KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

VETERINARY DRUG RESIDUES IN CHICKEN EGGS FROM SOME SELECTED POULTRY FARMS IN THE KUMASI METROPOLIS, KWABRE EAST AND OFFINSO DISTRICTS OF THE ASHANTI REGION.

BY

ODEI JUDITH

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF

MPHIL DEGREE IN ANALYTICAL CHEMISTRY.

SEPTEMBER, 2014

DECLARATION

I hereby declare that this submission is my own work towards the MPhil Degree in Analytical Chemistry and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Odei Judith, PG 6146411	……..	…….....
Student Name & ID	Signature	Date

Certified by:

Dr. John Kenneth Mensah	……..	…….....
Supervisor's Name	Signature	Date

Certified by:

Dr. Godfred Darko	……..	…….....
Supervisor's Name	Signature	Date

Certified by:

Dr. Godfred Darko	……..	…….....
Head of Department	Signature	Date

ABSTRACT

Multiresidue analyses of veterinary drugs were determined in commercial eggs from the Offinso, Kumasi Metropolis and the Kwabre East District in the Ashanti Region. A total of 200 table egg samples were collected for albendazole, chloramphenicol, levamisole, oxytetracycline, piperazine, sulphamethoxazole, sulphathiazole and tiamulin residues from 5 poultry farms. Whole eggs were homogenized in acetonitrile, extracts evaporated and residues dissolved in mobile phase. The fats were removed using hexane. Extracts were analyzed by solid phase reverse phase high performance liquid chromatography with photodiode array detector. Results showed that Albendazole was the most frequently detected in all the 200 samples taken from the five farms in the three districts which occurred at an average concentration of 116.1± 2.30 µg/kg. Chloramphenicol was also detected at an average concentration of 26.6 ± 0.39 µg/kg. Levamisole was recorded at an average concentration of 14.7 ± 0.26 µg/kg. Oxytetracycline residues registered an average concentration of 19.8 ± 0.47 µg/kg. Piperazine was detected at an average concentration of 40.00 ± 0.80 µg/kg. The two sulphonamides, sulphamethoxazole and sulphathiazole had detectable concentrations of 17.05 ± 0.34 µg/kg and 32.50 ± 0.42 µg/kg respectively. The least detectable residue in all the five farms was tiamulin which had a detectable concentration of 0.95± 0.04 µg/kg. Detectable levels of all the drug residues were below their maximum residual limit given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However the residues may accumulate to higher levels in human beings making them dangerous to human health.

DEDICATION

The Thesis is dedicated to my parents Mr. and Mrs. Odei and all those who stood strong by me when the going was tough.

ACKNOWLEDGEMENT

My greatest thanks go to God Almighty for his ever increasing grace and mercies, His love and protection and how far He has brought me. I am also grateful to my supervisors; Dr John Kenneth Mensah and Dr Godfred Darko for their contribution throughout my studies.

My heartfelt gratitude also goes to Mr and Mrs Apeti-Vikpeh for their unflinching suggestions and advice in the difficult moments.

I would also like to thank Mr Edward Ebo Kwaansa-Ansah (Spiritual) for allowing me to use his laboratory at all times including weekends for the extraction procedure. I am also grateful to Miss Alice Yamoah, Mr Alexander Amerh and Mr Reginald Wotse for their unflinching support towards this thesis. My thanks goes to the staff and members of the Chemistry Department, Kwame Nkrumah University of Science and Technology especially Mr Ofori-Agyei, Mr Elvis Baidoo and Mr Stephen Boandah for their assistance.

Last but not the least, I would like to thank my project group members for the criticisms, I had every Friday at the group meetings organized by Dr Darko which has made this work a success.

