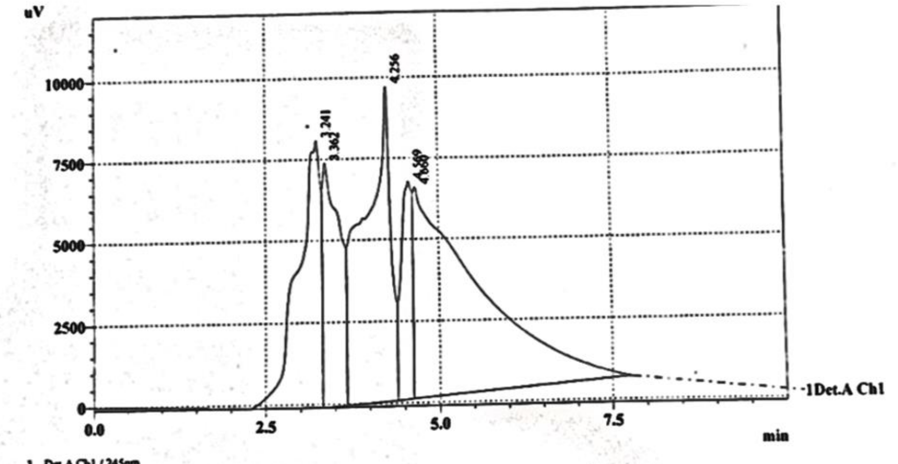
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.022	37849	2793	12.231	17.104
2	2.153	65927	2887	21.305	17.680
3	3.553	89401	6129	28.890	37.530
4	3.765	116271	4521	37.574	27.686
Total		309447	16331	100.000	100.000

Appendix (36): chromatogram from HPLC of detection of ivermectin in ghee sample from Al Hassainya district



Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.381	4814030	190106	38.085	34.343
2	3.551	2085800	203628	16.501	36.785
3	3.790	5740290	159823	45.413	28.872
Total		12640119	553556	100.000	100.000

Appendix (37): chromatogram from HPLC of detection of ivermectin in ghee sample from city center district

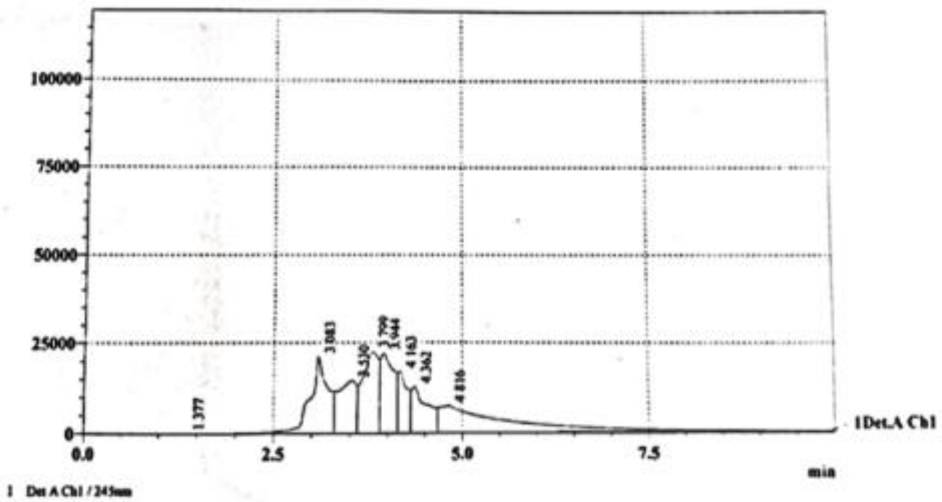


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.241	194797	8196	18.025	21.290
2	3.362	128103	7515	11.853	19.521
3	4.256	255605	9656	23.651	25.081
4	4.569	75229	6662	6.961	17.305
5	4.660	426999	6469	39.510	16.803
Total		1080733	38498	100.000	100.000

Appendix (38): chromatogram from HPLC of detection of ivermectin in ghee sample from Twairij district

