MILK QUALITY ASSESSMENT FOR ADULTERATION AND ORGANIC CONTAMINANTS IN JUJA AND GITHURAI MARKETS, KIAMBU COUNTY

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Milk Quality Assessment for Adulteration and Organic Contaminants in Juja and Githurai Markets, Kiambu County

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this thesis to my family and friends. Special gratitude to my aunt Nora Clementine Otieno, who believed in my dreams from childhood and who has been a great mentor over the years.

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ABREVIATIONS AND ACRONYMS

AchE	acetylcholinesterase
AOAC	Association of Official Analytical Chemists
API	Atmospheric pressure ionization
AVM	Automated vending machines
CAC	Codex Alimentarius Commission
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
EU	European Union
FAO	Food and Agriculture Organization
GC	Gas chromatography
GC-MS	Gas chromatography – mass spectrometry
HPLC	High performance liquid chromatography
IMS	Intermediate mixed standard
KEBS	Kenya Bureau of Standards
KNBS	Kenya National Bureau of Statistics
LC	liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLE	Liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass to charge ratio
MRL	Maximum residue limit
MRM	Multiple reaction monitoring
MS	Mass spectrometer
NP	Normal phase
OP	Organophosphate
RP	Reversed phase

SDP	Small scale diary project
SDGs	Sustainable Development Goals
S/N	Signal to noise ratio
SIM	Single ion monitoring
SPE	Solid phase extraction
SPME	Solid phase micro extraction
TIC	Total ion count
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

The annual milk consumption rate in Kenya is estimated at 50-150 liters per capita. Approximately 70% of milk marketed in Kenya is sold through a largely unregulated informal market. There are concerns about the quality of milk as some cases of adulteration with water, illegal preservatives and contamination with antibiotic residues have been reported. Thus, there is need to continuously assess the quality of milk in the market to ensure its safety for consumption. This study was carried out to investigate milk quality for adulteration and presence of organic contaminants in milk marketed in Juja and Githurai markets in Kiambu County, Kenya. Milk was grouped into three categories namely raw milk sold in shops (shop milk), automated vending machine milk (AVM) and packet milk. The adulterants of interest were water and hydrogen peroxide while the organic contaminants monitored were antibiotic and pesticide residues. The analysis was based on the Kenya Bureau of Standards bench marks for milk quality and the maximum residue limits (MRLs) for antibiotics and pesticide residues set by the Codex Alimentarius Commission and the European Union. A lactometer was used to evaluate milk adulteration with water. Added water was detected in 53 %, 78 % and 70 % of shop, AVM and packet milk samples, respectively. There was a significant difference in the mean densities for shop milk obtained from Githurai and Juja ($p = 0.0157$) at $p \leq 0.05$ significance using t-test. For AVM milk, there was no significant difference in the two mean values of densities of milk from Githurai and Juja ($p = 0.365$) at $p \leq 0.05$ level of significance using t-test. One-way ANOVA test for the mean densities for raw, AVM and packet milk showed that there was no statistically significant difference between the three groups ($p = 0.272$) at $p \leq 0.05$ level of significance. Hydrogen peroxide in the milk was measured using Quantofix peroxide test strips. Hydrogen peroxide was detected in 4 % and 20 % of AVM and packet milk, respectively while none of the shop milk samples had detectable levels of added hydrogen peroxide. Antibiotics residues (amoxicillin, cloxacillin, tetracycline, sulfamethoxazole and trimethoprim) were analyzed using Liquid chromatography tandem mass spectrometry (LC-MS/MS). Overall, 11 % of the samples had at least one antibiotic residue at concentrations above the MRLs while 22 % of the samples had detectable antibiotic residues at levels below the MRLs. Amoxicillin, cloxacillin and tetracycline were detected at concentrations above the respective MRLs in 2 %, 8 % and 2 % of the samples, respectively, while all the samples with detectable levels of sulfamethoxazole and trimethoprim were below the MRLs. Pesticide residues (amitraz, carbaryl, chlorpyrifos, cypermethrin and deltamethrin) were analysed using LC-MS/MS and gas chromatography –mass spectrometry (GC-MS). Overall, pesticides were detected in 14 % of the samples but none of the detected pesticides exceeded the MRLs. The results of this study provide evidence of adulteration and presence of organic contaminants in some milk sold within the selected study markets. Adulteration compromises the nutritional quality of milk. Consumption of milk containing organic contaminants such as pesticides and antibiotics may lead to bioaccumulation to harmful levels over time. Thus, increased market surveillance and milk safety awareness should be conducted to deter sale of adulterated and contaminated milk.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Milk is an important source of nutrients for many Kenyans. The Kenyan dairy industry is estimated to produce about five billion litres of milk annually (Nyokabi *et al.,* 2021). Kenya has one of the largest consumption rates of milk per capita in Africa, with an annual consumption rate of 50-150 litres per capita (Alonso *et al.,* 2018). The demand for milk and milk products has been estimated to grow at 5% per year due to rapid population growth, changing food preferences and urbanization (Nyokabi *et al.,* 2021; Ondieki *et al.,* 2017).

Due to the high demand for milk, there is a thriving and largely unregulated market. Farmers sell milk directly to consumers or through middlemen who supply to vendors who in turn sell to consumers. While formally processed milk undergoes routine screening to ensure it meets the required quality standard, there is no monitoring of milk quality in the informal channels. In recent times, there have been numerous allegations that some traders adulterate milk to increase profits, in contravention to section 140 of the *Food, Drug and Chemical Substances Act (*Cap 254) of Kenya which does not permit any additives in milk (Republic of Kenya, 2012).

Adulteration of milk poses a public health risk. Dilution of milk with water and other adulterants compromises the nutritional value of milk. Moreover, addition of unsafe water may introduce pathogenic microorganisms to milk as well as chemical contaminants including heavy metals, pesticides, and pharmaceuticals. The main pathway to pesticide residues in milk is through contaminated feeds and drinking water supplies. Chronic exposure to pesticide residues through consumption of contaminated milk may result to bioaccumulation to toxic levels over time (Jadhav *et al.,* 2019; Welsh *et al.,* 2019). Contamination of milk with pharmaceuticals primarily occur due to improper farming practices, especially noncompliance of recommended withdrawal periods following

treatment of lactating animals. Exposure to antibiotic residues through consumption of contaminated milk can lead to adverse health problems including allergic reactions, chronic toxicity and drug resistance (Bilandžić *et al.,* 2011; Hu *et al.,* 2021; Redding *et al.,* 2014). Without proper interventions, drug resistant infections are projected to become a global leading cause of death by 2050, with projections of ten million deaths per year and costing the global economy 100 trillion dollars (O'Neill, 2019).

This study aimed to assess milk quality for adulteration and presence of organic contaminants in milk marketed in Juja and Githurai markets in Kiambu County, Kenya. The adulterants of interest included water and hydrogen peroxide while the organic contaminants monitored included antibiotics (amoxicillin, cloxacillin, tetracycline, sulfamethoxazole, and trimethoprim) and pesticides (amitraz, carbaryl, chlorpyrifos, cypermethrin, and deltamethrin). The selected markets are densely populated metropolitan areas and are located within the catchment area of Githunguri Dairy, the largest dairy farmers' cooperative society within Kiambu County.

1.2 Statement of the problem

Milk is a highly perishable commodity and becomes quickly soured when stored at the ambient temperatures prevalent within tropical and subtropical climate in Eastern Africa (Kurwijila, 2006). In Kenya, there have been reports of milk vendors adding hydrogen peroxide and other chemical preservatives to milk to increase its shelf life (The Standard, 2018, January 30). However, the use of preservatives in milk is banned under the Food, Drugs and Chemical Substances Act (Republic of Kenya, 2012). Cases of milk vendors diluting milk with water to increase its volume have also been reported (The Standard, 2018, January 30). Addition of water to milk not only lowers its density but may also lower its microbial quality, posing a public health hazard (Kurwijila, 2006). Exposure to antibiotic residues in milk may trigger allergic reactions and development of antimicrobial resistance in the long run, necessitating the need for more expensive antibiotics. The contribution of livestock antibiotics to the emergence antimicrobial resistance is an issue of concern for global public health (Kosgey *et al.,* 2018). Drug-

resistant infections are a threat to the achievement of the Sustainable development goals (SDGs) of good health and wellbeing, no poverty and zero hunger (United Nations, 2016). If no action is taken, drug-resistant infections are projected to become a major cause of death by 2050, claiming approximately 10 million lives a year and costing the world economy 100 trillion USD (O'Neill, 2019).

Pesticides are used to control vectors that are responsible for many animal and crop health problems in the country. Failure to observe good application practices may result to high levels of pesticides residues in milk. Exposure to pesticide residues is associated with a variety of health risks such as disruption of reproductive and endocrine systems, impaired immunity and increased risk of various cancers (Lee *et al.*, 2014).

1.3 Justification of the study

This study is in line with the SDGs on good health and wellbeing, zero hunger and no poverty. Safety of milk must be ensured to protect the health of vulnerable consumers, especially children for whom milk is a major recommended dietary component (Akinyemi *et al.*, 2021). Access to safe milk is critical to achieving the health targets outlined in the SDGs. By assessing milk safety vital information can be generated for evidence-based interventions to protect consumers from the potential health risks of unsafe milk. Currently, there is scarce quantitative information regarding the safety levels of informally marketed milk consumed in Kiambu County, Kenya in terms of organic contaminants and adulterants hence an urgent need for the present study.

1.4 Hypothesis

i. Milk marketed in Juja and Githurai markets in Kiambu County, Kenya is adulterated with water and hydrogen peroxide.
ii. Milk marketed in Juja and Githurai markets in Kiambu County, Kenya contain antibiotic and pesticide residues at concentrations exceeding the recommended maximum residue limits.

1.5 Objectives

1.5.1 General objective

To investigate the levels of adulteration and organic contamination of milk marketed in Juja and Githurai markets in Kiambu County, Kenya.

1.5.2 Specific objectives

1. To determine the presence of added water in milk.
2. To determine the levels of hydrogen peroxide in milk.
3. To determine the presence of antibiotic residues (amoxicillin, cloxacillin, tetracycline, sulfamethoxazole and trimethoprim) in milk using liquid chromatography tandem mass spectrometry (LC-MS/MS).
4. To determine the presence of pesticide residues (amitraz, carbaryl, chlorpyrifos, cypermethrin and deltamethrin) in milk using gas chromatography-mass spectrometry (GC-MS) and LC-MS/MS.

1.6 Scope and Limitations

The current study focused on raw milk and processed milk sold in Juja and Githurai markets, Kiambu County. The study was limited to selected antibiotic and pesticide residues. Additional studies should be carried out to target more residues and cover more regions of the country to get a national outlook of the levels of antibiotic residues in the country's milk market.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Dairy industry in Kenya

Kenya has one of the biggest dairy industries in Sub-Saharan Africa (Alonso *et al.*, 2018). Dairy farming is an important source of livelihood for many Kenyans, generating approximately one million jobs at the farm level and accounts for approximately 6 – 8% of Kenya's $101 billion Gross Domestic Product (Odero-Waitituh, 2017; World Bank Group, 2022). The estimated annual milk production in Kenya is 5 billion liters per year (Nyokabi *et al.*, 2021). Small scale holders, with three or fewer cows dominate the milk industry, accounting for 80% of the total milk produced in the country (Small-scale Diary Project [SDP], 2004). An estimated 84% of the milk produced is sold as raw milk to consumers, both in rural and urban areas (Kamundi, 2014).

Marketing of milk is done through both formal and informal channels which accounts for 30% and 70% of the market milk, respectively (Alonso *et al.*, 2018). The formal milk value chain is characterized by branded, packaged milk products while the informal milk value chain primarily deals with raw milk and non-industrially pasteurized milk products. Milk in the informal value chain is sold directly to consumers through entities such as farm-gate shops, milk vending machines, mobile vendors, hawkers on the streets and corner-shops among others (Odero-Waitituh, 2017).

Nearly 84% of milk in the informal value chain is sold as raw milk to consumers both in rural and urban areas (Kamundi, 2014). Raw milk is preferred by consumers owing to its cost (30-50% cheaper) and high butter fat content compared to processed milk (Muriuki, 2011; SDP, 2004). The introduction of automated milk vending machines (AVMs) has seen an increasing trend in the proportion of pasteurized milk in the informal value chain. While regulations require AVMs to dispense pasteurized milk, some studies carried out within the country have determined that some vendors sell raw milk in AVMs (Omedo Bebe, 2020).

2.2 General composition of milk

Milk is rich in nutrients like proteins, vitamins, carbohydrates (lactose) and minerals. According to Grimaud *et al.,* (2007), the composition of milk is not constant but shows a wide variation depending on species of animal, breed and also individual animal. The composition of an animal's milk may vary from day to day depending on feeding, season of the year, age and stage of lactation of the animal. Table 2.1 shows the average milk composition from some animals used for human consumption (Pandey & Voskuil, 2011).

Table 2.1: Average composition of cow, goat, and sheep milk

	Cow	**Goat**	**Sheep**
Water	87.2%	85.8%	81.6%
Fat	4.0%	4.9%	6.5%
Protein	3.4%	4.3%	6.7%
Lactose	4.5%	4.1%	4.3%
Ash (minerals)	0.9%	0.9%	0.9%

Source: Pandey & Voskuil (2011)

2.2.1 Physicochemical properties of milk

2.2.1.1 Milk pH

The pH of milk is a measure of its acidity. Fresh milk is slightly acidic, with a pH range of 6.5 - 6.7. The natural acidity of milk is attributed to casein and phosphates. A pH above 6.7 indicates mastitis milk while a pH below 6.5 indicates bacterial deterioration (Aiello *et al.,* 2019). Mastitis is characterized by inflammation of the udder and mammary gland tissues. Extracellular fluids and blood from the inflamed tissues mix with the secreted milk, causing an increase in pH (Kandeel *et al.,* 2019). Action of bacteria on lactose in milk produces lactic acid, lowering the pH (Aiello *et al.,* 2019). Milk pH can be measured directly with a pH meter, titration or indicator dyes. It can also be estimated indirectly by the clot on boiling and alcohol tests.

2.2.1.2 Specific gravity of milk

Specific gravity is the density of a substance relative to that of water. Milk has a specific gravity of 1.028 – 1.036 (Kenya Bureau of Standards [KEBS], 2019). The specific gravity of milk is measured using a lactometer and is used as an indicator for milk adulteration. Addition of water and cream (fat) lowers the specific gravity of milk while addition of skim milk or removal of fat increases the specific gravity of milk.

2.2.1.3 Freezing point

Freezing point of a substance is the temperature at which it solidifies. The freezing point of milk is dependent on the number of solute particles present in the milk and is usually the most constant physical property of milk. Normal freezing point of milk is -0.525 to -0.550 ^{0}C (KEBS, 2019). The freezing point of milk is sensitive to added water which causes detectable elevation of the freezing point. Thus, it is usually used as a presumptive test for adulteration with water. Freezing point is measured using cryoscopy or calculated from lactometer measurements (Zagorska & Ciprovica, 2013).

2.2.2 Milk quality

Fresh milk is slightly acidic (pH 6.5 - 6.7). Upon souring, milk becomes more acidic due to formation of lactic acid by microbial action on lactose. Farm practices such as milking hygiene and handling equipments have been linked to the quality parameters of milk (Sraïri *et al.*, 2009). The microbial load of milk is a basic determinant of milk quality and gives an indication on the general milking conditions and health of the herd (Sraïri *et al.*, 2009).

Milk as it comes from the udder of a healthy cow contains negligible amounts of bacteria, typically <1000 total bacterial counts per millilitre of milk (Tegegne & Tesfaye, 2017). The main sources of milk contamination by microorganisms includes unclean teats, milking and transport equipment. Typical bacterial strains in milk comprise of lactic acid bacteria species such as *Lactobacillus, Lactoccocus, Leuconostoc, Enterococcus* and

Streptococcus species, which are responsible for the natural fermentation of milk (Quigley *et al.,* 2013). Milk leaves the udder with favourable temperature for microbial growth (35 ^{o}C), therefore, milk should be rapidly cooled to reduce multiplication of these organisms in fresh milk (Paludetti *et al.,* 2018). During cold storage, milk may still be proliferated with psychotropic bacteria species such *as Acinetobacter, Pseudomonas* and *Aeromonas spp.* (Quigley *et al.,* 2013). In addition, milk may become contaminated with pathogenic bacteria which pose health risk to humans. Some milk-borne bacteria of concern and their main pathways to milk contamination are summarized in Table 2.2.

Table 2.2: Major milk-borne pathogens and sources of contamination.

Organism	**Sources of milk contamination**	**Illness in humans**
Campylobacter jejuni	feces	gastroenteritis
listeria monocytogenes	water, soil	listeriosis
Mycobacterium bovis/tuberculosis	infected livestock	Lung disease
Escheria coli	Feces	Gastroenteritis
Salmonella spp.	Feces	Typhoid, Gastroenteritis
Coxiella burnetii	feces, urine	Q fever

Source: Oliver *et al.,* (2005)

2.3 Adulteration of milk

Adulteration refers to either addition or subtraction of any of the components of milk (Poonia *et al.,* 2017). Milk is highly perishable and sours quickly at room temperature. Scrupulous traders often adulterate milk either to increase profit margins or to reduce losses due to spoilage. Common forms of adulteration of milk involve addition of water to increase the volume of milk; addition of thickening agents to counter dilution effects; and addition of inhibitory substances to increase the shelf life of milk (Barham *et al.,* 2014).

2.3.1 Adulteration with water

Addition of water to raw milk is the most common form of adulteration. Adulteration with water depends on the amount of milk in supply, which is in turn influenced by the seasons. During the dry seasons, milk production is generally low and there is a tendency to dilute with water to increase the volume, and thereby maximize the profits. Dilution with water is often accompanied by addition of thickening agents like flour and starch to counter the dilution effects on milk appearance (Barham *et al.,* 2014).

Dilution with water decreases the nutritional value of milk. A more serious concern is introduction of pathogens to milk when untreated water is added. Untreated water can be a source of several pathogenic microorganisms such as *Escheria coli, Salmonella spp. and Campylobacteraceae jejuni* (Phelps, 2010). In addition, such water may contain chemical pollutants like heavy metals, pesticides, and pharmaceuticals amongst others (Shaker *et al.,* 2015).

2.3.2 Hydrogen peroxide and other preservatives

In Kenya, the use of preservatives, including Hydrogen peroxide is prohibited (Republic of Kenya, 2012). However, there are concerns that unscrupulous traders add hydrogen peroxide as a preservative to raw milk to prolong its shelf life (The Standard, 2018). Other toxic preservatives that have been detected in milk include boric acid, salicylic acid, formalin, bicarbonate and carbonates (Shaker *et al.,* 2015).

In small quantities, typically < 10 ppm, hydrogen peroxide activates the lactoperoxidase system (LPS). The LPS is a natural milk antibacterial protection system that involves a series of chemical reactions involving the enzyme lactoperoxidase, hydrogen peroxide and thiocyanate ions (Silva *et al.,* 2020). Lactorepoxidase enzyme catalyses the oxidation of thiocyanate ion by hydrogen peroxide, forming hypothiocyanite ion. The

chemical equation for this reaction is shown in equation 1. Hypothiocyanite alters bacterial metabolism, interfering with their ability to multiply (Silva *et al.,* 2020).

$$SCN^- + H_2O_2 \xrightarrow{lactoperoxidase} OSCN^- + H_2O \quad (1)$$

The low doses of hydrogen peroxide required for the LPS are difficult to measure accurately in a typical farm setup and could lead to overdosing. Hydrogen peroxide at concentrations 100 – 800 mg/L has direct bactericidal effects in milk, but disrupts milk proteins, causing problems in milk processing (Food and Agriculture Organization [FAO] & World Health Organization [WHO], 2005). Excessive amounts of hydrogen peroxide in milk have ill effects arising from its propensity for enhancing oxidative stress (Thandavan *et al.,* 2015). Hydrogen peroxide has detrimental effect on some digestive enzymes in the human gut (Valko *et al.,* 2007). However, hydrogen peroxide is not detectable in boiled milk as it breaks down to water and oxygen (Omore *et al.,* 2007).

2.4 Antibiotics residues in milk

Antibiotics are used to treat various infections such as mastitis in dairy animals. Cows treated with antibiotics produce milk containing antibiotic residues for certain duration after treatment. It is recommended that such animals be excluded from milking for a period to ensure the elimination of the drugs from their body system. Failure to observe the withdrawal periods result to unacceptable levels of residues in milk. Withdrawal periods vary for different drugs. Some examples include: 48 hours for cloxacillin, 60 hours for amoxicillin, 3 days for oxytetracycline and 6 days for ciprofloxacin (Anika *et al.,* 2019).

Exposure to antibiotic residues can cause allergic reactions in susceptible individuals. Long term exposure to antibiotics in milk may lead to development of drug resistant pathogenic bacteria (Gao *et al.,* 2012). Milk contamination with antibiotics is a serious problem for production of fermented dairy products. Antibiotics inhibit the growth of

starter culture leading to failure of fermentation and subsequent loss of product (Tasci *et al.,* 2021). In addition, culture failure allows for growth of some pathogens like *staphylococcus* and *salmonella*, posing a risk of disease outbreaks involving dairy products (Chye *et al.,* 2004; Sachi *et al.,* 2019).

2.4.1 Classification of antibiotics

Antibiotics are a class of drugs that are designed to either kill bacteria (bactericidal) or inhibit bacterial growth (bacteriostatic) (Walsh, 2003). Antibiotics can be classified based on their mode of action (bactericidal versus bacteriostatic), based on the spectrum of organisms they act on (broad versus narrow spectrum) or based on their chemical structures (Ebimieowei & Ibemologi, 2016).

The main classes of antibiotics according to their chemical structures include beta-lactams, tetracyclines, sulfonamides, Quinolones, macrolides, aminoglycosides, glycopeptides and oxazolidinones (Ebimieowei & Ibemologi, 2016). Antibiotics of the same class generally exhibit similar activity, potential side effects and toxicities. In Kenya, the common groups of antibiotics used to treat dairy cattle include beta-lactams, tetracyclines and sulphonamides (Darwish *et al.*, 2013).

2.4.1.1 Beta-lactams

The beta-lactam group of antibiotics contain the β-lactam ring in their core structure. The beta lactams interfere with bacterial cell wall synthesis through binding to the penicillin binding proteins (PBS), which are the bacterial enzymes essential for cell wall synthesis (Bush & Bradford, 2016). The β-lactams includes penicillins, cephalosporins, carbapenems and monobactams (Ebimieowei & Ibemologi, 2016). The basic core structures of the beta lactams are given Figure 2.1.