Finally, my deepest appreciation and gratitude is reserved for all the poultry farmers; Madam Leticia and Auntie Agnes from Kentinkrono in the Kumasi Metropolis, Asonaba, Mr Banahene of blessed memory from Offinso-South Municipality and Mr. Twum from Mamponteng in the Kwabre-East Districts for assisting me to obtain the samples and some standards.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABZ	Albendazole
PPZ	Piperazine
SDZ	Sulphathiazole
SMX	Sulphamethoxazole
LEV	Levamisole
OTC	Oxytetracycline
CMP	Chloramphenicol
TIA	Tiamulin
CAC	Codex Alimentarius Commission
EU	European Union
ELISA	Enzyme-Linked Immunosorbent Assay
EADI	Estimated Average Daily Intake
FPT	Four-Plate Test
GEADE	Global Estimated Acute Dietary Exposure
HPLC	High-Performance Liquid Chromatography
HPLCPDA	HPLC method equipped with a photodiode array detector
HPLC-UV	high-pressure liquid chromatographic with ultraviolet detection
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-ESI-MS/MS	Liquid chromatography electrospray ionisation-tandem mass spectrometry

LC	Liquid Chromatography
LOQ	Limit of Quantification
LOD	Limit of Detection
LC–MS/MS	Liquid Chromatography–Tandem Mass Spectrometric
MRLs	Maximum Residue Limits
PDA	Photodiode Array
RPHPLCPAD	Reverse Phase High Performance Liquid Chromatography with Photodiode Array Detector
RSD	Relative Standard Deviation
RP	Reverse Phase
SPE	Solid-Phase Extraction
TLC	Thin-Layer Chromatography
UHPLCMSMS	Ultra-High Pressure Liquid Chromatography–Tandem Mass Spectrometry
UPLC–ToF-MS	Ultra-Performance Liquid Chromatography Combined with Time-Of-Flight Mass Spectrometry

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Veterinary drugs are generally used on farm animals for therapeutic and prophylactic purposes and they include a large number of different types of compounds (Reig and Toldra, 2008). In modern agricultural practice, they are used on a large scale and administered as food additives or via the drinking water in order to prevent the outbreak of diseases. In addition, veterinary drugs can be used during dehydration of livestock (Stolker *et al*., 2007). Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives (Pavlov *et al*., 2008). Veterinary drugs are essential in poultry production to prevent and control infectious and non-infectious diseases, assist in reducing stress due to environmental changes, vaccination, debeaking and other management practices. In some instances, the drugs are used to promote growth or egg production (Kabir *et al*., 2004).

Residues of veterinary drugs may be found in edible tissues as well as in animal products such as eggs and milk which may be harmful to human health (Kabir *et al*., 2004). These residues may have direct toxic effects on consumers e.g. allergic reaction in hypersensitive individuals or antibiotics which may cause problems indirectly through induction of resistant strains of bacteria. For these purposes, the European Union (EU) has set maximum residue limits (MRLs) for a variety of veterinary drugs in tissues, milk and eggs (Nielen *et al*., 2008)

Drug residues in eggs may arise when laying hens are mistakenly given medicated feed and it is more likely in instances where the drug is fed continuously over a long period of time (Kabir *et al*., 2004). It may also occur when feed is contaminated at the mill during mixing or when drugs are given off-label or when eggs are harvested within the withdrawal period of

the drugs (Goetting *et al*., 2011). When these veterinary drugs reach the blood stream, they are distributed over the whole body especially the ovary with growing follicles and the oviduct where the egg white is formed and secreted and therefore may increase the incidence of unacceptable residues in eggs (Khattab *et al*., 2010). While a chicken lays an egg roughly every 24 hours, each egg takes several days to develop *in vivo* and some egg components are in existence months before the fully developed and shelled egg containing them is laid. Because of the protracted nature of egg development, many weeks may be required following treatment or exposure before eggs are free of drug residues (Goetting *et al*., 2011). Such residues may be reduced by establishing and adhering to withdrawal periods before slaughter, and by sometimes prohibiting the use of certain antibiotics in laying hens (Khattab *et al*., 2010).