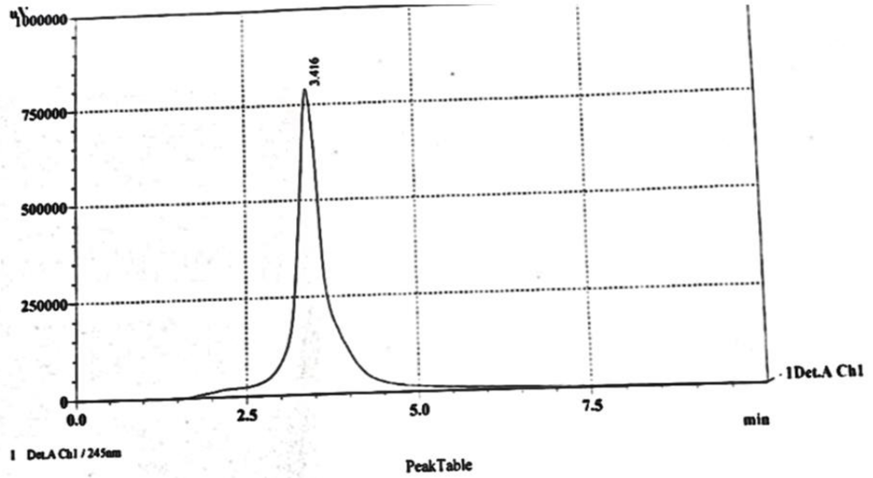


PeakTable

Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.377	1129	37	0.055	0.032
2	3.083	346471	21152	17.022	17.994
3	3.530	240757	14541	11.828	12.370
4	3.799	336774	22532	16.545	19.167
5	3.944	267680	22010	13.151	18.723
6	4.163	147258	17012	7.235	14.472
7	4.362	190674	12828	9.367	10.912
8	4.816	504746	7440	24.797	6.329
Total		2035491	117553	100.000	100.000

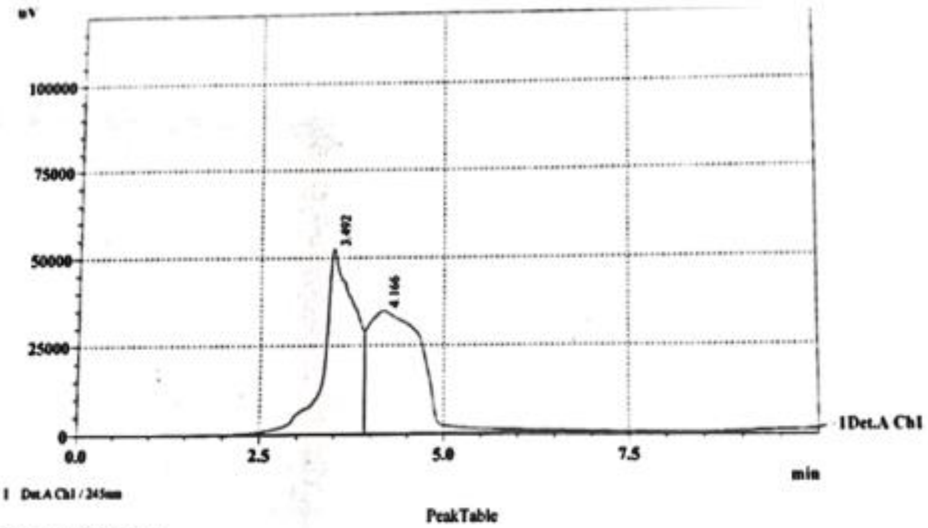
Appendix (39): chromatogram from HPLC of detection of ivermectin in ghee sample from Al Hur district



Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.416	25790138	790799	100.000	100.000
Total		25790138	790799	100.000	100.000

Appendix (40): chromatogram from HPLC of detection of ivermectin in ghee sample from Ain Al-Tumer district



Detector A Ch1 245nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.492	1564180	52306	46.414	60.060
2	4.166	1786012	34660	52.996	39.798
3	11.208	19885	124	0.590	0.143
Total		3370077	87090	100.000	100.000

المستخلص:

صممت هذه الدراسة للكشف عن بقايا بعض مبيدات الطفيليات الخارجية (السايبرمثرين والايفرمكتين) في 200 منتج غذائي حيواني المصدر (لحوم الابقاروالحليب والجبن والسمن الحيواني) بواسطة استخدام جهاز الكروماتوغرافيا السائلة (HPLC) والتي قورنت مع الحد الأقصى للمتبقيات الموضوع من قبل منظمة الصحة العالمية (WHO).