In dairy farming, beta-lactam antibiotics are extensively used in the treatment of mastitis in lactating cows. Narrow spectrum penicillins such as penicillin G, cloxacillin and amoxicillin are the first choice antibiotics for treatment of mastitis caused by *Streptococci* and *Staphylococci* species ((Pyörälä, 2009). Penicillins are not toxic to humans, but are known to cause allergic reactions in hypersensisitive individuals (Grunwald & Petz, 2003).

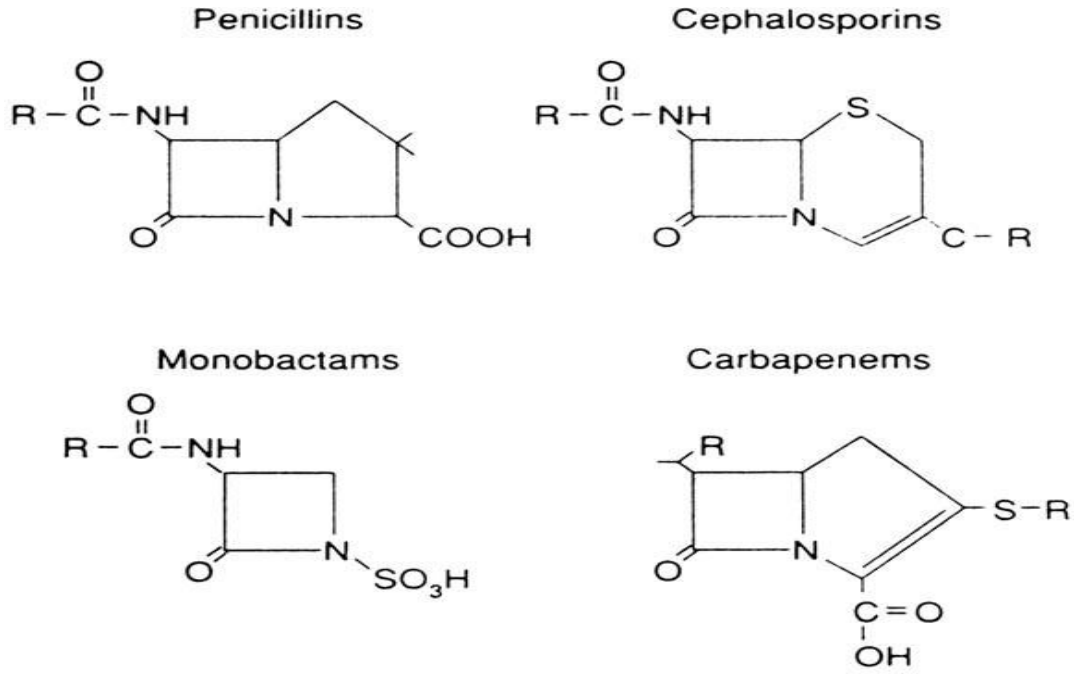
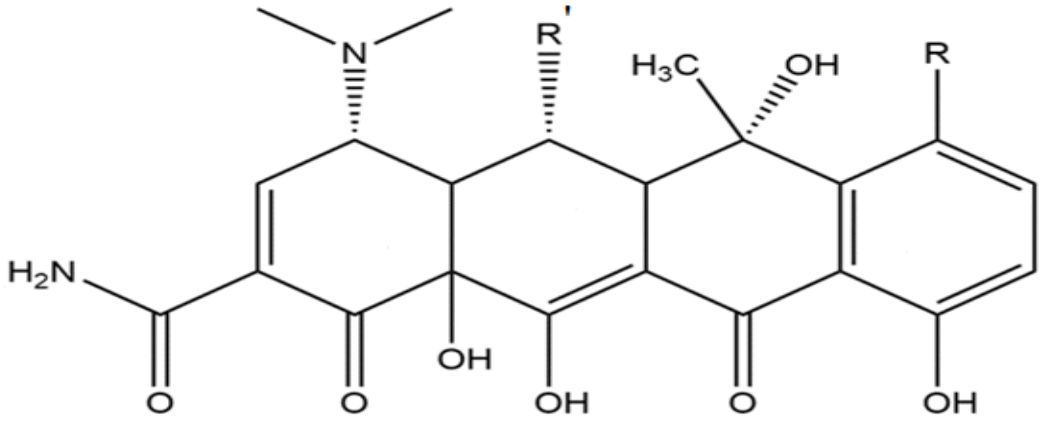


Figure 2.1: General structures of beta-lactam antibiotics (Ebimieowei & Ibemologi, 2016) .

2.4.1.2 Tetracyclines

The tetracycline antibiotics contain a fused linear tetracyclic nucleus in their structure onto which different functional groups are attached. The basic structure of tetracyclines is shown in Figure 2.2.

Tetracyclines inhibit protein synthesis in bacteria, affecting their ability to multiply (Ebimieowei & Ibemologi, 2016). Tetracyclines are active against broad spectrum of microorganisms, including gram positive, gram negative bacteria, aerobic and anaerobic bacteria, and some protozoa (Aalipour *et al.*, 2015).



Examples	R	R'
tetracycline	H	H
oxytetracycline	H	OH
chlortetracycline	Cl	H

Figure 2.2: General structure and some representative members of tetracyclines

Tetracyclines are widely used in animal husbandry for prevention and treatment of infectious diseases and as feed additives to promote growth (Aalipour *et al.*, 2015). Tetracycline, oxytetracycline and chlortetracycline are frequently used for treatment of bovine mastitis and the concentrations in milk can reach up to 70% of the serum concentration (Fritz & Zuo, 2007). Exposure to tetracyclines through consumption of contaminated milk may produce adverse health effects including tooth discoloration in children below 12 years of age, fetal abnormalities (teratogenicity) during pregnancy,

gastrointestinal discomfort and allergic reactions in some individuals (Aalipour *et al.*, 2015).

2.4.1.3 Sulphonamides

Sulphonamide antibiotics consists of an aniline base with a para-substituted sulphonamide group (-SO_2NHR). The basic structure of sulphonamides and some examples are given in Figure 2.3.

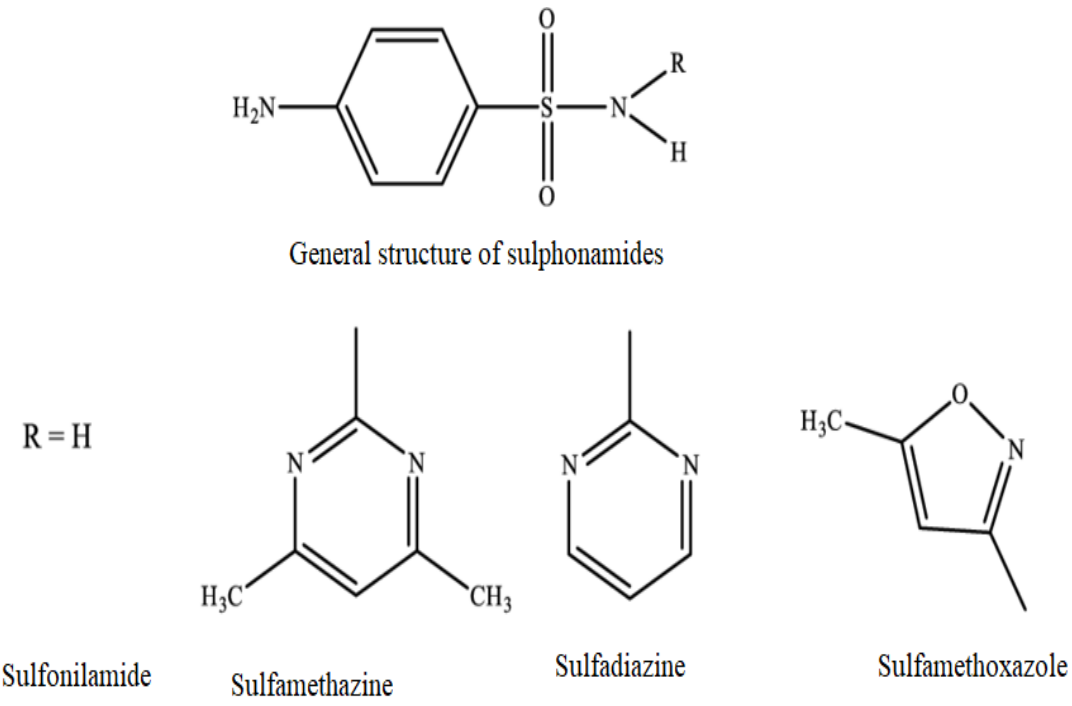


Figure 2.3: General structure and some representative members of sulphonamides.

Sulphonamides are structurally analogous to para-aminobenzoic acid and this forms the basis of their antibacterial activity. They competitively bind to the active sites of the bacterial enzyme dihydropteroate synthase, preventing the incorporation of para-aminobenzoic acid into the enzymatic reaction required for the synthesis of folic acid

(Prescott, 2013). Sulphonamides are broad spectrum antibiotics with bacteriostatic activity towards gram positive and gram negative bacteria as well as some protozoans (Ebimieowei & Ibemologi, 2016). Sulphonamides are often administered in combination with trimethoprim as a potentiator. Trimethoprim inhibits the enzyme activity of dihydrofolate reductase, which plays a key role in bacterial metabolism of folic acid (Chen *et al.*, 2017).

2.4.2: Maximum residue limits (MRLs) for antibiotics in milk

The MRL refers to the highest permissible concentration of a drug residue that is legally tolerated in food product derived from animals that have been treated with veterinary medicines (Alimentarius, 2017). MRLs for antibiotic residues are established by the Codex Alimentarius Commission (CAC) of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) using an internationally recognized multi-step scientific health risk assessment processes (Alimentarius, 2017). Within the European Union (EU), the regulatory control of antibiotics residues relies of the MRLs set by the European Medicine Agency (EU Commission Regulation, 2010). In Kenya, regulatory control of antibiotic residues in milk and milk products is guided by the Kenya Bureau of Standards (KEBS) milk specifications which stipulates that milk should comply with the CAC MRLs for veterinary drugs in milk (KEBS, 2019), (Table 2.3).

Table 2.3: MRLs of some antibiotic residues in milk

Drug	CAC MRL (µg/kg)	[b]EU MRL (µg/kg)
	Beta-lactams	
amoxicillin	4	4
ampicillin	4	4
cloxacillin	Not available	30
Penicillin-G	4	4
	Tetracyclines	
tetracycline	100	100
chlortetracycline	100	100
oxytetracycline	100	100
	Sulphonamides	
sulfamethazine	Not available	100*
sulfamethoxazole	Not available	100*
sulfadimidine	25	100*
	Others	
trimethoprim	Not available	50

***Sum of all sulfonamides present.**

2.5 Pesticide residues in milk

Pesticides are chemicals used to prevent, destroy, repel or mitigate pests (United States Environmental Protection Agency [USEPA], 2022). They include herbicides used for weed control, insecticides used for insect control, fungicides used against fungi, rodenticide used against rodents and acaricides used for control of acari.

In Kenya, ticks are a major pest affecting livestock farming. Tick and tick borne diseases lead to decreased productivity of livestock, resulting in negative impacts on farmers livelihoods (*Mutavi et al.,* 2021). Acaricides are used for control and prevention of tick

infestations in livestock. The active ingredients in acaricide products licensed for cattle use in Kenya are highlighted in Table 2.4.

Table 2.4: Active ingredients in acaricides for cattle use in Kenya (Pest Control Product Board, 2010).

Active ingredient	Chemical group	MRL (µg/kg) in milk*
carbaryl	carbamate	50
cypermethrin	pyrethroid	50
deltamethrin	pyrethroid	50
alpha-cypermethrin	pyrethroid	50
cyhalothrin	pyrethroid	200
chlorpyrifos	organophosphate	20
chlorphenvinphos	organophosphate	10**
amitraz	amidine	10

*** CAC MRL (Alimentarius, 2017) **EU MRL (EU Commission Regulation, 2010)**

2.5.1 Classification of acaricides

Acaricides are classified according to their chemical composition as organophosphates, carbamates, pyrethroids and amidines.

2.5.1.1 Organophosphates (OPs)

Organophosphates (OPs) are organic esters of phosphoric acid, with the general chemical structure as shown in Figure 2.4.

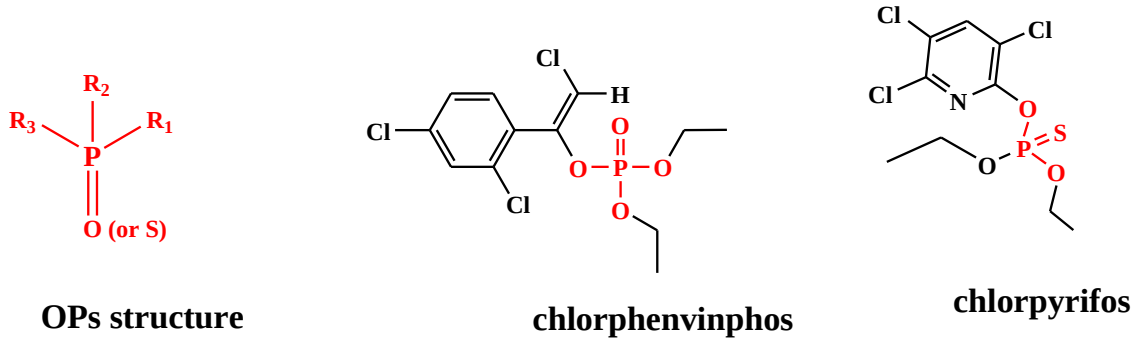


Figure 2.4: General structure of organophosphates and some examples.

Organophosphates affect the nervous system of organisms by inhibiting acetylcholinesterase (AchE), an enzyme involved in transmission of nerve signals. The inhibition of AchE prevents termination of nerve signals, leading to paralysis and eventual death of targeted organisms (Eicher, 2009).

Organophosphates are toxic to birds and fish as well. In humans, acute exposure to OPs causes dizziness, vomiting, respiratory depression, muscle twitching and hypersecretion (Roberts & Reigart, 2013). Long-term exposure may lead to neuropathy, memory loss, anxiety and personality changes (Eicher, 2009).

2.5.1.2 Carbamates

Carbamates are derivatives of carbamic acid, with the general structure shown in Figure 2.5.

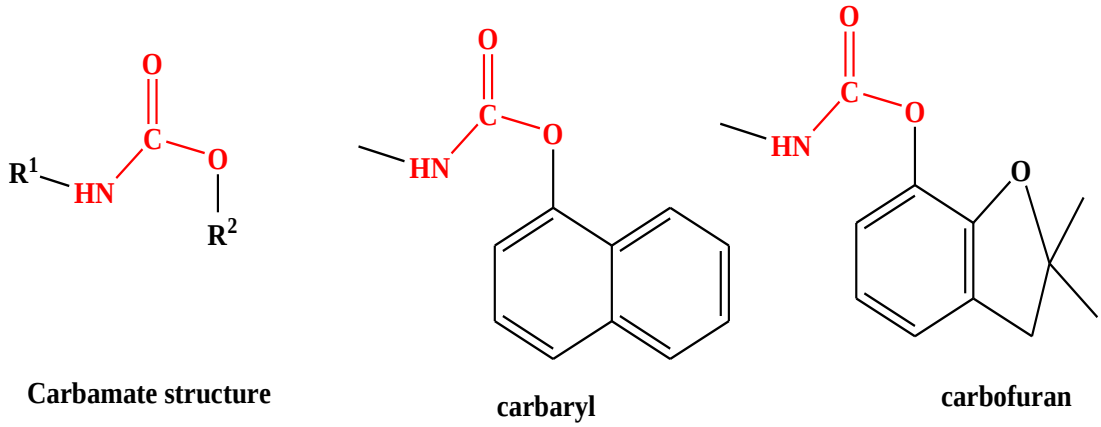


Figure 2.5: General structure of carbamates and some examples.

Carbamates are similar to OPs in their mechanism of action. Both are AchE inhibitors. However, carbamates bind reversibly to AchE, while organophosphates bind irreversibly. Thus, symptoms of carbamate poisoning last shorter duration than OPs (Roberts & Reigart, 2013).

Carbamates are classified as likely human carcinogens because they can be transformed into N-nitroso compounds (Mdeni *et al.*, 2022). Human exposure to carbamates occur primarily through inhalation and ingestion. Secondary exposure through dermal absorption is less toxic (Roberts & Reigart, 2013).

2.5.1.3. Pyrethroids

Pyrethroids are synthetic analogues of natural pyrethrins. Examples include cypermethrin, deltamethrin, permethrin and cyhalothrin (Figure 2.6).

Natural pyrethrins are extracted from the flowers of *Chrysanthemum* (pyrethrum) and have insecticidal and repellent properties. Natural pytherins degrade rapidly environment when exposed to sunlight. Synthetic pyrethroids have better insecticidal activity and improved stability in the natural environment compared to natural pyrethrins (Roberts & Reigart, 2013).

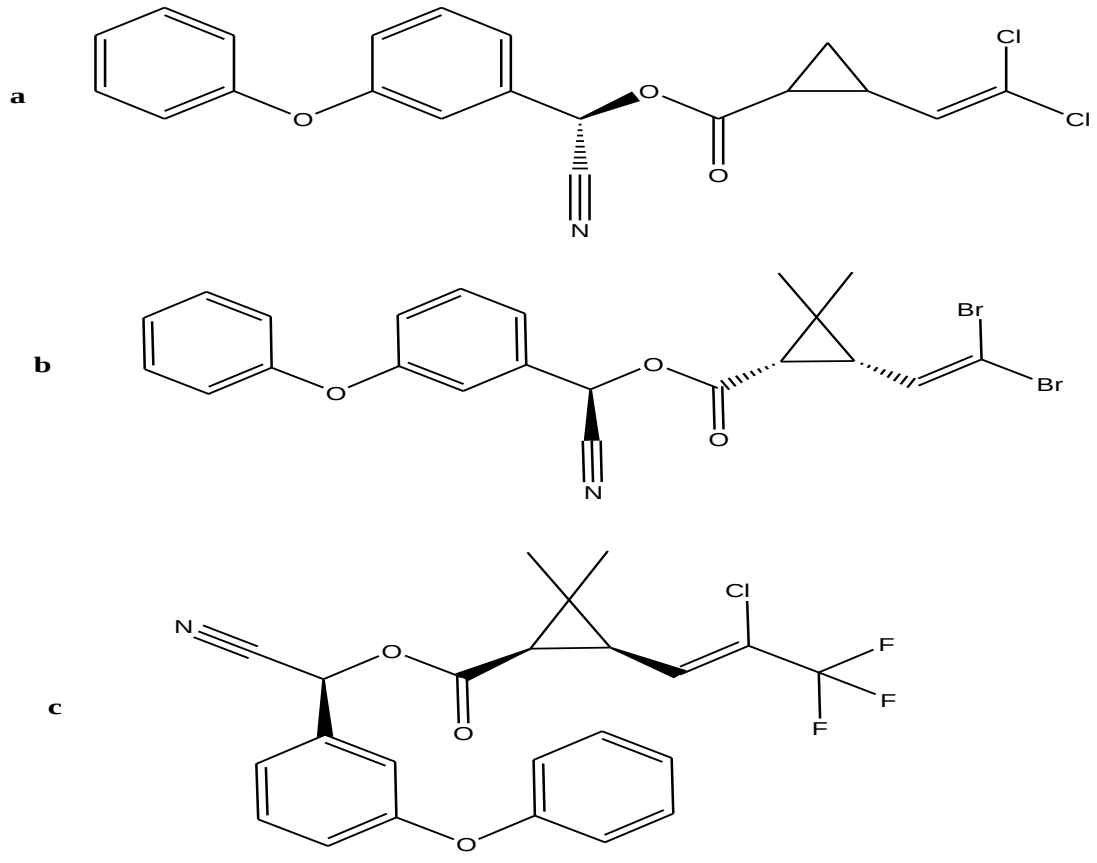


Figure 2.6: Structures of some pyrethroids. a) cypermethrin, b) deltamethrin and c) cyhalothrin

Pyrethroids target the central and peripheral nervous systems. They alter the voltage-gated sodium channels in the neurons, resulting in constant electrical excitation of the membranes which produces a knock-down effect (Field *et al.*, 2017). They are highly toxic to most agricultural pests and fish but exhibit relatively low mammalian toxicity (Field *et al.*, 2017).

2.5.1.4. Amidines

Amidines are derived from formamidine structure (NHCHNH2). Examples include amitraz, cymiazole and chlordimeform (Figure 2.7).

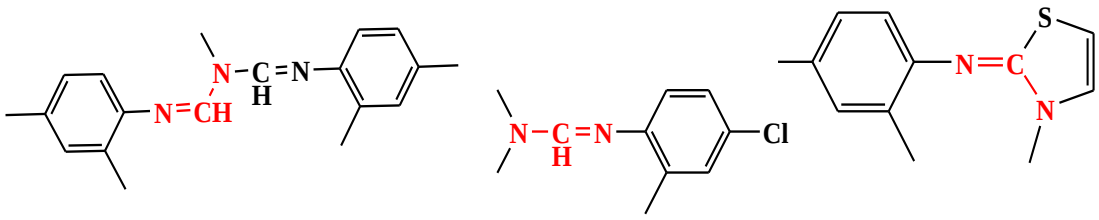


Figure 2.7: Structures of some amidines.

Amidines antagonize octopamine receptors in invertebrates. Octopamine is an important hormone and neurotransmitter in invertebrates involved in many physiological functions including feeding behavior, flight, locomotion, aggression, sleep, courtship and laying eggs (Costa, 2020).

2.5.2 Sources of pesticides in milk

A variety of pesticide residues have been documented in milk. Their presence in milk can be attributed to their lipophilic nature, relative environmental persistence and ability to bioaccumulate (Rodríguez-Díaz *et al.*, 2022). One pathway to pesticide contamination of milk is through dermal exposure during spraying/dips against ectoparasites. Rodrigues et al.(2011) found chlorpyrifos and ethion residues in cow milk up to 24 and 72 hours, respectively following treatment of ectoparasites. In another study, malathion and lindane were reported in milk up to seven days after skin application (Rodríguez-Díaz *et al.*, 2022). Another pathway to milk contamination is through contaminated drinking water, pastures and feeds. Chlorpyrifos, fipronil, deltamethrin, Aldrin, malathion, heptachlor epoxide, diazinon, DDT have been found in animal feeds and waters supplying livestock

farms and the same compounds were identified in cow milk from the farms (Bedi *et al.,* 2018; El Bahgy *et al.,* 2018; John *et al.,* 2001; Srivastava *et al.,* 2008).