Of the three main egg components (yolk, albumen, and shell), the yolk has the longest development time (Goetting *et al*., 2011). Before an egg is laid, the yolk undergoes a stage of rapid growth, in which it increases in size exponentially over 10 days. Drugs that deposit in the yolk will rapidly accumulate during this time and can be present in successive eggs for 10 or more days following treatment. Following yolk maturation, the albumen or 'egg white' is laid down over a period of 2 to 3 hours and can also serve as a residue accumulation site. The egg shell is added after albumen proteins are deposited and diluted with water (Goetting *et al*., 2011) Many drugs deposit preferentially in the yolk or albumen, depending on the drug's and physicochemical properties. Some characteristics that affect the distribution of residues are the drug's tendency to bind to plasma proteins, hydrophobicity or hydrophilicity, and the ability to move through different tissue types (Goetting *et al*., 2011). Enrofloxacin and sarafloxacin have been approved for treatment of bacterial infections in poultry but are forbidden for use in laying hens because of the possibility that residues of these antibiotics transfer to and accumulate in eggs (Stolker *et al*., 2007).

1.2 CLASSIFICATION OF VETERINARY DRUGS

Veterinary drugs are classified either by their chemical structure or mechanism of action. According to their chemical structure, the most commonly used veterinary drugs in food-producing animals can be grouped into five major classes. These include the β-lactams (e.g. penicillins and cephalosporins), tetracyclines (e.g. oxytetracycline, tetracycline and chlortetracycline), aminoglycosides (e.g. streptomycin, neomycin and gentamicin), macrolides (e.g. erythromycin) and sulfonamides (e.g. sulfamethazine) (RUYCK, 2003).

1.3 STATEMENT OF THE PROBLEM

Although veterinary drugs are of immense benefit in the poultry industry, there are reasonable concerns regarding harmful concentrations of drug residues which may be present in eggs (Donoghue, 2003) .

The ever increasing demand for eggs by the growing fast food industry and the use of eggs as a source of animal protein place a lot of pressure on the few poultry farms. The poultry farmers are therefore compelled to use veterinary drugs indiscriminately to boost production thus failing to observe the recommended withdrawal periods. The abuse or misuse of veterinary drugs is the main source of drug residues in animal products (Idowu et al., 2010a). Hens treated with drugs would produce eggs containing residues for some time even after drug withdrawal. This is because poultry rearing does provide a continuous supply of eggs (Khattab *et al*., 2010)

Much work has not been done on veterinary drug residues in Ghana.

1.4 AIMS AND OBJECTIVES

1.4.1 Main objectives

- To determine residues of selected veterinary drugs in chicken eggs.

1.4.2 Specific objectives

- To determine residues of chloramphenicol, sulphathiazole, sulphamethoxazole, oxytetracycline, tiamulin, levamisole, piperazine and albendazole in chicken eggs in the Kumasi metropolis and its immediate environs.
- To compare the levels of the drug residues to that of the MRL given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

1.5 JUSTIFICATION

In recent years, food safety issues have become a recurring phenomenon (Stolker and Brinkman, 2005). Hens treated with drugs would produce eggs containing residues for some time even after drug withdrawal (Kabir *et al*., 2004). Veterinary drug residues are considered public health hazards. For most veterinary drugs, presence of drugs in blood, faeces or urine at the time of slaughter indicates presence of drugs in edible tissues and in eggs at lower concentrations (Kabir *et al*., 2004).

Skin allergies could occur following consumption of poultry products such as eggs containing high concentrations of sulphonamide residues. The human health problems that could result from the intake of sub-therapeutic levels of tetracycline include gastrointestinal disturbances, poor foetal development and staining of the teeth of young children (Idowu et al., 2010a). Reported side effects of ivermectin include fever, dizziness, headache and hypotension (Mushtaq *et al*., 2010). Chloramphenicol is known to exert several side effects in humans such as allergic reactions, gastrointestinal disorders, dose dependent bone marrow

depression and grey syndrome in newborns. The most serious and potentially lethal effect of chloramphenicol is aplastic anaemia (Mehdizadeh *et al*., 2010). It seems there is no official residue monitoring program and consumer response at the moment to elucidate the dangers posed by residues (Kabir *et al*., 2004).