جميع العينات تم جمعها من الأسواق المحلية ومحلات القصابة في مختلف الوحدات الإدارية (الحسينية ومركز المدينة والحر وطوريج وعين التمر) في محافظة كربلاء. أظهرت نتائج فحص بقايا السايبرمثرين زيادة معنوية على مستوى معنوية (P≥0.0001) في عينات لحوم الابقار من (الحسينية 0.1569ومركز المدينة 5.408والحر 6.409وطوريج5.509 وعين التمر 5.408) PPM على التوالي.

وكذلك أظهرت بقايا السايبرمثرين في عينات حليب الابقار زيادة معنوية عالية على مستوى معنوية (P≥0.001) في (الحسينية 5.651 ومركز المدينة4.778 والحر3.073 وطوريج 0.1569وعين التمر0.1026) PPMعلى التوالي مقارنة مع منظمة الصحة العالمية (WHO).

بينما جميع العينات الاجبان المجموعة من مختلف الوحدات الإدارية في محافظة كربلاء (الحسينية0.3271 ومركز المدينة5.750 والحر6.127 وطوريج0.07613 وعين التمر4.625) على التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لمتبقيات السايبرمثرين. كذلك جميع عينات السمن الحيواني المجموعة من مختلف الوحدات الإدارية لمحافظة كربلاء (الحسينية0.06863ومركزالمدينة5.632والحر0.1700 وطوريج2.788 وعين التمر0.0452) على التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لمتبقيات السايبرمثرين.

على نفس الجانب أظهرت نتائج فحص بقايا الايفرمكتين بواسطة استخدام جهاز الكروماتوغرافيا السائلة (HPLC) والتي قورنت مع الحد الأقصى للمتبقيات الموضوع من قبل منظمة الصحة العالمية (WHO)اقل معنوية مقارنة مع نتائج فحص بقايا السايبرمثرين حيث أظهرت نتائج فحص لحوم الابقار لبقايا الايفرمكتين المجموعة من مختلف الوحدات الإدارية (الحسينية0.4293 ومركز المدينة 0.1969والحر0.2425 وطوريج0.4461 وعين التمر0.7081)PPMعلى التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لبقايا الايفرمكتين في لحوم الابقار.

جميع عينات الحليب المجموعة من مختلف الوحدات الإدارية في محافظة كربلاء سجلت (الحسينية0.6843 ومركز المدينة 1.065والحر0.04360 وطوريج0.3634 وعين التمر0.4288)PPM على التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لبقايا الايفرمكتين في الحليب.

وكذلك جميع عينات الاجبان المجموعة من مختلف الوحدات الإدارية في محافظة كربلاء سجلت (الحسينية0.4262 ومركز المدينة 0.0075والحر0.03336 وطوريج0.2453 وعين التمر0.0052)PPM على التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لبقايا الايفرمكتين في الاجبان وأيضا جميع عينات السمن الحيواني سجلت المجموعة من مختلف الوحدات الإدارية في محافظة كربلاء سجلت (الحسينية0.4023 ومركز المدينة 0.3786والحر0.3595 وطوريج3.858 وعين التمر0.1362)PPM على التوالي لديها زيادة معنوية على مستوى معنوية (P≥0.0001) مقارنة مستوى اقصى حد لبقايا الايفرمكتين في السمن الحيواني PPM (0.4)حسب الحد الأقصى للمبيدات الحشرية في مؤشر منظمة الصحة العالمية لعام 2022. إن ارتفاع مستويات المبيدات الحشرية (السايبرمثرين والايفرمكتين) في بعض المنتجات الغذائية ذات الأصل الحيواني (اللحوم، الحليب، الجبن، والسمن الحيوانية) التي وجدت في نتائج هذه الدراسة يعزى إلى استخدام هذه المبيدات لغرض القضاء على انتشار القراد بصورة عشوائية وعدم الالتزام بالجرعات وفترات الاعطاء وفترة التحريم للقضاء على الوسيط الناقل للحمى النزفية في المحافظات العراقية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية الطب البيطري
فرع الصحة العامة البيطرية

الكشف عن بقايا مبيدات الطفيليات الخارجية في منتجات الابقار (لحوم ، الحليب ومنتجات الحليب) في محافظة كربلاء

رسالة مقدمة الى

مجلس كلية الطب البيطري كجزء من متطلبات نيل درجة الماجستير في الطب البيطري/فرع الصحة العامة

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