2.6. Methods for detection of adulteration and presence of organic contaminants in milk

2.6.1 Methods for detection of added water in milk

Exogenous water causes notable changes in physical properties like density and freezing point of milk. The principal methods used for detection of added water in milk involve either measuring the specific gravity or the freezing point depression using a lactometer or cryoscopy, respectively.

2.6.1.1 Lactometer

A lactometer is a special type of a hydrometer designed to give readings related to the specific gravity of milk. Figure 2.8 gives an illustration of lactometer operation.

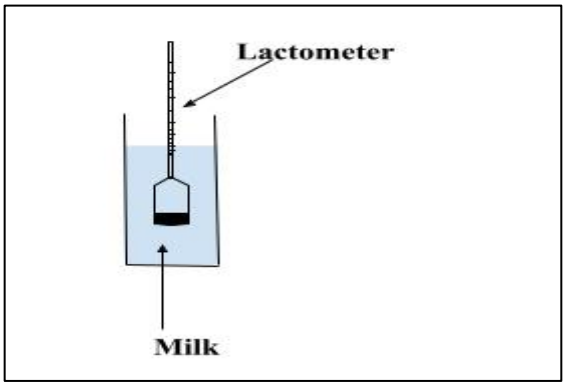


Figure 2.8: Lactometer immersed in milk

A lactometer consists of a cylindrical stem made of glass and a bulb filled with mercury. The lactometer operates based on Archimedes principle that a free-floating body displaces an amount of liquid of the same weight as the floating body. The milk sample

to be tested is placed in a measuring cylinder and the hydrometer lowered into the cylinder such that it floats freely without touching the sides or bottom of the cylinder. The depth of the lactometer in the sample is given by a scale located on the stem, such that the reading is taken at the point where the liquid surface touches the stem; and the specific gravity of milk is calculated from the lactometer reading.

2.6.1.2 Cryoscopy

A cryoscope measures the freezing point depression of milk. Normal milk has a freezing point between -0.525 ^{0}C and -0.550 ^{0}C (KEBS, 2019). Addition of water to milk, even in small amounts dilutes the soluble components of milk, causing its freezing point to rise towards that of water (Shaker *et al.,* 2015). The osmotic pressure of a solution is proportional to its freezing point depression. Thus, the depression of the freezing point is a measure of the osmolality of the solution.

In a cryoscopy, the test sample is super cooled to an appropriate temperature and crystallization induced, causing instantaneous heat release with an accompanying warming of the sample. The corresponding temperature rise is monitored until a temperature plateau is obtained. This plateau is taken as the freezing point of the sample. The instrument is calibrated using sodium chloride solutions of known osmolality, since the freezing point depression of a solution is proportional to its osmolality (Barham *et al.,* 2014).

2.6.2 Methods for detection of hydrogen peroxide in milk

Several analytical methods have been developed for the determination of hydrogen peroxide residues in milk, including, spectroscopic methods, electrochemical methods, flow injection analysis and batch injection analysis.

Abbas *et al.,* (2010) developed a fluorimetric method for the determination of hydrogen peroxide in milk. In this method, hydroxyl radicals generated in a fenton reaction oxidizes non-fluorescent coumarin to 7-hydroxycoumarin with a fluorescence peak at 456nm that is used as a H_2O_2 probe. However, this method suffers a limitation of long sample preparation time (nine minutes) rendering it unsuitable for routine applications.

Electrochemical methods have gained attention due to their high sensitivity, speed, ease of miniaturization and simple instrumentation. Silva *et al.,* (2012) developed a rapid analytical method based on batch injection analysis with amperometric detection using a Prussian blue modified graphite electrode. The combination of batch injection analysis with electrochemical sensing makes this method advantageous in terms of speed, precision, selectivity and improved sensitivity. This method does not require a prior sample preparation step thus suitable for routine applications (Silva *et al.*, 2012).

2.6.3 Methods for detection of antibiotic residues in milk

Several methods have been developed for the determination of antibiotic residues in milk samples. These methods are of two types: screening methods and confirmatory methods. Screening tests are rapid, inexpensive tests with high sample throughput used to sift through large number of samples to identify suspected samples that are taken for further confirmatory tests. The commonly used screening tests include microbial inhibition assays, immunoassays, reporter gene assays and enzymatic assays (Sachi *et al.*, 2019).

Confirmatory methods are instrumental methods used to identify and quantify the specific antibiotics present in samples. These methods are more selective, expensive and require more time for analysis (Sachi *et al.*, 2019). Therefore, only samples that test positive in the screening test are subjected to confirmatory test to save time. The most commonly used instrumental methods for antibiotic residue analysis are liquid chromatography using diode array detector, fluorescence detector and mass-spectrometry (MS); and Gas

chromatography using electron capture detector, flame ionization detector and MS detection.

Traditionally, antibiotic residues in foods including milk were analyzed based on single class methods for ease of optimization of both sample extraction and instrumental conditions due to the similar physical/chemical properties of the same class compounds. However, the development of gas chromatography- mass spectrometry (GC-MS) and LC-MS/MS systems have made it possible to develop multiclass methods capable of analyzing hundreds of analytes belonging to different chemical families in a single run (Hu *et al.,* 2021).

In multi-class antibiotic residue analysis, sample extraction and cleanup is the most vital step. Milk consists of a complex matrix with fats and proteins that may bind antibiotics interfering with their extraction. For analysis of milk, acetonitrile is the most reported solvent used for extraction due to its efficacy in protein denaturation, high extraction recoveries and minimal co-extraction of lipids (Chiaochan *et al.,* 2010). The cleanup methods commonly applied in multi-class antibiotic residue analysis include Solid-Phase Extraction (SPE), QuEChERS, and ultrasonic assisted matrix solid phase dispersion (Han *et al.,* 2015).

2.6.4 Available methods for detection of pesticide residues in milk

Pesticide residues in food samples are found at trace concentrations. Pesticide residue analysis involves two main steps: sample treatment and analytical determination.

Sample treatment involves the extraction of the target analytes from the matrix followed by purification of the extract (Štajnbaher & Zupančič-Kralj, 2003). Traditional sample treatment methods include liquid-liquid extraction (LLE) and solid phase extraction (SPE). However, these methods suffer the drawbacks of consumption of large volumes of toxic organic solvents, generation of large amounts of wastes, and time consuming

(Lehotay *et al.,* 2007). Modern sample preparation techniques have been developed to overcome these challenges. Recent developments are geared towards methods that are fast, solventless, inexpensive and amenable to automation (Tran-Lam *et al.,* 2021). Common modern sample preparation techniques include Solid phase micro extraction (SPME) and QuEChERS.

2.6.4.1 Solid Phase Micro-Extraction (SPME)

SPME is a modified form of SPE that combines both extraction and concentration of analytes in a single solventless step. A fused silica fiber coated with an appropriate material is dipped into a liquid sample/ liquid extract. Analytes are adsorbed on the coating in an equilibrium process that is controlled by the equilibrium distribution coefficient of the analyte between the coating and the sample matrix. Subsequently, the analytes are thermally desorbed directly into the GC column for analysis. The amount of analytes adsorbed is proportional to the initial concentration of the sample.

2.6.4.2 QuEChERS

QuEChERS, an anagram for Quick, Easy, Cheap, Efficient, Rugged and Safe, is a new approach for extracting a wide range of pesticides from non-fatty and low-fat (<20%) food matrixes with high water content (Anastassiades *et al.,* 2003). The procedure requires a very small amount of sample (10 – 15 g). A single extraction is done in acetonitrile and a large excess of salts (typically anhydrous $MgSO_4$ and NaCl) are added to improve the extraction of both polar and non-polar pesticides. The extract is subjected to dispersive solid phase extraction to remove residual water and matrix interference. The resulting extract is directly analyzed by GC-MS or by LC-MS/MS after proper dilution.

2.7 Instrumentation

2.7.1 Gas Chromatography - Mass Spectrometry (GC-MS)

GC-MS is an analytical technique that combines gas chromatography (GC) and Mass spectrometry (MS) used for separation and identification of components of complex mixtures in relatively short analytical times. GC is used for separation while the MS is used for identification of the separated components. GC is a type of column chromatography where the mobile phase is a gas (carrier gas) while the stationary phase is either a solid or a liquid held on a solid support. It is used for the analysis of thermally stable volatile organic compounds. The sample is injected into the system through a heated injector port and immediately vaporized. The vaporized sample is transported by the carrier gas into the column where separation takes place. The column is located in a thermostated oven. Proper temperature control is critical for a high degree of separation. It is common to employ temperature programming, with continuous or stepwise temperature rise. Sample components are separated according to their boiling points (Snyder *et al.,* 2010) . The components leaving the column pass through a transfer line that connects the GC and the MS instruments.

The MS is operated at high vacuum conditions. Its main components include an ion source, mass analyzer and detector. The ion source contains a heated filament in an electric field that generates a beam of fast moving electrons (70eV). The electrons collide with sample from the GC outlet resulting into ionization of the sample molecules. The ions are accelerated by the electric field towards the mass analyzer. The ions reaching the mass analyzer are separated according to their mass to charge ratio (m/z). The detector counts the separated ions and generates an electrical signal that is processed to give a mass spectrum of relative ion intensity versus m/z ratio. The ion of the highest intensity is assigned 100% (base peak) and other ions peaks are expressed relative to the base peak (Skoog *et al.,* 2018).

2.7.2 High Performance Liquid Chromatography (HPLC)

HPLC is an improved type of liquid chromatography that uses high pressure to force the mobile phase through a column packed with very fine particles for high-resolution separations in shorter optimum run times (Skoog *et al.*, 2018).

A typical HPLC system consists of a solvent delivery system with a high pressure pump, sample injection port, a column, a detector and a computer for system control and display of results. Many systems include a thermostated oven to regulate the column temperatures and a guard column to protect the main column from impurities. The sample (liquid/solution) is injected into the system through the injection port and is carried by the mobile phase through the column where separation takes place due to the different degree of interactions between the sample components with the stationery and the mobile phases (Skoog *et al.,* 2018). A proper choice of both the stationery phase (column) and the mobile phase is essential for good separations. The column is the heart of the HPLC system where separation takes place. The separated components are detected as they leave the column. Different detectors can be used with HPLC depending on the sample properties. The most common detectors include UV/VIS, Diode Array, Electrochemical, Fluorescence and Mass Spectrometric detectors.

Two modes of chromatography are commonly used with HPLC namely normal phase (NP) and reversed phase (RP) chromatography. NP chromatography employs polar stationary phase (e.g. silica) and non-polar mobile phase while RP chromatography employs non polar stationary phase (e.g. C18 and C8) and polar mobile phase such as acetonitrile, water and methanol (Corradini, 2011).

Eluent strength plays a critical role in the HPLC separation process. The greater the eluent strength of the chosen mobile phase, the faster the solutes will be eluted from the column. A more polar solvent has higher eluent strength in NP chromatography while a less polar solvent has higher eluent strength in RP chromatography (Corradini, 2011). Two modes of elution are commonly applied in HPLC to achieve the desired eluent strength: Isocratic

and Gradient. Isocratic elution mode uses a single solvent or a solvent mixture of constant composition while in gradient elution, the solvent composition changes steadily during the run to increase eluent strength (Eugster & Wolfender, 2012).

2.7.3 Liquid chromatography tandem mass spectrometry (LC-MS/MS)

LC-MS/MS is an analytical technique that combines the separating power of liquid chromatography (LC) with the detection power of mass spectrometry (MS). LC-MS/MS is very versatile, enabling the analysis of a wide range of compounds, including non-volatile and thermally unstable compounds that are not amenable to GC-MS. Any substance can be analyzed using LC-MS/MS provided it can be dissolved in a suitable mobile phase (Corradini, 2011).

A typical LC-MS/MS system consists of a HPLC unit, atmospheric pressure ionization (API) unit, vacuum system, mass analyzer, detector and a computer system for system control and data processing. Separation of the sample components takes place in the HPLC column by differential partitioning between the mobile and stationary phases. The separated components leaving the column then enter the API unit where they are converted into gas phase ions and the mobile phase is pumped to waste. The ions are separated according to their m/z ratio in the mass analyzer and the detector 'counts' the ions leaving the analyzer and converts the information to a signal that is then processed by the computer system to give an output (mass spectrum). Mass analysis and detection is carried out under a vacuum system (Corradini, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling

Sampling was done from June to August 2020. Milk samples were purchased directly from vendors in Juja and Githurai markets, Kiambu County. The sample size was estimated using Cochran's formula with the desired confidence level of 95% (Z-score = 1.96, precision = 5%). The proportion of milk not meeting the recommended quality standards was assumed to be 4.1% based on the findings by Orwa *et al.,* (2017) and the resulting sample size was 60 samples.

Sampling was done by simple random sampling of milk from vendors on randomly chosen days within the study period. Three sample categories (shop milk, automated vending machine (AVM) milk and packet milk) were chosen because they were the main types of milk sold within the region. The distribution of samples collected is summarized in Table 3.1.

Table 3.1: Distribution of samples

Location	shop	AVM	packet milk	Total
Juja	17	11	5	33
Githurai	15	12	5	32
Total	32	23	10	65

Shop and AVMs milk samples were collected in sterilized plastic bottles (250 ml), while packaged milk were purchased in 500 ml packages. Samples were transported in a cool box to the laboratory within 2 hours of collection and analysis was performed within 6 hours of arrival. When samples could not be analyzed upon arrival, they were frozen at -20 ^{o}C and analyzed on the following day. Frozen samples were thawed at room temperature prior to analysis.

3.2 Equipment

- GC-MS: Shimadzu GCMS-QP 2010 SE was used for separation and quantification of some pesticide residues. The system was controlled by Shimadzu GCMSsolutions software.
- LC-MS/MS: An Agilent HP1100 LC system coupled to a Waters Quattro-ultima mass spectrometer was used for separation and quantification of antibiotic residues and some pesticide residues. The system was controlled by Waters MassLynx software.
- Lactometer was used for measuring of specific gravity of milk.
- Thermometer was used for measuring the temperature of milk samples.
- SPE manifold with vacuum pump were used for loading samples into SPE cartridges and subsequent elution of the analytes from the cartridges.
- Vaccum concentrator: Mi-Vac 23050-A00 DNA pre-concentrator was used to evaporate the solvent from sample extracts.

3.3 Chemicals and reagents

Hydrogen Peroxide test strips (Quantofix 25) were purchased from Marcherey-Nagel, Germany through ChemoQuip (Kenya). Amitraz, carbaryl, chlorpyrifos, cypermethrin, deltamethrin, amoxicillin, cloxacillin, sulfamethoxazole and ciprofloxacin standards were purchased from Sigma Aldrich (Germany) through Kobian Scientific (Kenya). Trimethoprim and tetracycline standards were obtained from Universal Pharmaceuticals Corporation (Kenya). Methanol, acetonitrile, formic acid, acetic acid and water used were HPLC grade. Citric acid monohydrate, anhydrous sodium phosphate dibasic (Na_2HPO_4), oxalic acid, disodium ethylenediaminetetraacetic acid dihydrate ($Na_2EDTA.2H_2O$), anhydrous magnesium sulphate and anhydrous sodium acetate were reagent grade.

3.4 Preparation of standards and extraction solutions

3.4.1 Antibiotics standards

Individual stock solutions at a concentration of 1 mg/mL were prepared in methanol and stored at -20 ^{o}C. Intermediate mixed standard (IMS) solutions (0.02 mg/mL) were prepared in methanol and stored at -20 ^{o}C. Working standards solutions were prepared by appropriate dilutions of the IMS in 9:1 water: methanol. Working standard solutions were prepared daily, IMS weekly and stock solutions every 3 months.

3.4.2 Pesticide standards

Stock solutions for individual pesticides at a concentration of 1mg/ml were prepared in methanol and stored at – 20 ^{o}C. IMS solutions (0.02 mg/mL) were prepared in methanol and stored at -20 ^{o}C. Two sets of IMS were prepared: one containing amitraz, carbaryl, and cypermethrin for LC-MS/MS analysis and the other one containing cypermethrin and deltamethrin for GC-MS analysis. Working standards were prepared by appropriate dilutions of the IMS in water: methanol (8:2) for LC-MS/MS analysis and methanol for GC-MS analysis. Working standard solutions were prepared daily, IMS weekly and stock solutions every 3 months.

3.4.3 EDTA/Mcllavaine buffer preparation

Anhydrous sodium phosphate dibasic salt (28.41 g) was dissolved in a 1 L volumetric flask to make a solution of 0.2 M Na_2HPO_4. In a separate flask, 0.1M citric acid solution was prepared by dissolving 21.01 g of citric acid monohydrate in a 1L volumetric flask and making up the volume to 1L. The citric acid solution (1L) was combined with the Na_2HPO_4 solution (625 ml) and 60.50 g of EDTA disodium salt added. The solution was sonicated until all the salt had dissolved.

3.5 Optimization of analytical methods

3.5.1 Optimization of LC-MS/MS system

The MS detection parameters optimization for each compound was performed by direct infusion of 1 µg/ml standard solution into the mass spectrometer using a syringe pump set at a flowrate of 5 µl/min. A Tee connector was used to infuse the syringe pump flow stream into the LC flow stream to ensure stable continuous infusion. The MS was operated on electrospray ionization (ESI) positive ion mode. The capillary voltage was set at 3.0 kV, source temperature at 100 ^{o}C, desolvation temperature at 350 ^{o}C and desolvation gas flowrate at 700 L/hr. The desolvation gas was nitrogen while argon gas was used as a collision gas.

Separation was carried out using Kinetex EVO C18 column (100 mm x 3.0 mm, 5 µm particle size, 100 A^{o}). The elution gradient, flow rates and temperature were optimized by varying the conditions until satisfactory peak separation was achieved.

3.5.2 Optimization of GC-MS system

Separation was carried out on a BP-X5 capillary column (length: 30 m, ID: 0.25 mm, film thickness 0.25 µm). The separation conditions were optimized by varying the injection temperature and the oven temperature program until satisfactory separation of the target analytes were achieved. The source temperature and the interface temperature were held constant at 200 ^{o}C and 250 ^{o}C respectively.

3.5.3 Method performance characteristics

Method performance characteristics of selectivity, linearity, detection limits and recovery were evaluated for both the LC-MS/MS and GC-MS analyzed compounds.

3.5.3.1 Selectivity

Selectivity was assessed by analysis of blank milk samples. Six replicate blanks were analyzed and the resulting chromatograms checked for any interfering compounds within the expected retention intervals of the analytes in the samples.

3.5.3.2 Linearity

Calibration curves were used to investigate the linear range of the analytical methods. Calibration curves were prepared by injecting a series of standards of varying concentrations ranging from 2 µg/L to 100 µg/L for LC-MS/MS analysis and 10 µg/L to 1000 µg/L for GC-MS analysis.

3.5.3.3 Detection limits

The limit of detection (LOD) and limit of quantification (LOQ) for each compound was calculated based on the principle that the LOD is the analyte concentration that gives a signal to noise ratio of 3 while the LOQ is the analyte concentration that gives signal to noise ratio of 10. LOQs and LOQs were calculated from the linear calibration curves using equations 4 and 5 respectively.

$$LOD = \frac{3\delta}{S} \quad (4)$$

$$LOQ = \frac{10\delta}{S} \quad (5)$$

Where δ is the standard deviation of the blank; and S is the slope of the regression equation.

3.5.3.4 Recovery

Recovery was evaluated by comparing the concentrations of spiked samples (pre- and post-extraction spikes). Previously analyzed blank milk samples were spiked at a concentration of 2.5 µg/L, 10 µg/L and 25 µg/L (equivalent to 10 µg/L, 40 µg/L and 100

µg/L respectively in the final extract) for amoxicillin, cloxacillin, sulfamethoxazole, tetracycline and trimethoprim; and a concentration of 10 µg/L, 25 µg/L and 50 µg/L (equivalent to 20 µg/L, 50 µg/L and 100 µg/L in the final extract) for amitraz, carbaryl, chlorpyrifos, cypermethrin and deltamethrin. The recovery was calculated using equation 2.

$$\text{Relative recovery} = \frac{[pre-extraction\ spike]}{[post-extraction\ spike]} \times 100\% \quad (2)$$

3.6 Determination of added water

Milk density was determined as an indicator for added water. A lactometer was used to determine the density of milk samples. Milk (100 mL) was poured in a measuring cylinder and the lactometer lowered into the milk such that it floated freely in the milk without touching the bottom or sides of the cylinder. The lactometer reading was recorded (^{o}L). Temperature correction was done for the lactometer readings. For each ^{o}C below the calibration temperature, 0.2 ^{o}L was subtracted from the lactometer reading and for each ^{o}C above the calibration temperature, 0.2 ^{o}L was added to the lactometer reading. The milk densities were calculated using equation 3.

$$\text{Specific gravity} = 1 + \frac{corrected\ lactometer\ reading}{1000} \quad (3)$$

3.7 Determination of hydrogen peroxide

The concentration of hydrogen peroxide was determined using Quantofix hydrogen peroxide test strips (Quantofix 25) from Marcherey-Nagel, Germany. The test strip was dipped in the milk sample for one second then removed and excess milk shaken off. It was allowed to stand for 15 seconds for the color to develop and the resulting color compared on the color scale. In presence of hydrogen peroxide, the strips formed a blue color whose intensity is dependent on the concentration of hydrogen peroxide. For very concentrated samples, appropriate dilutions were made, and the diluted sample tested with a fresh test strip.

3.8 Determination of Antibiotic residues

The workflow diagram for the determination of antibiotic residues in milk sample is given in Figure 3.1.

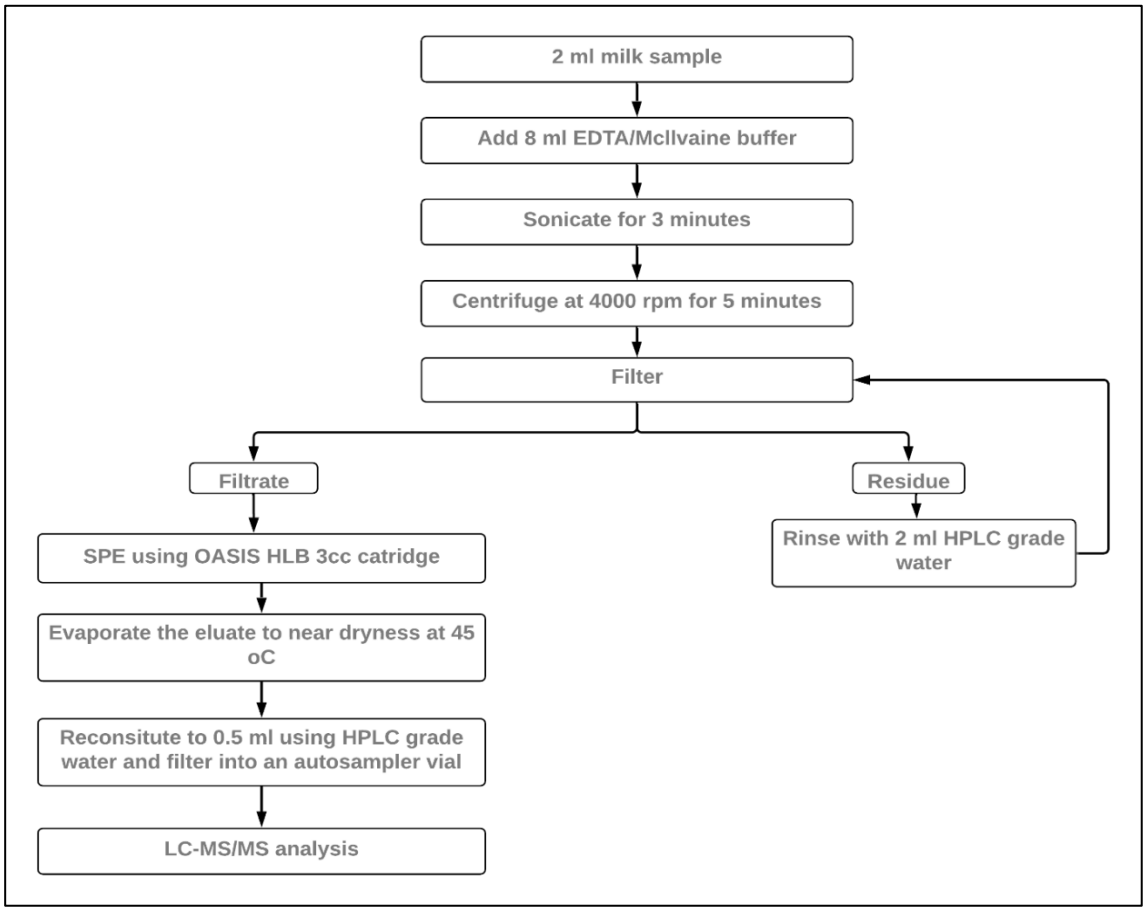


Figure 3.1: Workflow diagram for the determination of antibiotic residues in milk

A single multi-class extraction was performed. 2 mL of milk sample was transferred into a 15 mL centrifuge tube. 8 mL of EDTA/McIlvaine buffer was added to tube. The mixture was shaken for 30 seconds, sonicated for 3 minutes and centrifuged at 4000 rpm for 5 minutes. The supernatant was filtered using a fast filter paper onto a 15 mL glass test-tube. The residue was rinsed with 2 mL of water and added to the filtrate. The filtrate was then taken for SPE cleanup.

SPE cleanup was performed using OASIS HLB SPE cartridges, 3cc. The SPE cartridges were conditioned using 3 mL of methanol, followed by 3 mL of water. The filtrate from the extraction step was loaded at a flowrate of approximately 2 mL per minute. Once loading was complete, the cartridge was rinsed with 1 mL of water and allowed to dry under vacuum for 5 minutes. Elution was done using 1 mL of methanol at a flowrate of 1 mL per minute. The solvent was evaporated to near dryness in a miVac pre-concentrator with the temperature set at 45 ^{o}C and reconstituted to 0.5 mL with water. Finally, the extract was filtered using a 0.22 µm syringe filter into an auto-sampler vial and analyzed using LC-MS/MS.

3.9 Determination of pesticide residues

Sample extraction was done using the QuEChERS method (Anastasiades *et al.*, 2003).

Milk sample (10 mL) was transferred into a clean 50 mL centrifuge tube. 10 mL of acetonitrile was added to the tube and shaken for 1 minute. Anhydrous magnesium sulfate (4.0 g) and anhydrous sodium acetate (1.0 g) was then added to the tube and immediately the contents shaken vigorously for one minute. The contents were centrifuged at 5000 rpm for 5 minutes in order to separate the solid material from the extract. Two -1 mL aliquots of the extract were taken in separate glass vials. Both aliquots were evaporated to dryness in a miVac sample pre-concentrator. One vial was reconstituted with 500 µL of methanol and analyzed with GC-MS while the other was reconstituted with 500 µL of 10% acetonitrile in water and analyzed with LC-MS/MS. Both reconstituted extracts were filtered with 0.45 µm syringe filter prior to instrumental analysis.

The workflow for extraction of milk samples for the determination of pesticide residues is given in Figure 3.2.

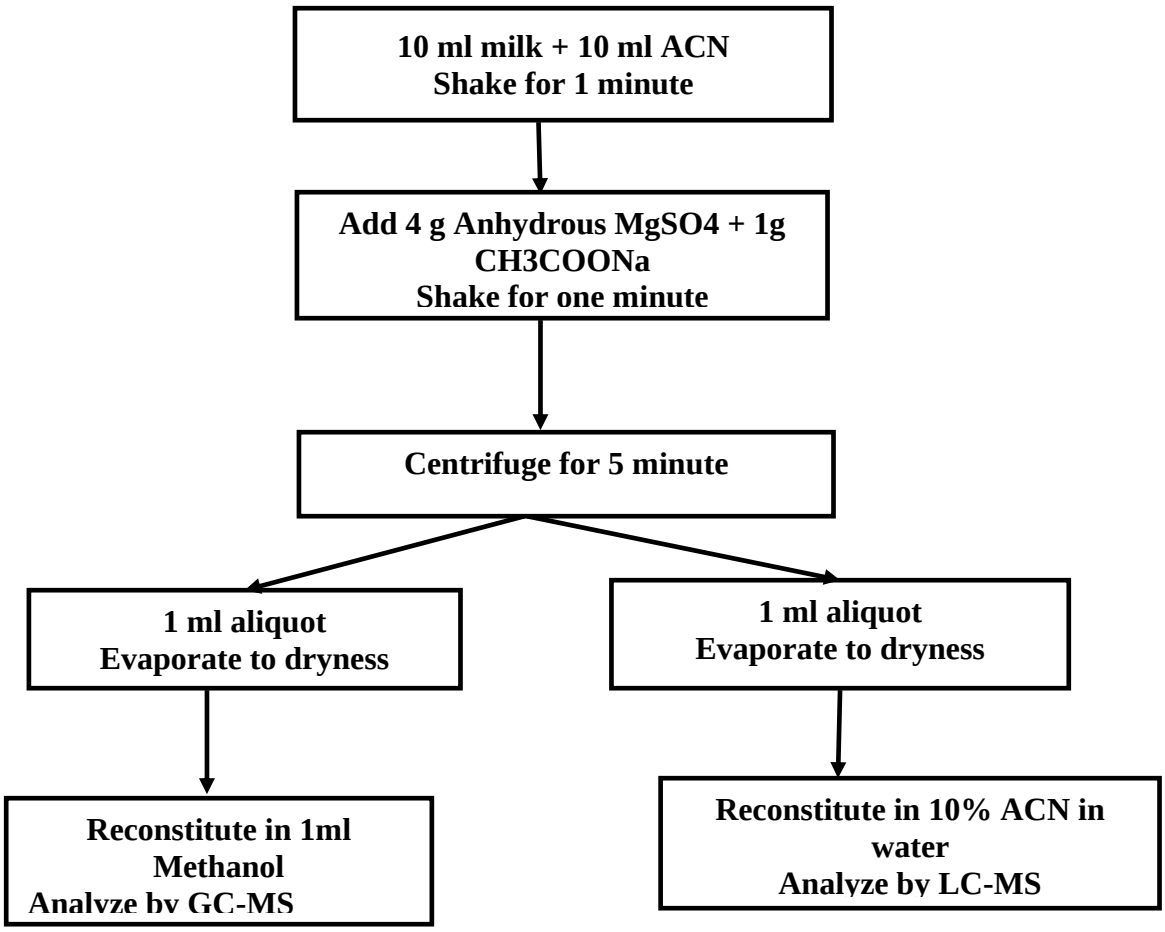


Figure 3.2: Workflow diagram for the determination of pesticide residues in milk

3.10 Data analysis

Data processing was done using various software. MassLynx software was used to analyze LC-MS/MS data. GCMS solution software was used to analyze GC-MS data. Microsoft-Excel software was used to analyze data from lactometer readings. Statdisk software was used to perform statistical tests on the processed data. The 2-proportion Z-Tests was used to compare proportions of non-compliant samples for antibiotic residues and water adulteration while One-way ANOVA and 2-sample T-Tests were used to compare mean milk densities. All comparisons were made at $p \leq 0.05$ level of significance.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Optimization of analytical systems

4.1.1. Optimization of the LC-MS/MS system

The optimized multiple reaction monitoring (MRM) parameters for the compounds analyzed by LC-MS/MS are given in Table 4.1.

Table 4.1: Optimized MRM parameters for LC-MS/MS analysis

compound	*monoisotopic mass (Da)	parent ion (m/z)	cone voltage (V)	product ions (m/z)	collision energy (CE)
amoxicillin	365.1	366	20	349	13
				114	20
cloxacillin	435.1	436	20	277	18
				160	21
tetracycline	444.2	445	25	410	20
				154	25
sulfamethoxazole	253.1	254	28	156	20
				92	25
trimethoprim	290.1	291	35	230	30
				123	35
amitraz	293.2	294	25	163	20
				122	30
carbaryl	201.1	202	20	145	22
				127	25
chlorpyrifos	348.9	350	35	198	30
				97	30

*** Monoisotopic mass is the mass of a molecule comprising entirely of the most abundant isotopes for each element present in the molecule**.

Acceptable resolution was obtained using a C18 column (Kinetex Evo C18, 100 mm x 3 mm, 5µm). The mobile phase comprised of 0.1% formic acid in water (solvent A) and acetonitrile acidified with 0.1% formic acid (solvent B). Antibiotics and pesticide residues were analyzed using separate chromatographic runs. For both runs, gradient separation was performed at 30 ^{o}C temperature and 0.45 mL/min flow rate. The gradient program used for separation of antibiotic residues was as follows: 0-2 min (10 % B); 2-7 min (increase to 100% B); 7-7.5 min (decrease to 10 % B), 7.5-10 min (10 % B). The gradient program used for separation of pesticide residues was as follows: 0- 0.5 min (30 % B); 0.5 -5 min (increase to 100 % B), 5-7 min (100 % B), 7-7.5 min (decrease to 30 % B), 7.5-10 min 30 % B). Sample total ion count (TIC) chromatograms obtained under these conditions are given in Figures 4.1 and 4.2 for antibiotics and pesticide residues, respectively.

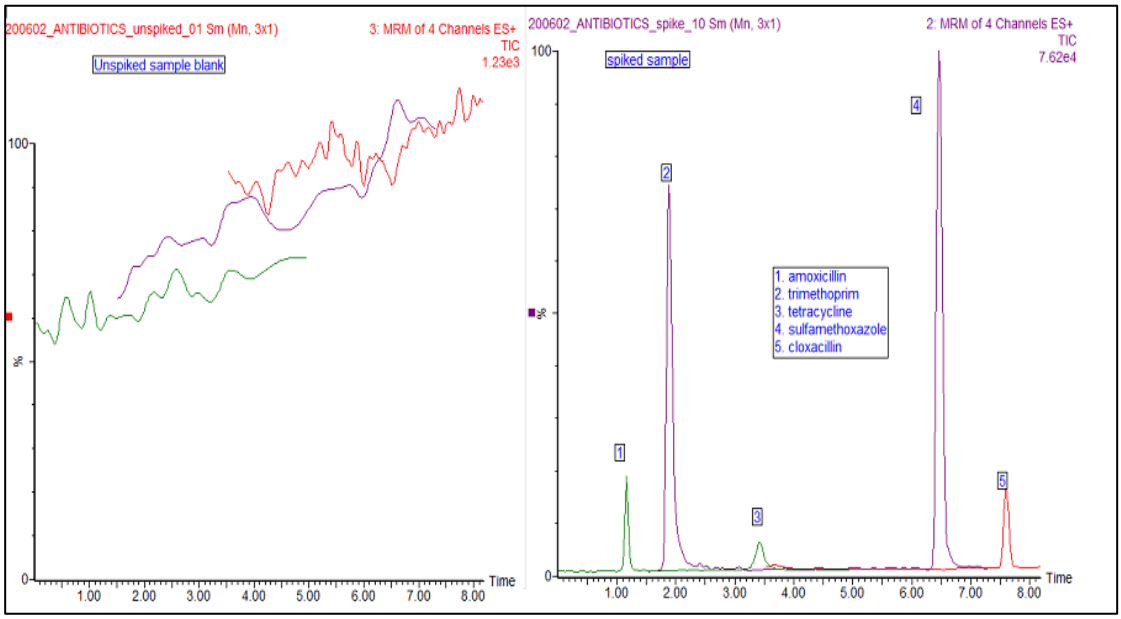


Figure 4.1: Overlay TIC chromatograms for antibiotic residues. Left: Blank milk sample. Right: Milk spiked with 10 µg/L of each antibiotic drug. The acquisition parameters for function 1 (green), function 2 (purple) and function 3 (red) are given in Appendix I.

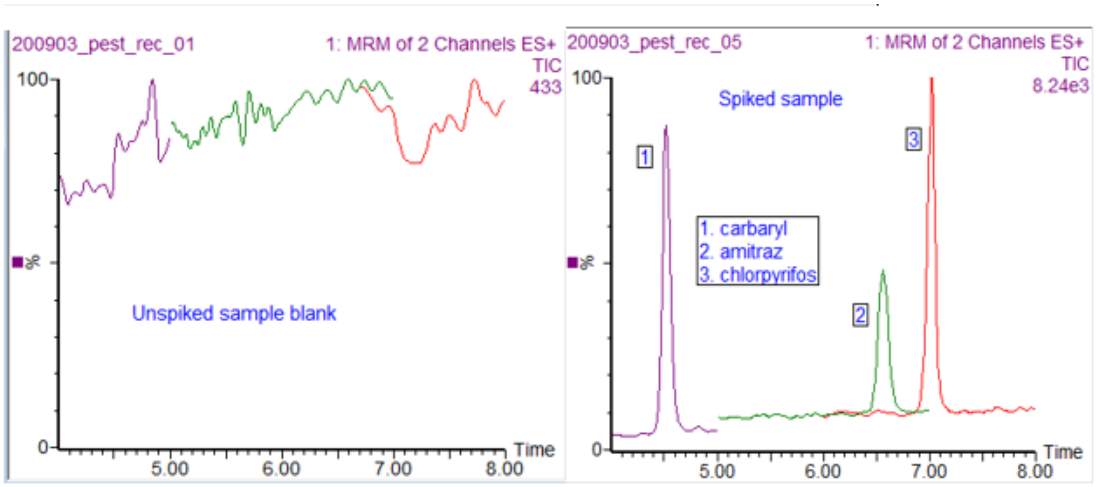


Figure 4.2: Overlay TIC chromatograms for pesticide residues. Left: blank milk sample. Right: milk sample spiked with carbaryl (20µg/L), amitraz (10 µg/L) and chlorpyrifos (10 µg/L). The acquisition parameters for parameters for function 1 (purple), function 2 (green) and function 3 (red) are given in Appendix I.

4.1.2. Optimization of the GC-MS system

The results for optimization of GC temperature program for elution of deltamethrin and cypermethrin was as follows: 50 ^{o}C initial temperature; 30 ^{o}C/min to 200 ^{o}C; 4^{o}C/min to 300 ^{o}C; hold at 300 ^{o}C for 5 min. The total run time for the program was 36 minutes and injection was done in split mode. The optimized injection temperature was 250 ^{o}C. A scan mode chromatogram was used to identify the retention windows of the target compounds and their identity confirmed by performing a library search on the eluted peaks. A sample chromatogram obtained under these optimized conditions showing cypermethrin and deltamethrin peaks is given in Figure 4.3 while sample mass spectrum identifying the compounds is in Figure 4.4.

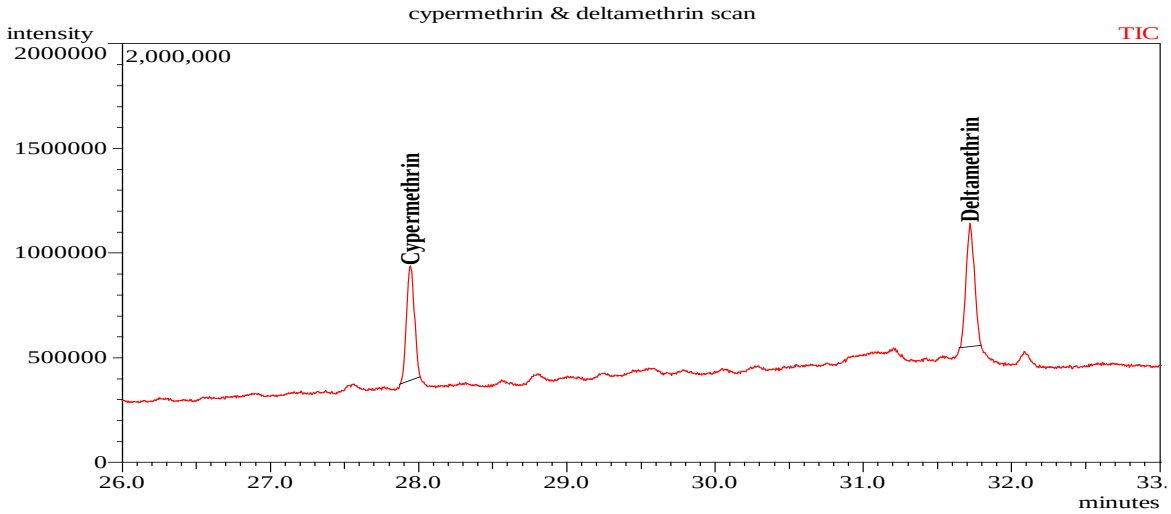


Figure 4.3: Sample scan chromatogram for cypermethrin and deltamethrin at a concentration of 2 µg/mL. The retention times were 27.945 and 31.720 minutes, respectively.

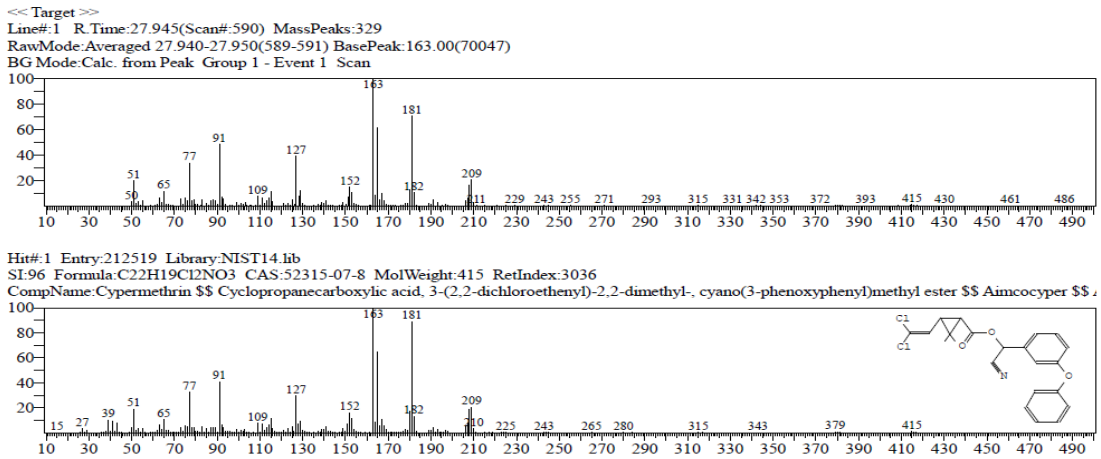


Figure 4.4: Mass spectrum of cypermethrin. The Target Line #1 is the spectrum from the injected sample while the Hit #1 spectrum is the library match identifying the target compound as cypermethrin. The mass spectrum for deltamethrin is given in Appendix II

In scan mode, the MS is set to continuously scan a pre-defined mass range and records the full mass spectra. Scan mode is useful in identification of components using the mass spectra and for determination of retention windows and suitable mass fragments for single ion monitoring (SIM) mode (Shimadzu Corporation, 2007).

In SIM mode, the mass spectrometer is set to record only the ion current of selected mass fragments that are characteristic of the compounds of interest. SIM mode offers higher selectivity, sensitivity and specificity, allowing better detection limits for quantitative analysis in comparison to scan mode (Turner *et al.*, 2019). For this work, SIM mode was used for quantitative analysis. Two mass fragments were selected for each compound and data acquisition was done only during the expected retention window for the compound initially established from the scan chromatogram. The selected m/z were 163 and 181 for cypermethrin; 181 and 253 for deltamethrin. Sample SIM chromatograms are given in Figures 4.5 and 4.6 for cypermethrin and deltamethrin, respectively.

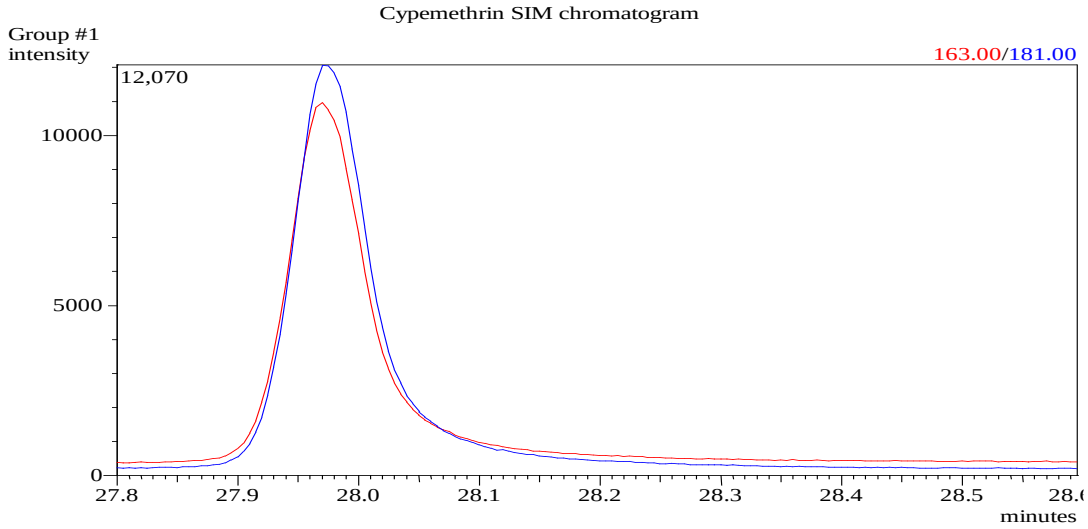


Figure 4.5: Sample SIM chromatogram for cypemethrin (50 ng/L). The monitored mass fragments were m/z 163 and 181.

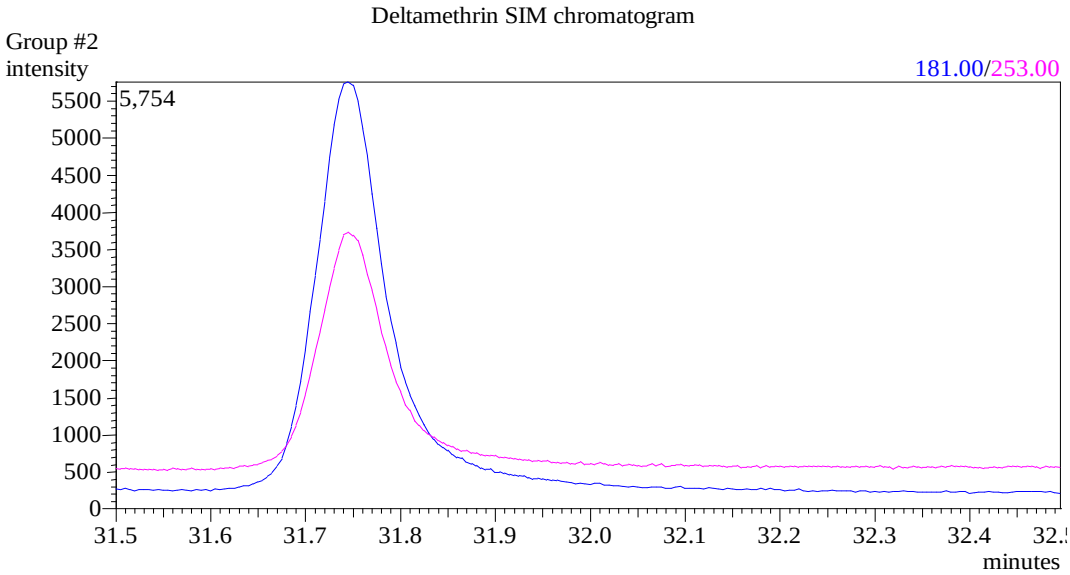


Figure 4.6: Sample SIM chromatogram for deltamethrin (50 µg/L). The monitored mass fragments were m/z 181 and 253.

4.1.3 Method performance characteristics

4.1.3.1 Selectivity

Blank milk samples were analyzed and the resulting chromatograms checked for any interfering peaks within the expected retention windows of the target analytes. For all the analytes, there were no interfering peaks in the expected retention windows for the blank samples analyzed as shown in Figures 4.1 and 4.2 for antibiotics and pesticide residues, respectively. Peaks were only observed when the blanks were spiked with the targeted analytes, indicating that the analytical methods had sufficient selectivity.

4.1.3.2 Linearity

Calibration curves were plotted using the peak areas versus the concentrations for each target compound. Sample calibration curves are given in Figure 4.7. Calibration curves were linear with correlation coefficients greater than 0.998 for all the analytes, indicating linear correlation in the tested concentration ranges. The results of the regression analysis are summarized in Table 4.2.

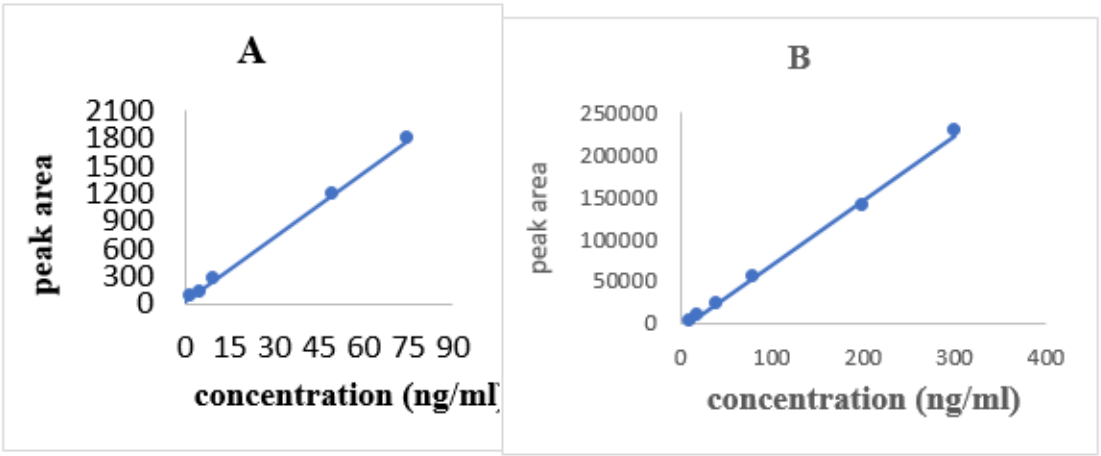


Figure 4.7: Sample calibration curves.

A: Amoxicillin and B: Cypermethrin. Calibration curves for other compounds are given in Appendix III

Table 4.2: Summary of method performance characteristics

	instrumental technique	calibration range (µg/L)	Pearson Correlation Coefficient (R)	LOD (µg/L)	LOQ (µg/L)	Relative recovery	MRL (µg/L)	[c] C.F
amoxicillin	LC-MS/MS	2 – 75	0.9998	1.41	4.69	90 ± 5	[a]4	4
cloxacillin	LC-MS/MS	2 – 75	0.9997	1.44	4.80	90 ± 5	[b]30	4
tetracycline	LC-MS/MS	2 – 100	0.9997	2.34	7.80	118 ± 6	[a]100	4
sulfamethoxazole	LC-MS/MS	2 – 100	0.9995	2.52	8.42	86 ± 4	[a]100	4
trimethoprim	LC-MS/MS	5 – 100	0.9996	2.41	8.03	108 ± 7	[b]50	4
amitraz	LC-MS/MS	10 – 100	0.9981	4.91	16.37	79 ± 5	[a]10	2
carbaryl	LC-MS/MS	20 – 400	0.9996	10.52	35.07	96 ± 6	[a]50	2
chlorpyrifos	LC-MS/MS	10 – 200	0.9998	3.22	10.74	106 ± 7	[a]20	2
cypermethrin	GC-MS	10 – 300	0.9990	9.70	32.33	89 ± 9	[a]50	2
deltamethrin	GC-MS	10 – 300	0.9995	7.25	24.16	92 ± 5	[a]50	2

a CAC MRLs (Alimentarius, 2017) . b EU MRLs (EU Commission Regulation, 2010). c C.F -preconcentration factor in sample preparation steps prior to analysis to increase sensitivity of detection in milk samples

4.1.3.3 Detection limits

The LOD is lowest analyte concentration that can be detected while the LOQ is the lowest analyte concentration that can be quantified. The LODs and LOQs for the antibiotics and pesticide analyzed are summarized in Table 4.2.

The calculated LODs and LOQs were lower than the MRLs in milk for all the targeted compounds except for amoxicillin and amitraz as shown in Table 4.2. To ensure detection of analytes concentrations up to the MRLs, the sample preparation methods were designed in such a way that the final extract had a concentration factor of four (4X) for antibiotic residues and concentration factor of two (2X) for pesticide residues.

4.1.3.4: Recoveries

The mean recoveries for this study ranged from 79 -118 % as summarized in Table 4.2.

Analyte recovery is an essential parameter for determining the efficiency of extraction in analytical trace analysis (Ngumba, 2018). According to the Association of Official Analytical Chemists (AOAC), recoveries within the range 70 -120 % are acceptable (Chandrakar, 2020). The recoveries for all analytes targeted in this study were within the acceptable range.

4.2 Determination of added water in milk

The specific gravity of milk can be used as an indicator for milk adulteration with water.

The distribution of milk densities is shown in Figure 4.8

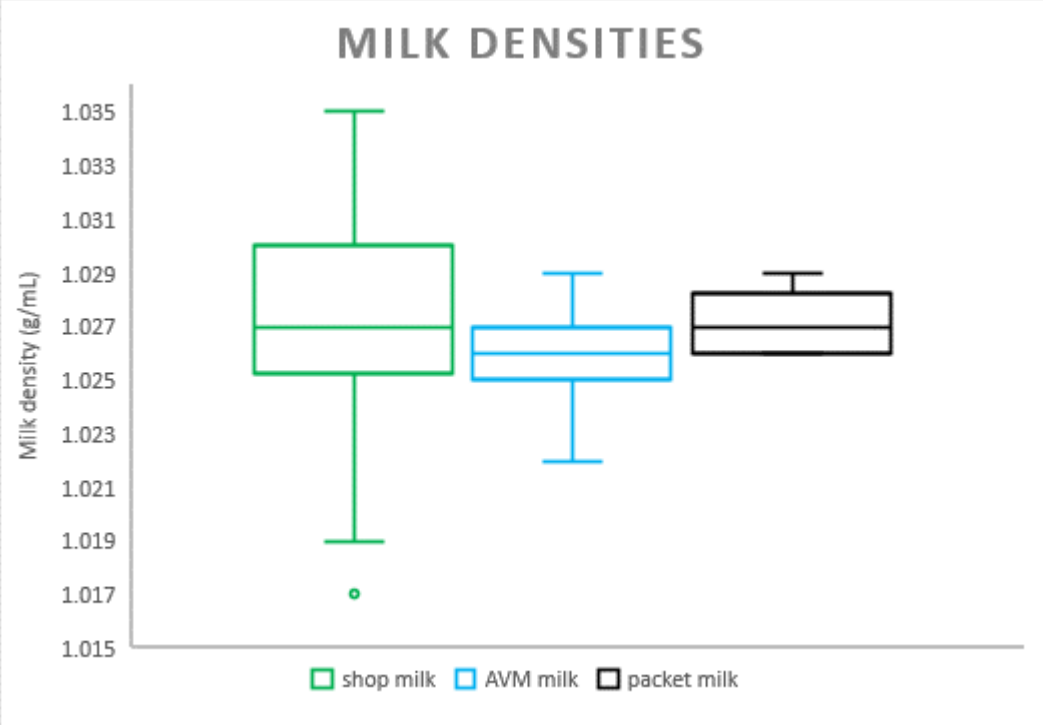


Figure 4.8: Distribution of density by type of milk. The densities for individual milk samples are given in Appendix IV.

Addition of water lowers the specific gravity of milk. According to the Kenya Bureau of Standards (KEBS) milk specification standards for raw and pasteurized cow milk (KS EAS 67: 2019; KSEAS: 69:29), the specific gravity of both raw and pasteurized cow milk should be 1.028 – 1.036 g/mL at 20 ^{o}C.

The distribution of milk density among shop milk samples showed greater range compared to both AVM and packet milk samples. Packet milk had the least range of distribution of densities. This could be attributed to the fact that packaged milk comprise of bulked samples from many different farms. Many of the shops handled low volumes

of milk (5-20 Liters) which could have possibly been sourced from single farms/ distributor leading to the big variations in densities between samples.

The mean densities for the different types of milk samples are summarized in Table 4.3.

Table 4.3: Summary of milk density by type of samples.

Sample group	Number of samples analyzed	Samples with acceptable density	Mean density ±SD
Shop milk	32	15 (47 %)	1.027 ± 0.004
AVM milk	23	5 (22 %)	1.026 ± 0.002
Packet milk	10	3 (30 %)	1.027 ± 0.001
Overall	65	23 (35 %)	1.027 ± 0.03

The milk densities ranged between 1.017 -1.035 g/mL and the overall mean density for all the samples together was 1.027 ± 0.003 g/mL. There was no statistically significant difference between the mean densities of shop, AVM and packet milk using one-way ANOVA test (F_{cal} (2, 62) = 1.3279, p = 0.272) as compared to F_{tab} (2,62) = 3.1453 at $p \leq 0.05$ level of significance.

Overall, 35 % of samples were within acceptable density range of 1.028 -1.036 g/mL. All the non-compliant samples had densities below 1.028 g/mL pointing towards possible adulteration with water.

The results indicate that adulteration of milk with water was widespread as nearly two-thirds of all the samples analyzed did not meet the recommended density range. Milk vendors dilute raw milk with water to increase the profit margins from the increased volume. Adulteration with water has been reported by other studies in Kenya. Orregard (2013) found that 31% of raw milk samples in the informal value chain did not fall within the acceptable density range. Ndung'u *et al.* (2016) reported that adulteration levels of up to 37 % while Wanjala *et al.* (2018) reported 13.6%. The extent of adulteration varies seasonally and with the targeted market. It has been reported that adulteration is more

prevalent during the dry seasons (Orregard, 2013) and in urban slum areas (Wanjala *et al.,* 2018). However, some of the existing literature is based on the old standards, where the acceptable range was 1.026 - 1.032 g/ mL. Based on the old standards, the level of adulteration for this present study would be 29.23% which is similar to some previous studies within Kenya.

4.2.1 Density of shop milk

The densities for shop milk ranged between 1.017 -1.035 g/mL. The mean density was 1.027 ± 0.004 g/mL. Figure 4.9 shows a comparison between the mean densities for shop milk in Juja and Githurai areas

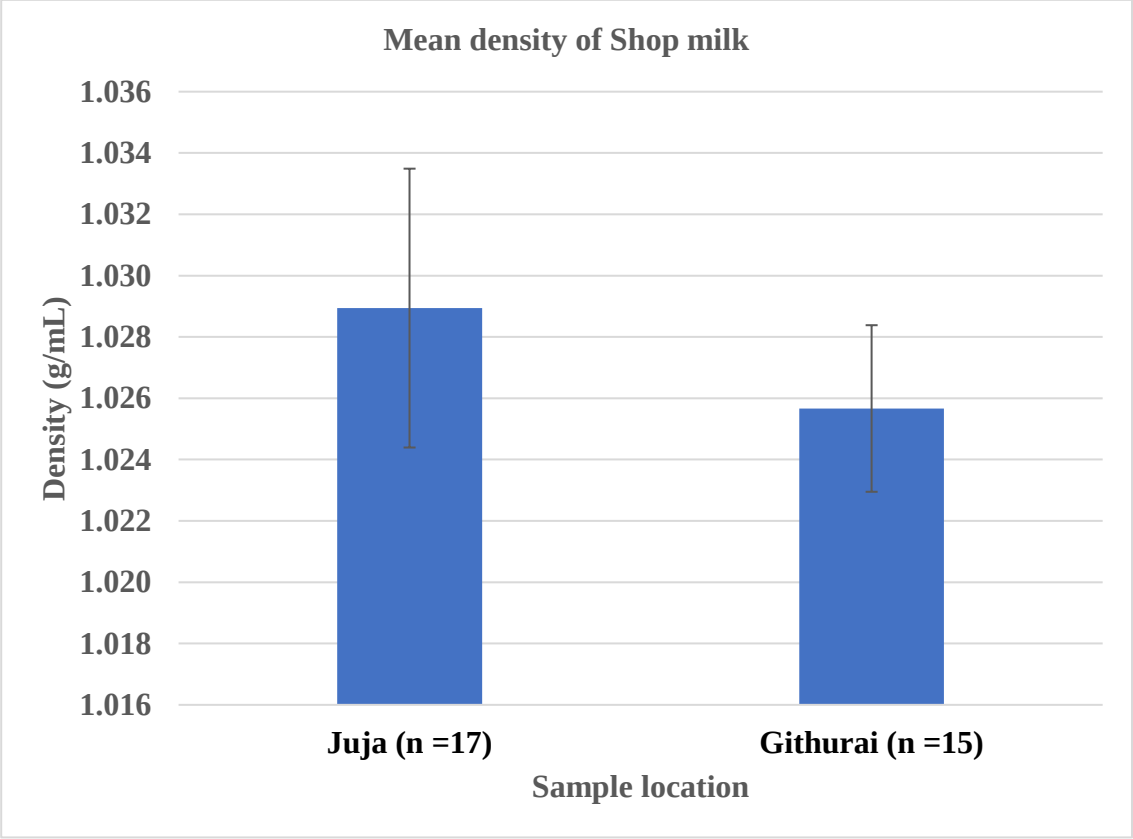


Figure 4.9: Mean density for shop milk

The mean density for shop milk was 1.029 ± 0.005 g/mL and 1.026 ± 0.003 g/mL for Juja and Githurai, respectively. Statistical tests at 95% CL ($p \leq 0.05$ level of significance) indicated a statistically significant difference between the two means (t-test for two independent samples, p-value = 0.0187). 41 % and 67 % of shop milk samples in Juja and Githurai, respectively did not meet the acceptable density limits stipulated by KEBS specifications for cow milk. However, this difference in proportions of shop milk violating the density specifications in the two places was not statistically significant (two-sample proportion z-test, p-value = 0.149).

4.2.2 Density of automated vending machines (AVM) milk

The densities for AVM milk ranged between 1.022 -1.029 g/mL. The mean density was 1.026 ± 0.002 g/mL. Figure 4.10 shows a comparison between the mean densities for AVM milk in Juja and Githurai areas.

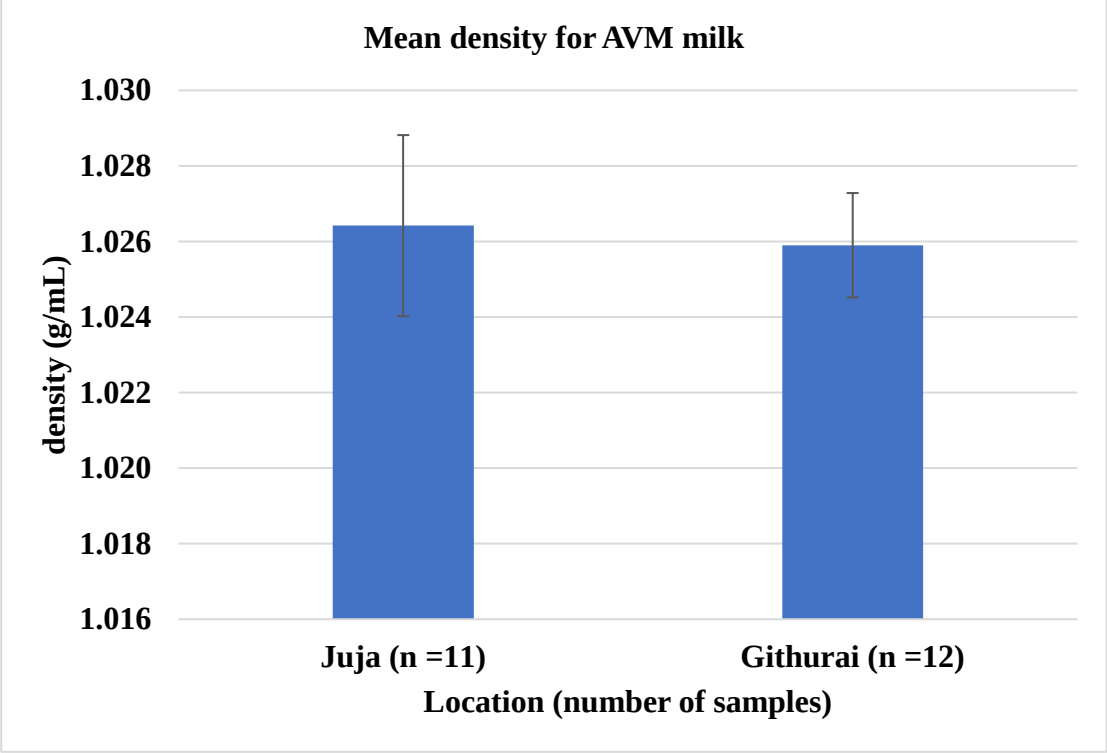


Figure 4.10: Mean density for AVM milk

The mean density for AVM milk was 1.026 ± 0.002 g/mL and 1.026 ± 0.001 g/mL for Juja and Githurai, respectively. Statistical tests at 95% CL ($p \leq 0.05$ level of significance) indicated that there was no statistically significant difference between the two means (t-test for two independent samples, p-value = 0.367). 55 % and 100 % of the AVM milk samples in Juja and Githurai, respectively did not meet the acceptable density limits stipulated by KEBS specifications for cow milk. The proportion of AVM milk samples violating the density specifications was significantly higher in Githurai than Juja (2-proportion z-test, p-value = 0.0041). The higher violations in Githurai could be attributed to a higher demand for milk in Githurai, occasioned by the high population density in the area, which drives traders to dilute the milk to meet the high market demand for milk while maximizing profits. According to the Kenya National Bureau of Statistics (KNBS) census of 2019, the population density in Githurai and Juja were 7906 and 880 people per sq. km, respectively (KNBS, 2019).

4.2.3 Milk density for packet milk

The densities for packet milk ranged between 1.026 -1.029 g/mL. The mean density was 1.027 ± 0.001 g/mL. 70% of the packet milk samples did not meet the acceptable density specification of 1.028 -1.036 g/mL. However, it is important to note that when tested against the old specifications that stipulated the acceptable density range between 1.026 -1.032 g/mL, 100% of the tested packet milk samples would be within acceptable density limits. It is possible that some milk processors were still relying on the old standards for their acceptance criteria. Similar studies conducted in Nairobi did not find any packaged milk with a density below 1.026 g/mL (Wanjala *et al.*, 2018).

4.3 Determination of hydrogen peroxide in milk

Three milk samples from the three categories of milk samples analyzed (65 samples) forming overall 5% had detectable level of hydrogen peroxide ranging from 0.5 -250 mg/L. Table 4.4 gives a summary of results on the proportion of samples with detectable levels of hydrogen peroxide among the different sample categories.

Table 4.4: Hydrogen peroxide in different types of milk samples

Sample group	Number of samples analyzed	Samples with detectable hydrogen peroxide
Shop milk	32	0 (0%)
AVM milk	23	1(4%)
Packet milk	10	2 (20%)
Overall	65	3 (5%)

Hydrogen peroxide was not detected in any of the shop milk samples analyzed. The low incidence of hydrogen peroxide in shop milk samples could be attributed to the fact that many of the vendors kept their milk refrigerated. Prevalence of hydrogen peroxide was 4 % and 20 %, in AVM and Packet milk samples, respectively. The results of this study agree with a similar study conducted in Nairobi, which reported that pasteurized milk were likely to be contaminated with hydrogen peroxide than raw milk (Wanjala *et al.*, 2018). Other studies have reported a prevalence ranging from 0% in raw milk to up to 70% in automated vending machines (Ongarora & Karwimbo, 2020; Orregård, 2013; Wanjala *et al.*, 2018).

Among the positive samples, the concentration of hydrogen peroxide ranged from 0.5 – 250 mg/L. Possible sources of hydrogen peroxide in the milk could be due to intentional addition by farmers/traders to prolong the shelf-life of milk, and residual hydrogen peroxide from the disinfection of milk processing facilities. The Food and Agriculture Organization recommends the use of hydrogen peroxide (10 mg/L) to activate the

Lactoperoxidase enzyme to prolong the shelf-life of milk in areas where there are no cooling facilities (Martin *et al.,* 2014). Kenyan law prohibits the addition of preservatives, including hydrogen peroxide in raw milk (KEBS, 2019). Hydrogen peroxide is used as a disinfectant for cleaning milk handling equipment and may leave residues in the milk (Wanjala *et al.,* 2018). However, upon boiling of milk, the peroxide decomposes and thus not detectable in boiled milk (Omore *et al*., 2007).

4.4 Antibiotic Residues in milk

The Kenya Bureau of Standards (KEBS) standards for cow milk stipulates that milk should comply with the CAC MRLs for veterinary drugs in milk (KEBS, 2019). The MRLs in milk are set at 100 µg/kg for tetracyclines and 4 µg/kg for penicillins (Alimentarius, 2017). The sum of all sulfonamides in milk should not exceed 100 µg/kg (Alimentarius, 2017) while the MRL for trimethoprim in milk is 50 µg/kg (EU Commission Regulation, 2010). Summary of results for the prevalence of antibiotics residues by location and sample type is given in Table 4.5.

Table 4.5: Prevalence of antibiotic residues by location and sample type

Location -Type	# Samples analyzed	# Positive for antibiotic residues	# Samples above MRL
Juja -AVM	11	2 (18 %)	0 (0 %)
Juja -Shop	17	5 (29 %)	0 (0 %)
Githurai -AVM	12	4 (33 %)	1 (8 %)
Githurai -Shop	15	10 (67%)	6 (40 %)
Packet	10	0 (0 %)	0 (0 %)
Total	**65**	**21 (32 %)**	**7 (11 %)**

Eleven percent of the samples had at least one antibiotic residue above the MRLs, 22 % of the samples had at least one antibiotic residue at concentrations below the MRLs and

68 % of the samples had no detectable levels of the targeted antibiotic residues. There were detectable levels of antibiotic residues in 52 % and 25 % of milk samples from Githurai and Juja, respectively. Twenty-six percent of the positive samples from Githurai were above the MRLs while none of the positive samples from Juja violated the MRLs for the detected antibiotics. The overall prevalence of antibiotics residues in Githurai was significantly higher than Juja (2-proportion Z-Test, $\alpha = 0.05$, p-value = 0.0202). This could be explained by the high demand for milk in Githurai occasioned by the high population density compared to Juja. According to data from the KNBS, Githurai has a population density of 7906 people per square kilometer while Juja has a population density of 880 people per square kilometer (KNBS, 2019). Due to the high demand, farmers likely sell milk without waiting for the recommended withdrawal period after treatment of lactating cows with antibiotics.

There were detectable levels of all the antibiotic residues targeted in this study. Amoxicillin and tetracycline were detected at concentrations above the MRLs in 2 % of the samples, respectively while cloxacillin in 8% of the samples. All the samples with detectable levels of sulfamethoxazole and trimethoprim were below the MRLs. There findings are comparable to similar studies conducted in Kibera, which reported a prevalence of 7% and 3% for beta-lactams and tetracyclines, respectively (Brown *et al.*, 2020). A summary of the concentrations of the detected antibiotics are presented in Table 4.6. Sample chromatograms of samples with detectable levels of some antibiotics are given in Figure 4.11.

Table 4.6: Summary of the detected concentrations (µg/L) for individual antibiotic residues

Drug	Detection frequency % (n =65)	[a] Min conc.	[a]Max conc.	% above MRL	MRL (µg/L)
amoxicillin	2	6.7 ± 0.39	6.7 ± 0.39	2	[b]4.0
cloxacillin	17	0.45 ± 0.04	200 ± 17	8	[c]30.0
tetracycline	18	1.1 ± 0.16	134 ± 15	2	[b]100.0
sulfamethoxazole	14	1.4 ± 0.25	12 ± 0.11	-	[b]100
trimethoprim	8	1.3 ± 0.53	14 ± 0.69	-	[c]50

[a]Concentration in µg/L ± standard deviation (n=2). [b]CAC MRLs (Alimentarius, 2017), [c]EU MRLs (EU Commission Regulation, 2010).

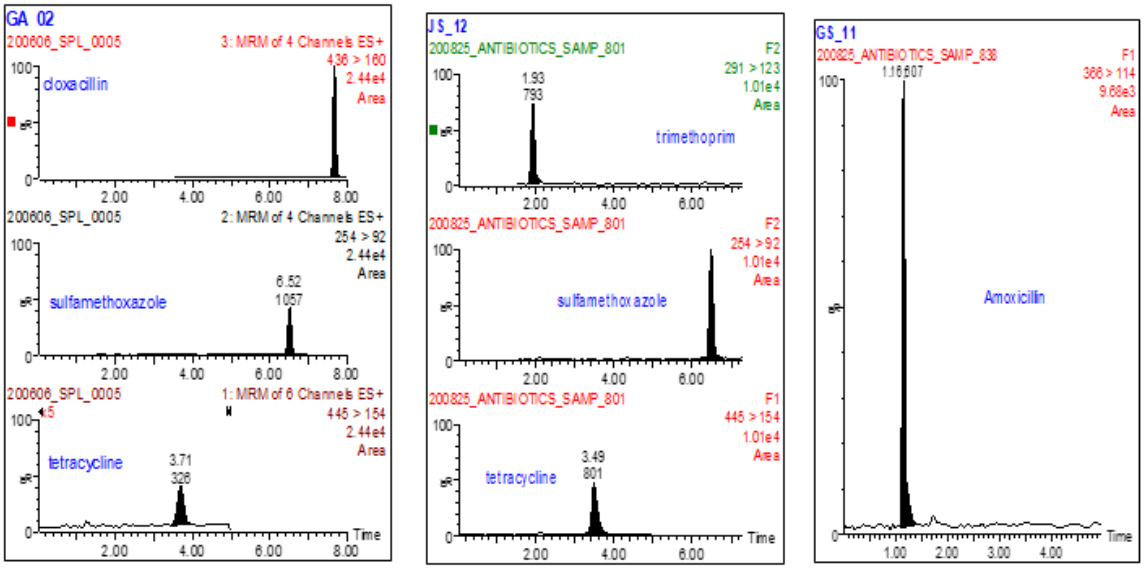


Figure 4.11: Sample chromatograms showing detected antibiotics in some samples. From L –R, Sample GA_02 had cloxacillin (17 µg/L), sulfamethoxazole (1.7 µg/L) and tetracycline (12 µg/L). Sample JS_12 had trimethoprim (2.0 µg/L), sulfamethoxazole (2.0 µg/L) and tetracycline (25 µg/L). Sample GS_11 had amoxicillin (6.7 µg/L)

4.4.1 Antibiotic residues in shop milk

There were detectable levels of all the targeted antibiotics in shop milk samples. The concentrations of the detected antibiotics in shop milk samples are given in Table 4.7.

Table 4.7: Concentrations (mean ± SD) of detected antibiotics in shop milk samples

Sample ID	Concentration (µg/L)				
	amoxicillin	**cloxacillin**	**tetracycline**	**sulfametho-xazole**	**trimetho-prim**
GS_01	-	127 ± 9.9	-	4.5 ± 0.32	-
GS_02	-	66 ± 3.2	30 ± 2.4	-	-
GS_03	-	0.99 ± 0.30	-	-	-
GS_05	-	0.45 ± 0.035	134 ± 15	-	-
GS_06	-	-	-	1.4 ± 0.25	-
GS_09	-	-	1.1±0.16	-	-
GS_11	6.7 ± 0.39	-	-	-	-
GS_12	-	67 ± 0.34	-	-	-
GS_13	-	-	-	11.7 ± 0.11	2.4 ± 0.51
GS_15	-	105 ± 2.0	-	-	-
JS_01	-	0.59 ± 0.30	2.7 ± 0.39	-	11 ± 2.8
JS_02	-	1.46 ± 0.23	2.3 ± 0.16	2.2 ± 0.14	14 ± 0.69
JS_12	-	-	25 ± 0.92	2.2 ± 0.55	2.0 ± 0.35
JS_13	-	-	37 ± 5.4	-	-
JS_14	-	-	3.3 ± 1.4	9.5 ± 0.19	-

GS- Githurai shop, JS –Juja shop. The number of samples analysed were 17 and 15 from Juja and Githurai, respectively.

Eight out of the 15 positive samples (53%) had detectable levels of more than one drug, with one sample (JS_02) testing positive for 4 different drugs. This could be linked to indiscriminate use of vetirenerary antibiotics without prescription *(Mbindyo et al.*, 2021). In a study about perceptions and practices among dairy farmers in Central Kenya, Nyokabi *et al.*, 2021 reported that majority of farmers had limited knowledge on the risks of antibiotic residues and do not strictly comply to withdrawal periods.

Comparative prevalence of different antibiotic residues in shop milk samples from Githurai and Juja areas is shown in Figure 4.12.

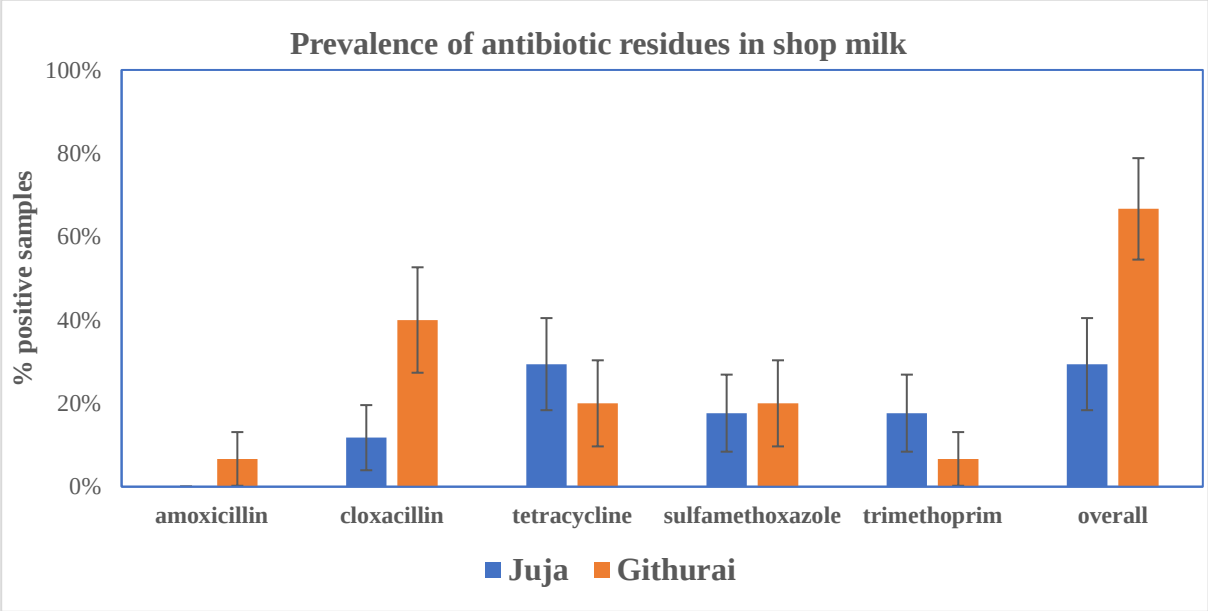


Figure 4.12: Comparative prevalence of antibiotic residues (%) in shop milk. The error bars represent the standard deviations.

Amoxicillin was not detected in any shop milk sample from Juja. Cloxacillin and tetracycline were detected in 12% and 29% of the samples, respectively while sulfamethoxazole and trimethoprim were detected in 18% of the samples. In Githurai, amoxicillin, cloxacillin, tetracycline, sulfamethoxazole and trimethoprim were detected in 7%, 40%, 20%, 20% and 7% of shop milk samples, respectively. The overall prevalence rate for antibiotic residues was 29% (95% CI: 18 - 40%) and 67% (95% CI:

55 – 79%) for shop milk samples from Juja and Githurai, respectively. The proportion of shop milk samples with detectable levels of at least one of the targeted antibiotic residues was significantly higher in Githurai than Juja (2-proportion z-test, 95% CL, p-value = 0.016).

Among the positive sample from Juja, none exceeded the MRL for the respective antibiotics. In Githurai, the percent of samples with antibiotic residues levels exceeding the set MRLs were 27% for cloxacillin and 7% for amoxicillin and tetracycline, respectively. None of the samples in Githurai exceeded the MRLs for sulfamethoxazole and trimethoprim.

4.4.2 Antibiotic residues in AVM milk

All the targeted antibiotics, except amoxicillin, were detected in AVM milk samples. The concentrations of the detected antibiotics in AVM milk samples are presented in Table 4.8.

Table 4.8: Concentrations (mean ± SD) of detected antibiotics in AVM milk samples

	Concentration (µg/L)				
Sample ID	**amoxicillin**	**cloxacillin**	**tetracycline**	**sulfamethoxa-zole**	**trimethop-rim**
GA_01	-	200 ± 17	3.1 ± 1.2	2.1 ± 0.48	-
GA_02	-	17 ± 3.0	12 ± 0.11	1.7 ± 0.48	-
GA_08	-	-	57 ± 5.4	-	-
GA_11	-	0.55 ± 0.07	58 ± 12	-	-
JA_06	-	-	-	9.9 ± 4.9	-
JA_11	-	-	-	-	1.3 ± 0.53

GA- Githurai AVM, JA –Juja AVM. The number of samples analysed were 11 and 12 from Juja and Githurai, respectively.

Three out of the six positive AVM milk samples (50%) had detectable levels of more than one antibiotic residue. Two of the three samples with multiple drug residues also had at least one drug exceeding the MRL. A similar trend was observed among shop milk samples (Table 4.7), indicating possible misuse of livestock antibiotics among dairy farmers. The comparative prevalence of antibiotic residues in AVM milk samples is shown in Figure 4.13.

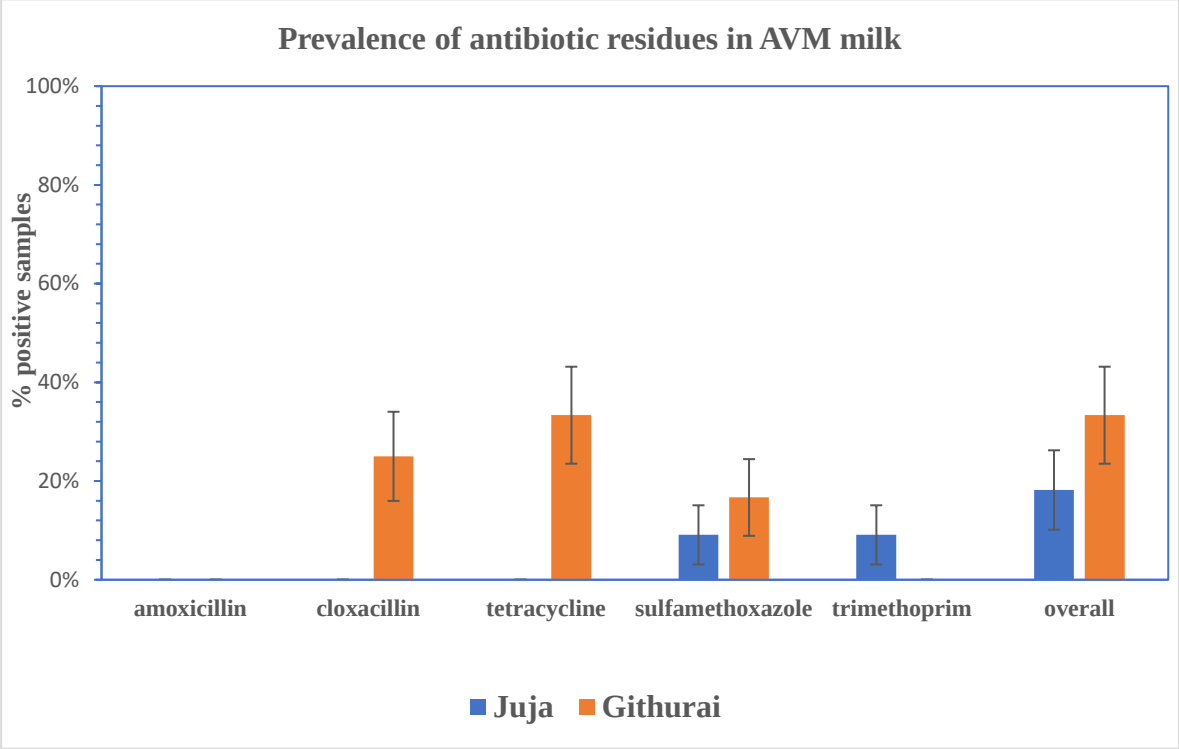


Figure 4.13: Comparative prevalence of antibiotic residues in AVM milk. The error bars represent the standard deviations.

Amoxicillin, cloxacillin and tetracycline were not detected in any AVM milk sample from Juja. Sulfamethoxazole and trimethoprim were detected in 9 % of AVM milk samples from Juja, respectively. In Githurai, cloxacillin, tetracycline and sulfamethoxazole were detected in 25%, 33% and 17% of AVM milk samples, respectively. Amoxicillin and trimethoprim were not detected in any AVM milk sample from Githurai. The overall prevalence rate for antibiotic residues was 18% (95% CI: 8 – 26%) and 33 % (95 % CI: 23 – 43 %) in AVM milk samples from Juja and Githurai, respectively. This difference

was not statistically significant at 95 % confidence level (2-proportion z-test, p-value =0.408).

None of the AVM samples from Juja exceeded the MRL for any of targeted antibiotics. In Githurai, 8% of the samples exceeded the MRL for cloxacillin while none of the samples exceeded the MRLs for amoxicillin, tetracycline, sulfamethoxazole and trimethoprim.

Overall, the prevalence of antibiotic residues in AVM milk (95% CI: 17 -35%) was lower than shop milk samples (95% CI: 38 – 56%) was lower than shop milk samples. This could be due to bulking effects in the AVMs. Milk vending machines available in Kenya have capacities ranging from 200 – 1500 L whereas shops handle small volumes of milk (2-20 L). Majority of AVMs source their milk from milk processors and cooperatives, which bulk milk from different farms (Ingasia *et al.*, 2020). Bulking results in dilution of residues whereby contaminated milk is mixed with non-contaminated milk, lowering the drug concentrations to below detectable levels.

4.4.3 Antibiotic residues in packet milk

All of the packet milk samples analyzed had no detectable levels of antibiotic residues. This is because milk processors partake routine screening of incoming raw milk samples for wholesomeness, bacteriological and chemical quality prior to acceptance at the collection centers (Merwan *et al.*, 2018). The presence of antibiotic residues, even at low concentrations presents serious challenges to the production of fermented dairy products. Antibiotics inhibit the growth of starter culture leading to failure of fermentation and subsequent loss of product (Sachi *et al.*, 2019).

4.4.4 Comparison with similar studies in Kenya

Studies in Kenya have reported mixed results on antibiotic residues in milk. A study of milk from smallholder farms detected antibiotic residues in milk using screening tests but could not confirm any of the residues using HPLC (Ahlberg *et al.*, 2016), whereas in this study, antibiotic residues were confirmed by LC-MS/MS.

In another study, 24% of the milk vending machines within Eldoret tested positive for at least one antibiotic residue (Kosgey *et al.*, 2018). In this study, the prevalence of antibiotic residues in AVM samples was 26 % (95% CI: 17 -35%). A study of milk from peri-urban areas in Nakuru County reported sulfonamides levels above the MRLs but did not detect any tetracycline residues in milk samples using HPLC for confirmatory analysis (Orwa *et al.*, 2017). However, for this present study, tetracycline, amoxicillin and cloxacillin were detected at levels above the MRL in some samples while sulfamethoxazole and trimethoprim did not exceed the MRLs. The findings of this study were comparable to a similar study in Kibera, which reported the prevalence of tetracyclines and beta-lactams at 7 % and 3 %, respectively (Brown *et al.*, 2020). This study employed LC-MS/MS, which is a more sensitive analytical method, capable of detecting much lower levels of antibiotic residues compared to qualitative screening methods.

4.5 Pesticide residues in milk

The KEBS standards stipulate that cow milk should comply with the MRLs for pesticide residues in milk set by CAC (KEBS, 2019). The MRLs in milk are set at 10 µg/kg for amitraz, 20 µg/kg for chlorpyrifos, 50 µg/kg for carbaryl, 50 µg/kg for deltamethrin and 50 µg/kg for cypermethrin.

A total of 65 milk samples were analyzed for pesticide residues. The samples comprised of 28 samples from Juja (17 shop + 11 AVM), 27 samples from Githurai (15 shop + 12 AVM) and 10 packet milk samples. The concentrations of the detected pesticides are

presented in table 4.9 and sample chromatograms from positive samples are given in Figures 4.14 and 4.15.

Table 4.9: Concentrations (mean ± SD) of detected pesticides milk samples

Sample ID	Concentration (µg/L)				
	amitraz	**carbaryl**	**chlorpyrifos**	**cypermethrin**	**deltamethrin**
GA_03	-	-	2.3 ± 0.10	-	-
GS_03	-	-	-	-	13 ± 1.3
GS_05	8.2 ± 0.42	-	-	-	-
JA_01	-	-	-	17 ± 3.0	-
JS_10	-	-	-	-	30 ± 3.5
JS_12	-	-	28 ± 1.2	-	-
JS_13	-	19 ± 6.6	-	-	-
JS_14	-	-	-	17 ± 1.3	-
JS_17	-	-	-	17 ± 4.2	-

GA – Githurai AVM, GS – Githurai shop, JA – Juja AVM, JS – Juja shop.

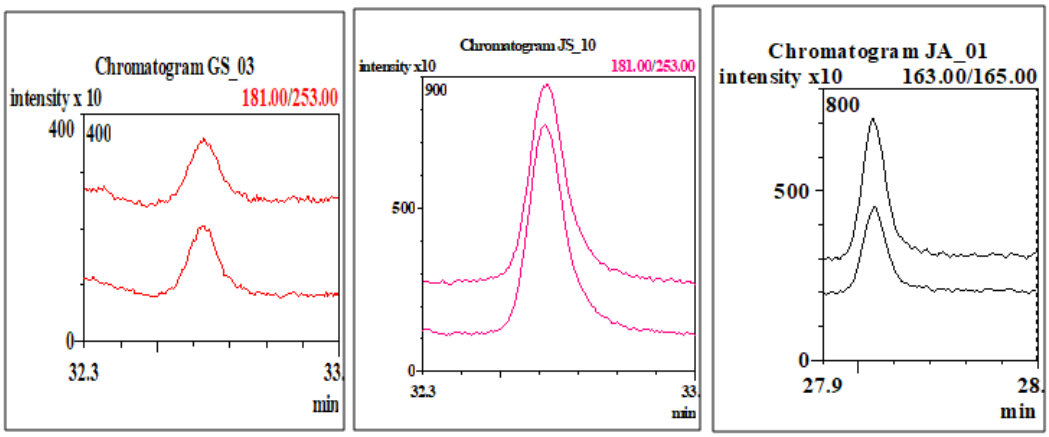


Figure 4.14: Sample SIM chromatograms showing deltamethrin and cypermethrin in milk samples. Samples GS_03 and JS_10 had deltamethrin at concentration 13 and 30 µg/L, respectively. Sample JA_01 had cypermethrin at concentration 17 µg/L

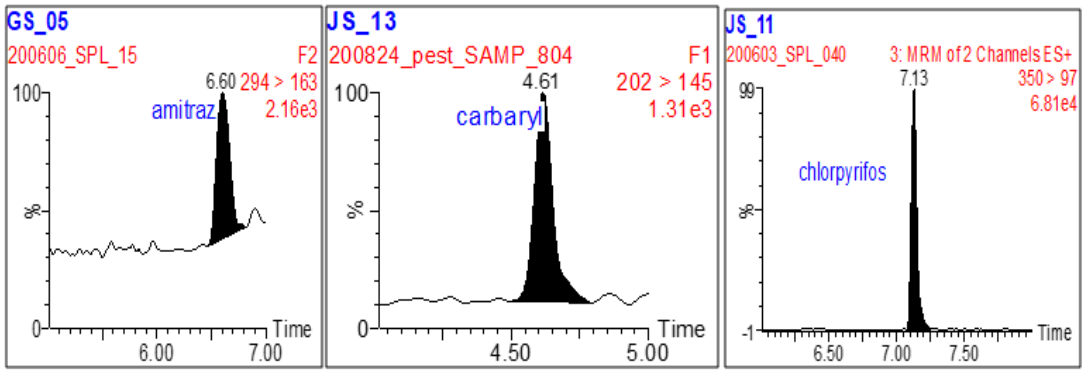


Figure 4.15: Sample MRM chromatograms showing amitraz, carbaryl and chlorpyrifos in some milk samples. Sample GS_05 had amitraz (8 µg/L), sample JS_13 had carbaryl 28 µg/L and sample JS_11 had chlorpyrifos 30 µg/L.

There were detectable levels of at least one of the targeted pesticides in 14 % of the analysed milk samples (9 samples). The concentrations of all detected pesticides were below the MRLs. All milk samples analysed fulfilled KEBS requirement for pesticide residues, hence the concentration levels of pesticide residues in milk samples should not cause any alarm.

The results for this study are similar to other studies conducted on milk samples from other parts of the world. In a study carried out in Punjab, India, chlorpyrifos and cypermethrin were detected in 10 % and 9 % of milk samples, respectively and the mean residue concentrations were all lower than the MRLs (Bedi *et al.,* 2018). In another study, organophosphate residues were detected in 7 % of milk samples at concentrations ranging between 5-18 mg/kg. Chlorpyrifos was reported as one of the main contaminants but the levels of contamination were lower than the MRL's for all the analysed organophosphates (Gill *et al.,* 2020). In a study in Spain, carbaryl was detected in 8 % of milk samples while a similar study in brazil did not detect any carbamates in milk (Bedi *et al.,* 2018).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Adulteration of milk with water

The results of this study indicate widespread adulteration of milk with water as only 35 % of the samples were within the acceptable density range of 1.028 -1.036 g/mL All the non-compliant samples had densities below 1.028 g/mL Addition of water to milk lowers its density thus, the low densities observed in non-compliant samples point towards widespread dilution of milk with water. The percentage of samples adulterated with water was 53% for raw milk sold in shops, 78% for AVM and 70% for packet milk samples. It was noted all the non-compliant packet milk samples and many of the AVM milk samples would be acceptable based on the old standards, when acceptable density range was 1.026 – 1.032 g/mL. It is possible that milk processors are still relying on the old standards, which should not be the case.

5.1.2 Adulteration of milk with hydrogen peroxide

Hydrogen peroxide was detected in 5 % of the milk samples overall. Hydrogen peroxide was not detected in any of the raw shop milk samples analyzed. The percentage of samples adulterated with hydrogen peroxide was 4% for AVM and 20% for packet milk samples, respectively. The concentrations of hydrogen peroxide in the positive samples ranged from 0.5 mg/L to 250 mg/L. The presence of hydrogen peroxide at low concentrations in AVM and packet milk could be due to residual contamination from the milk processing facilities for which hydrogen peroxide is used as a disinfectant. It is also possible that hydrogen peroxide was intentionally added to milk samples, to prolong its shelf-life contrary to the Kenyan Law regulations which prohibit addition of hydrogen peroxide and other preservatives in milk.

5.1.3 Contamination of milk with antibiotic residues

Antibiotic residues were detected in some milk samples. 11 % of the samples had antibiotic residues at levels above the permissible maximum residue limits while 20 % of the samples had detectable levels of antibiotics but did not exceed the maximum residue levels.

Antibiotic residues were detected in 29% of raw milk sold in shops and 18% of AVM milk samples. 53% of the positive AVM milk and 50% of the positive shop milk samples had detectable levels of multiple drug residues, pointing towards indiscriminate use of antibiotic medications without veterinary prescription.

Antibiotic residues were not detected in any packet milk sample. This is because milk processors undertake routine screening of incoming milk samples at the collection centers. Based on these findings, packet milk is safe from antibiotic contamination.

5.1.4 Contamination of milk with pesticide residues

Pesticide residues were detected in 14% of the milk samples. None of the positive samples violated the permissible maximum residue limits for the detected pesticides. All the milk samples fulfilled the KEBS requirements for pesticide residues indicating that the milk was safe from pesticide contamination.

5.2 Recommendations

1. There is need for increased monitoring of milk marketed across the country and stringent enforcement of existing regulations to curb adulteration of milk.
2. Farmers should be educated on the correct usage of antibiotic drugs in dairy farming and the importance of observing the recommended withdrawal periods following treatment of animals.
3. Milk vendors should be trained on milk safety screening to ensure they only receive and sell milk that meets the required safety standards
4. Further research should be carried out in other parts of the country and involving monitoring of more adulterants to get a more comprehensive overview on the status of milk quality in the country to inform future regulatory policy framework.
5. Further research should be carried out to investigate the presence of antibiotic resistance genes in milk and how these relate to drug resistant infections in the country.

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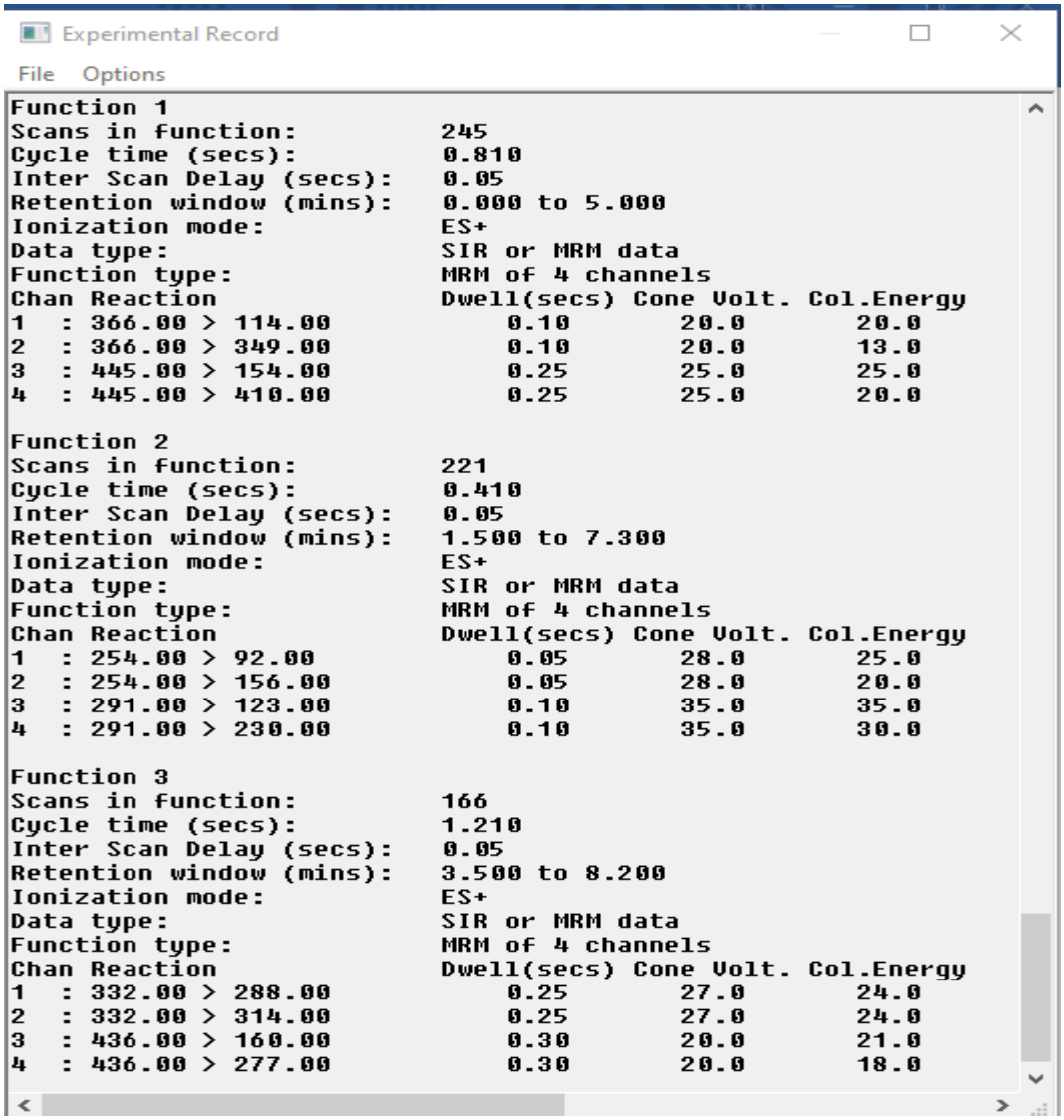
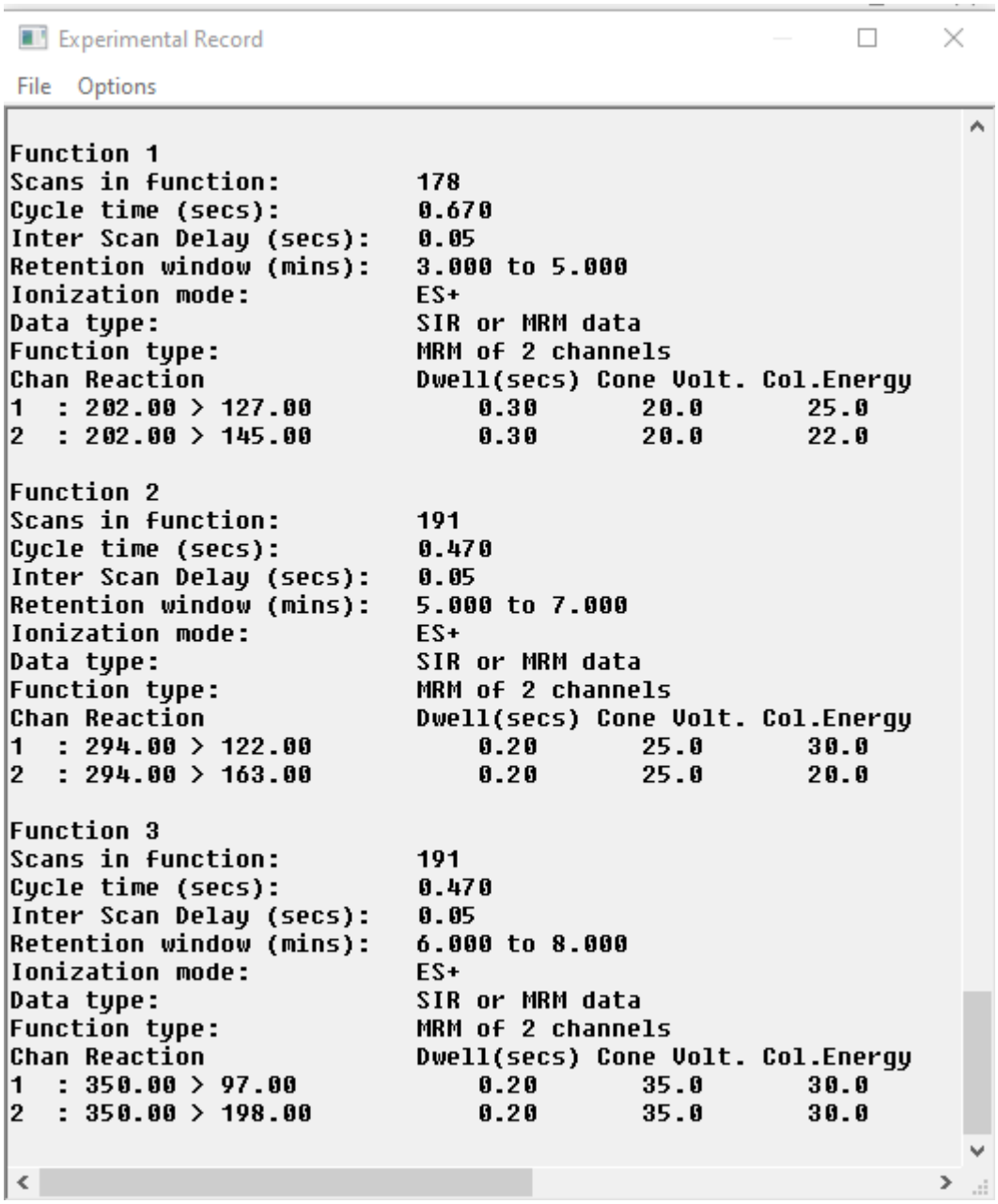
APPENDICES

APPENDIX I: MRM ACQUISITION FUNCTIONS

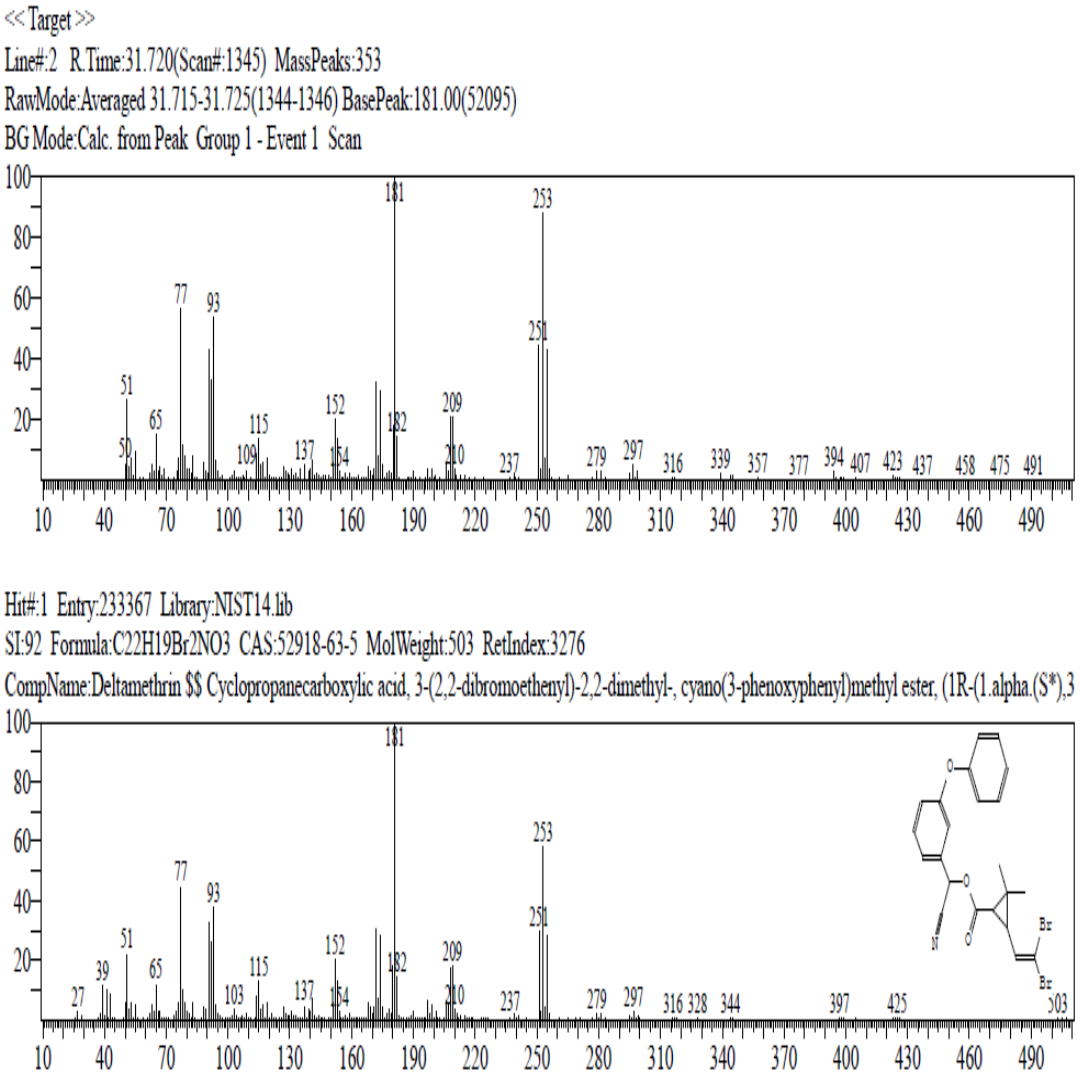


MRM acquisition functions used for LC-MS/MS analysis of antibiotic residues in milk. Amoxicillin (366) and tetracycline (445) were monitored in function 1, sulfamethoxazole (254) and trimethoprim (291) in function 2, and cloxacillin (436) in function 4.



MRM acquisition functions used for LC-MS/MS analysis of pesticide residues in milk. Carbaryl was monitored in function 1, amitraz (291) in function 2 and chlorpyrifos in function 3.

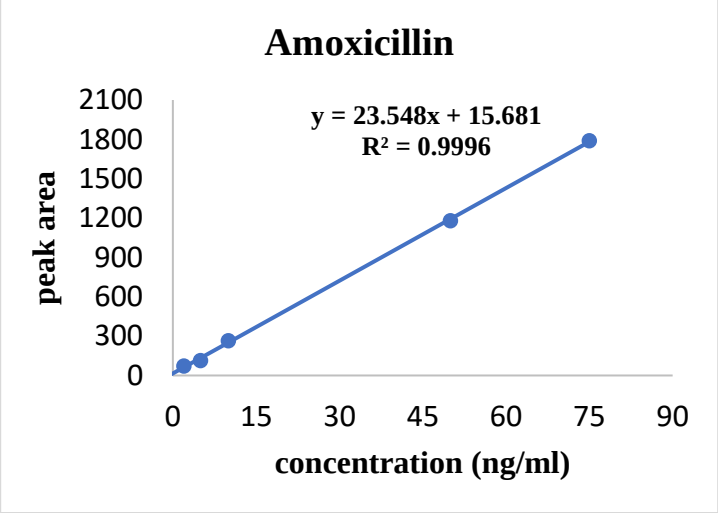
APPENDIX II: Mass spectra

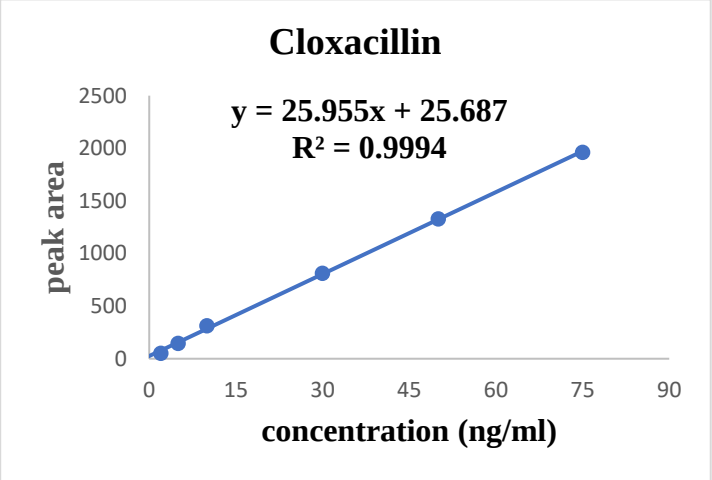


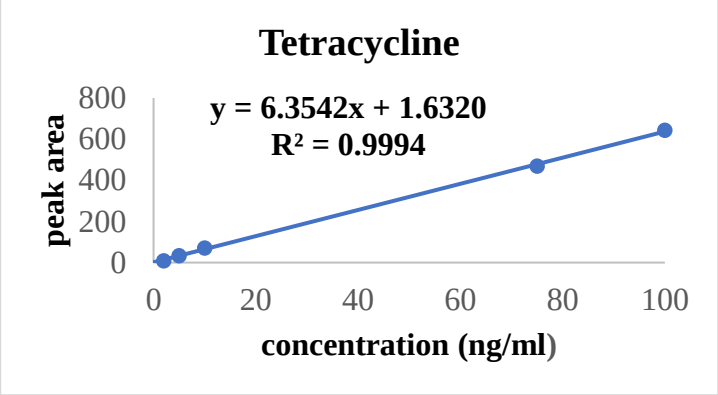
Mass spectrum of deltamethrin. The upper spectrum (Target) is the spectrum from the analyzed sample while the lower spectrum (Hit #1) is the library match identifying the target compound as deltamethrin.

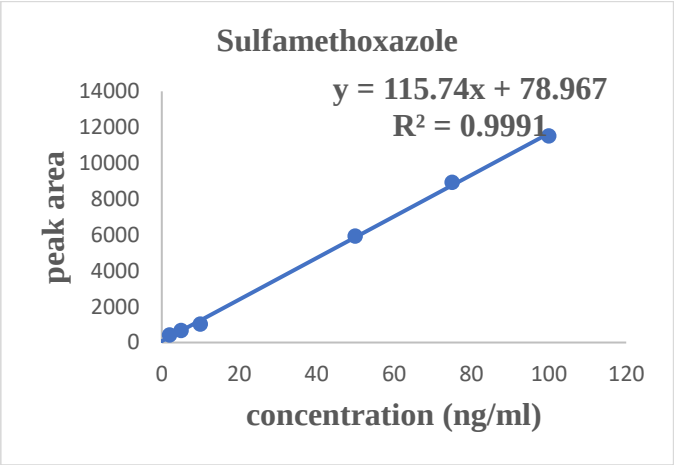
APPENDIX III: Calibration curves of:

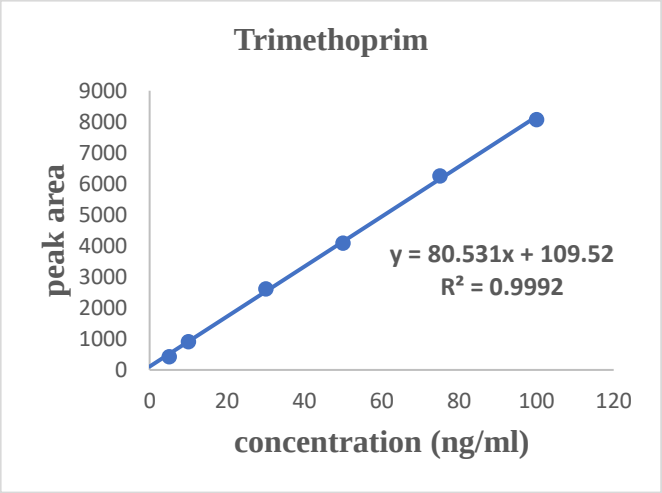
a) antibiotic residues



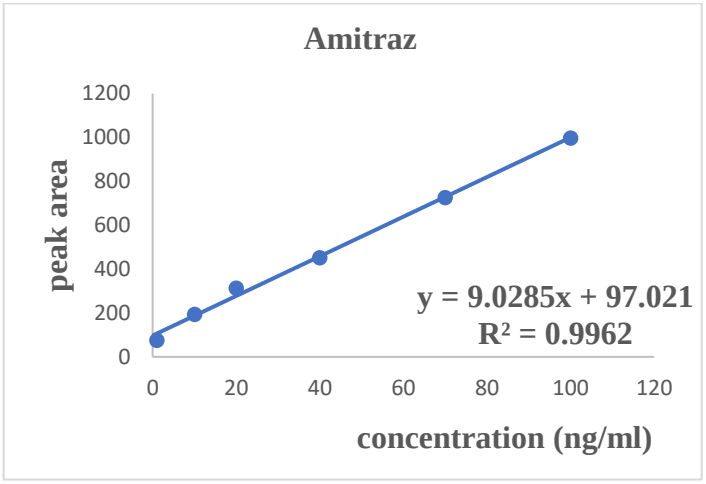


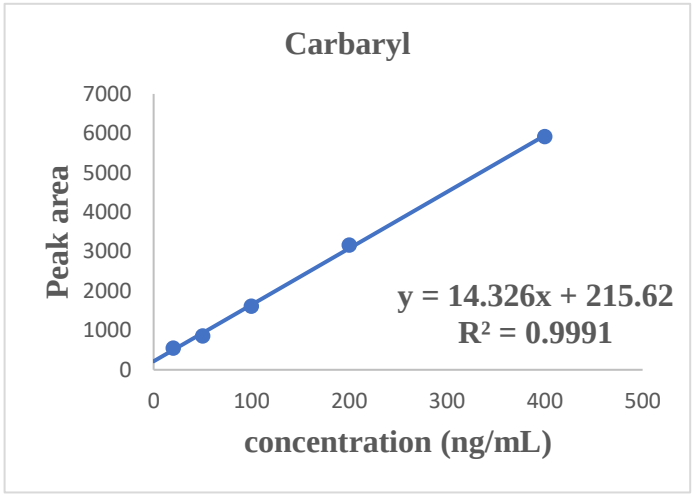


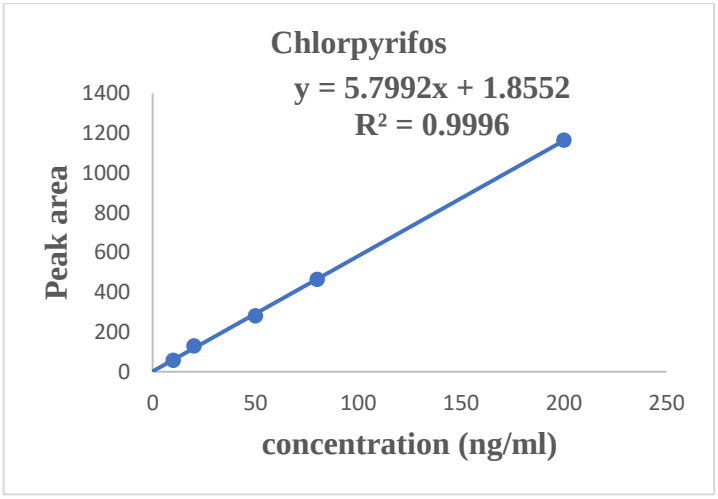

Sulfamethoxazole
y = 115.74x + 78.967
R² = 0.9991
peak area
14000
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120
concentration (ng/ml)

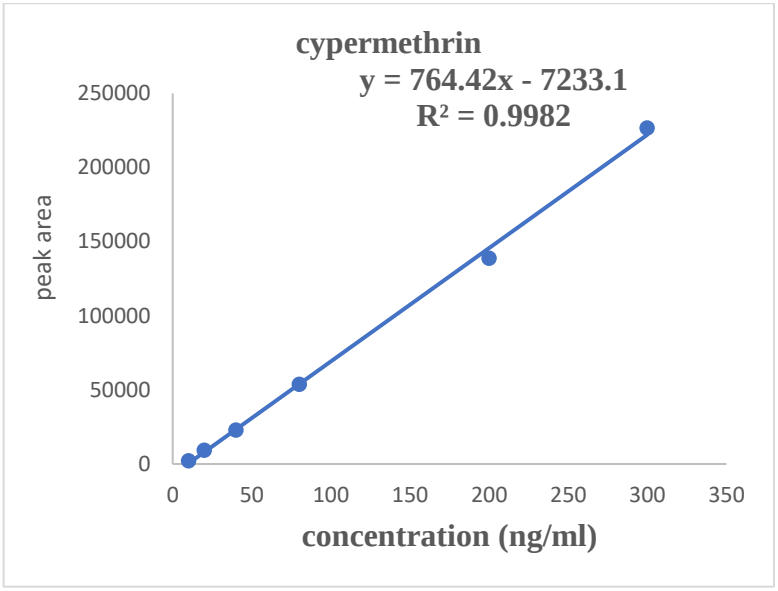

Trimethoprim
peak area
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
y = 80.531x + 109.52
R² = 0.9992
0
20
40
60
80
100
120
concentration (ng/ml)

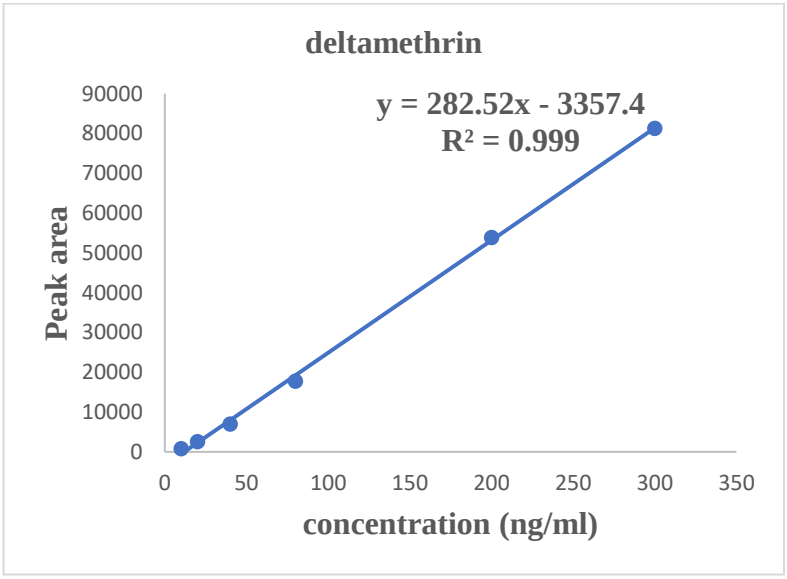
b) Pesticide residues








cypermethrin
y = 764.42x - 7233.1
R² = 0.9982
peak area
250000
200000
150000
100000
50000
0
0
50
100
150
200
250
300
350
concentration (ng/ml)


deltamethrin
y = 282.52x - 3357.4
R² = 0.999
Peak area
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
50
100
150
200
250
300
350
concentration (ng/ml)

APPENDIX IV: Density and hydrogen peroxide data

S/N	Sample Code	Location	Type	density (g/mL)	H_2O_2 (mg/L)
1	GA_01	Githurai	AVM	1.026	0.5
2	GA_02	Githurai	AVM	1.026	ND
3	GA_03	Githurai	AVM	1.027	ND
4	GA_04	Githurai	AVM	1.026	ND
5	GA_05	Githurai	AVM	1.027	ND
6	GA_06	Githurai	AVM	1.027	ND
7	GA_07	Githurai	AVM	1.026	ND
8	GA_08	Githurai	AVM	1.025	ND
9	GA_09	Githurai	AVM	1.027	ND
10	GA_10	Githurai	AVM	1.026	ND
11	GA_11	Githurai	AVM	1.025	ND
12	GA_12	Githurai	AVM	1.022	ND
13	GS_01	Githurai	Shop	1.019	ND
14	GS_02	Githurai	Shop	1.028	ND
15	GS_03	Githurai	Shop	1.028	ND
16	GS_04	Githurai	Shop	1.026	ND
17	GS_05	Githurai	Shop	1.029	ND
18	GS_06	Githurai	Shop	1.028	ND
19	GS_07	Githurai	Shop	1.026	ND
20	GS_08	Githurai	Shop	1.028	ND
21	GS_09	Githurai	Shop	1.022	ND
22	GS_10	Githurai	Shop	1.027	ND
23	GS_11	Githurai	Shop	1.023	ND
24	GS_12	Githurai	Shop	1.025	ND
25	GS_13	Githurai	Shop	1.026	ND
26	GS_14	Githurai	Shop	1.024	ND

27	GS_15	Githurai	Shop	1.026	ND
28	GP_01	Githurai	Packet	1.027	ND
29	GP_02	Githurai	Packet	1.026	ND
30	GP_03	Githurai	Packet	1.026	250
31	GP_04	Githurai	Packet	1.027	ND
32	GP_05	Githurai	Packet	1.029	ND
33	JA_01	Juja	AVM	1.029	ND
34	JA_02	Juja	AVM	1.024	ND
35	JA_03	Juja	AVM	1.023	ND
36	JA_04	Juja	AVM	1.023	ND
37	JA_05	Juja	AVM	1.028	ND
38	JA_06	Juja	AVM	1.025	ND
39	JA_07	Juja	AVM	1.028	ND
40	JA_08	Juja	AVM	1.026	ND
41	JA_09	Juja	AVM	1.026	ND
42	JA_10	Juja	AVM	1.028	ND
43	JA_11	Juja	AVM	1.029	ND
44	JS_01	Juja	Shop	1.035	ND
45	JS_02	Juja	Shop	1.035	ND
46	JS_03	Juja	Shop	1.026	ND
47	JS_04	Juja	Shop	1.027	ND
48	JS_05	Juja	Shop	1.031	ND
49	JS_06	Juja	Shop	1.030	ND
50	JS_07	Juja	Shop	1.017	ND
51	JS_08	Juja	Shop	1.032	ND
52	JS_09	Juja	Shop	1.027	ND
53	JS_10	Juja	Shop	1.034	ND
54	JS_11	Juja	Shop	1.030	ND

55	JS_12	Juja	Shop	1.030	ND
56	JS_13	Juja	Shop	1.025	ND
57	JS_14	Juja	Shop	1.033	ND
58	JS_15	Juja	Shop	1.025	ND
59	JS_16	Juja	Shop	1.029	ND
60	JS_17	Juja	Shop	1.026	ND
61	JP_01	Juja	Packet	1.029	ND
62	JP_02	Juja	Packet	1.028	ND
63	JP_03	Juja	Packet	1.026	ND
64	JP_04	Juja	Packet	1.027	25
65	JP_05	Juja	Packet	1.027	ND

ND – not detected.

APPENDIX V: One-Way ANOVA summary for mean milk densities

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
shop milk	32	32.877	1.027406	1.68E-05
AVM milk	23	23.599	1.026043	3.41E-06
packet milk	10	10.272	1.0272	1.29E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.59709E-05	2	1.3E-05	1.32794	0.272454	3.145258
Within Groups	0.000606275	62	9.78E-06			
Total	0.000632246	64				

APPENDIX VI: Antibiotic residues in milk samples

S/N	Sample Code	Location	Type	amoxicillin (µg/L)	cloxacillin (µg/L)	tetracycline (µg/L)	sulfamethoxazole (µg/L)	trimethoprim (µg/L)
1	GA_01	Githurai	AVM	ND	200.5	3.1	2.1	ND
2	GA_02	Githurai	AVM	ND	16.7	12.2	1.7	ND
3	GA_03	Githurai	AVM	ND	ND	ND	ND	ND
4	GA_04	Githurai	AVM	ND	ND	ND	ND	ND
5	GA_05	Githurai	AVM	ND	ND	ND	ND	ND
6	GA_06	Githurai	AVM	ND	ND	ND	ND	ND
7	GA_07	Githurai	AVM	ND	ND	ND	ND	ND
8	GA_08	Githurai	AVM	ND	ND	56.7	ND	ND
9	GA_09	Githurai	AVM	ND	ND	ND	ND	ND
10	GA_10	Githurai	AVM	ND	ND	ND	ND	ND
11	GA_11	Githurai	AVM	ND	0.6	57.6	ND	ND
12	GA_12	Githurai	AVM	ND	ND	ND	ND	ND
13	GS_01	Githurai	Shop	ND	127.2	ND	4.5	ND
14	GS_02	Githurai	Shop	ND	65.7	30.2	ND	ND
15	GS_03	Githurai	Shop	ND	1.0	ND	ND	ND
16	GS_04	Githurai	Shop	ND	ND	ND	ND	ND
17	GS_05	Githurai	Shop	ND	0.5	133.7	ND	ND

18	GS_06	Githurai	Shop	ND	ND	ND	1.4	ND
19	GS_07	Githurai	Shop	ND	ND	ND	ND	ND
20	GS_08	Githurai	Shop	ND	ND	ND	ND	ND
21	GS_09	Githurai	Shop	ND	ND	1.1	ND	ND
22	GS_10	Githurai	Shop	ND	ND	ND	ND	ND
23	GS_11	Githurai	Shop	6.7	ND	ND	ND	ND
24	GS_12	Githurai	Shop	ND	66.8	ND	ND	ND
25	GS_13	Githurai	Shop	ND	ND	ND	11.7	2.4
26	GS_14	Githurai	Shop	ND	ND	ND	ND	ND
27	GS_15	Githurai	Shop	ND	105.3	ND	ND	ND
28	GP_01	Githurai	Packet	ND	ND	ND	ND	ND
29	GP_02	Githurai	Packet	ND	ND	ND	ND	ND
30	GP_03	Githurai	Packet	ND	ND	ND	ND	ND
31	GP_04	Githurai	Packet	ND	ND	ND	ND	ND
32	GP_05	Githurai	Packet	ND	ND	ND	ND	ND
33	JA_01	Juja	AVM	ND	ND	ND	ND	ND
34	JA_02	Juja	AVM	ND	ND	ND	ND	ND
35	JA_03	Juja	AVM	ND	ND	ND	ND	ND
36	JA_04	Juja	AVM	ND	ND	ND	ND	ND
37	JA_05	Juja	AVM	ND	ND	ND	ND	ND

38	JA_06	Juja	AVM	ND	ND	ND	9.9	ND
39	JA_07	Juja	AVM	ND	ND	ND	ND	ND
40	JA_08	Juja	AVM	ND	ND	ND	ND	ND
41	JA_09	Juja	AVM	ND	ND	ND	ND	ND
42	JA_10	Juja	AVM	ND	ND	ND	ND	ND
43	JA_11	Juja	AVM	ND	ND	ND	ND	1.3
44	JS_01	Juja	Shop	ND	0.6	2.7	ND	10.8
45	JS_02	Juja	Shop	ND	1.5	2.3	2.2	14.3
46	JS_03	Juja	Shop	ND	ND	ND	ND	ND
47	JS_04	Juja	Shop	ND	ND	ND	ND	ND
48	JS_05	Juja	Shop	ND	ND	ND	ND	ND
49	JS_06	Juja	Shop	ND	ND	ND	ND	ND
50	JS_07	Juja	Shop	ND	ND	ND	ND	ND
51	JS_08	Juja	Shop	ND	ND	ND	ND	ND
52	JS_09	Juja	Shop	ND	ND	ND	ND	ND
53	JS_10	Juja	Shop	ND	ND	ND	ND	ND
54	JS_11	Juja	Shop	ND	ND	ND	ND	ND
55	JS_12	Juja	Shop	ND	ND	25.1	2.1	2.0
56	JS_13	Juja	Shop	ND	ND	37.4	ND	ND
57	JS_14	Juja	Shop	ND	ND	3.3	9.5	ND

58	JS_15	Juja	Shop	ND	ND	ND	ND	ND
59	JS_16	Juja	Shop	ND	ND	ND	ND	ND
60	JS_17	Juja	Shop	ND	ND	ND	ND	ND
61	JP_01	Juja	Packet	ND	ND	ND	ND	ND
62	JP_02	Juja	Packet	ND	ND	ND	ND	ND
63	JP_03	Juja	Packet	ND	ND	ND	ND	ND
64	JP_04	Juja	Packet	ND	ND	ND	ND	ND
65	JP_05	Juja	Packet	ND	ND	ND	ND	ND

ND – not detected.

APPENDIX VII: Pesticide residues in milk

S/N	Sample Code	Location	Type	amitraz (µg/L)	carbaryl (µg/L)	chlorpyrifos (µg/L)	cypermethrin (µg/L)	deltamethrin (µg/L)
1	GA_01	Githurai	AVM	ND	ND	ND	ND	ND
2	GA_02	Githurai	AVM	ND	ND	ND	ND	ND
3	GA_03	Githurai	AVM	ND	ND	2.3	ND	ND
4	GA_04	Githurai	AVM	ND	ND	ND	ND	ND
5	GA_05	Githurai	AVM	ND	ND	ND	ND	ND
6	GA_06	Githurai	AVM	ND	ND	ND	ND	ND
7	GA_07	Githurai	AVM	ND	ND	ND	ND	ND
8	GA_08	Githurai	AVM	ND	ND	ND	ND	ND
9	GA_09	Githurai	AVM	ND	ND	ND	ND	ND
10	GA_10	Githurai	AVM	ND	ND	ND	ND	ND
11	GA_11	Githurai	AVM	ND	ND	ND	ND	ND
12	GA_12	Githurai	AVM	ND	ND	ND	ND	ND
13	GS_01	Githurai	Shop	ND	ND	ND	ND	ND
14	GS_02	Githurai	Shop	ND	ND	ND	ND	ND
15	GS_03	Githurai	Shop	ND	ND	ND	ND	ND
16	GS_04	Githurai	Shop	ND	ND	ND	13.1	ND
17	GS_05	Githurai	Shop	8.2	ND	ND	ND	ND

18	GS_06	Githurai	Shop	ND	ND	ND	ND	ND
19	GS_07	Githurai	Shop	ND	ND	ND	ND	ND
20	GS_08	Githurai	Shop	ND	ND	ND	ND	ND
21	GS_09	Githurai	Shop	ND	ND	ND	ND	ND
22	GS_10	Githurai	Shop	ND	ND	ND	ND	ND
23	GS_11	Githurai	Shop	ND	ND	ND	ND	ND
24	GS_12	Githurai	Shop	ND	ND	ND	ND	ND
25	GS_13	Githurai	Shop	ND	ND	ND	ND	ND
26	GS_14	Githurai	Shop	ND	ND	ND	ND	ND
27	GS_15	Githurai	Shop	ND	ND	ND	ND	ND
28	GP_01	Githurai	Packet	ND	ND	ND	ND	ND
29	GP_02	Githurai	Packet	ND	ND	ND	ND	ND
30	GP_03	Githurai	Packet	ND	ND	ND	ND	ND
31	GP_04	Githurai	Packet	ND	ND	ND	ND	ND
32	GP_05	Githurai	Packet	ND	ND	ND	ND	ND
33	JA_01	Juja	AVM	ND	ND	ND	ND	ND
34	JA_02	Juja	AVM	ND	ND	ND	ND	ND
35	JA_03	Juja	AVM	ND	ND	ND	ND	ND
36	JA_04	Juja	AVM	ND	ND	ND	ND	ND
37	JA_05	Juja	AVM	ND	ND	ND	ND	ND

38	JA_06	Juja	AVM	ND	ND	ND	ND	ND
39	JA_07	Juja	AVM	ND	ND	ND	ND	ND
40	JA_08	Juja	AVM	ND	ND	ND	ND	ND
41	JA_09	Juja	AVM	ND	ND	ND	ND	ND
42	JA_10	Juja	AVM	ND	ND	ND	ND	ND
43	JA_11	Juja	AVM	ND	ND	ND	ND	ND
44	JS_01	Juja	Shop	ND	ND	ND	ND	17.3
45	JS_02	Juja	Shop	ND	ND	ND	ND	ND
46	JS_03	Juja	Shop	ND	ND	ND	ND	ND
47	JS_04	Juja	Shop	ND	ND	ND	ND	ND
48	JS_05	Juja	Shop	ND	ND	ND	ND	ND
49	JS_06	Juja	Shop	ND	ND	ND	ND	ND
50	JS_07	Juja	Shop	ND	ND	ND	ND	ND
51	JS_08	Juja	Shop	ND	ND	ND	ND	ND
52	JS_09	Juja	Shop	ND	ND	ND	ND	ND
53	JS_10	Juja	Shop	ND	ND	ND	ND	ND
54	JS_11	Juja	Shop	ND	ND	ND	29.5	ND
55	JS_12	Juja	Shop	ND	ND	28.0	ND	ND
56	JS_13	Juja	Shop	ND	19.3	ND	ND	ND
57	JS_14	Juja	Shop	ND	ND	ND	ND	17.1

58	JS_15	Juja	Shop	ND	ND	ND	ND	ND
59	JS_16	Juja	Shop	ND	ND	ND	ND	ND
60	JS_17	Juja	Shop	ND	ND	ND	ND	ND
61	JP_01	Juja	Packet	ND	ND	ND	ND	ND
62	JP_02	Juja	Packet	ND	ND	ND	ND	ND
63	JP_03	Juja	Packet	ND	ND	ND	ND	ND
64	JP_04	Juja	Packet	ND	ND	ND	ND	ND
65	JP_05	Juja	Packet	ND	ND	ND	ND	ND

ND – not detected.