This project has therefore been necessitated by the fact that drug residues in chicken eggs are of health concern since most Ghanaians use eggs as a major source of protein in their foods and also it would help the regulatory authorities to quarantine the eggs that contain high levels of residues in them.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 CLASSIFICATION OF VETERINARY DRUGS

Veterinary drugs are classified either by their chemical structure or mechanism of action. According to their chemical structure, the most commonly used veterinary drugs in food-producing animals can be grouped into five major classes. These include the β-lactams (e.g. penicillins and cephalosporins), tetracyclines (e.g. oxytetracycline, tetracycline and chlortetracycline), aminoglycosides (e.g. streptomycin, neomycin and gentamicin), macrolides (e.g. erythromycin) and sulfonamides (e.g. sulfamethazine) (Ruyck, 2003).

2.1.1 Albendazole residue in eggs

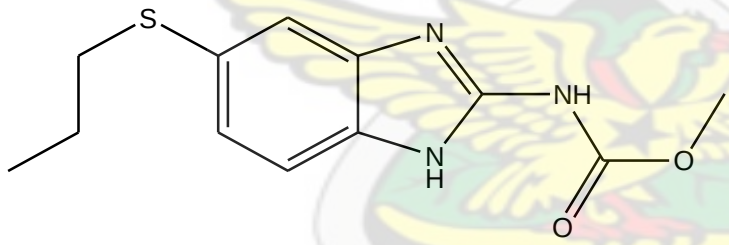


Fig 2.1: Chemical structure of Albendazole (5-(propylthio)-1H-benzimidazol-2-yl)

Few anthelmintic drugs are commercially available for use in avian production systems due to an increasing prevalence of roundworm parasites in poultry, particularly in litter-based housing systems. Bistoletti *et al*., (2012) reported the anthelmintic efficacy of albendazole in poultry. The goal of this work was to characterize the albendazole and metabolites plasma disposition kinetics after treatment with different administration routes in laying hens. Twenty-four laying hens, Plymouth Rock Barrada, were distributed into three groups and treated with Albendazole (ABZ) as follows: intravenously, orally and in medicated feed all at

10 mg⁄kg everyday for 7 days. Blood samples were taken up to 48 hour post treatment and up to10 days post start feed medication. The collected plasma samples were analyzed using high-performance liquid chromatography (HPLC). Albendazole and its metabolites were recovered in plasma after albendazole administration. Albendazole parent compound showed an initial concentration of 16.4 ± 2.0 l g⁄mL being rapidly metabolized into the metabolites. The work reported provided useful information on the pharmacokinetic behavior of Albendazole after oral administrations in hens which is a useful first step to evaluate its potential as an antihelmintic tool for use in poultry.

2.1.2 Chloramphenicol residue in eggs

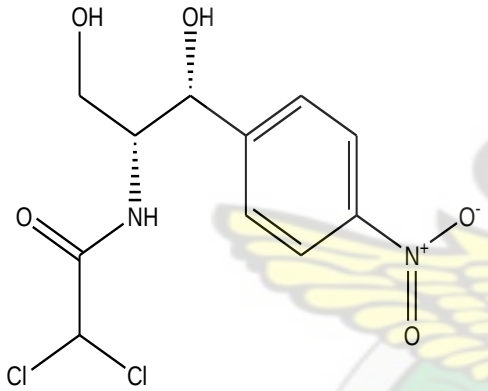


Fig 2.2: Chemical structure of chloramphenicol (2, 2-dichloro-N-[1, 3-dihydroxy-1-(4-nitrophenyl) propan-2-yl-acetamide)

Omeiza *et al*., (2012) used a special thin-layer chromatography to assess the occurrence of chloramphenicol residues in commercial eggs. The egg yolk was separated from the egg white for general antibiotic screening. Acetonitrile was used to extract chloramphenicol from the egg yolk followed by the addition of n-hexane to chloramphenicol, distilled water followed by centrifugation after which ethyl acetate was added to the lower aqueous/residue and dried. The dried extract was resuspended in ethanol after which the resulting mixture was

spotted on a thin-layer chromatography (TLC) pre-coated plates and was run in a solvent system containing 90 parts of chloroform, 9 parts of methanol and 1.5 ammonium hydroxide. The dry plate was sprayed with 1.0 % tin (ii) chloride solution followed by 1.0% p-dimethyl aminobenzaldehyde solution for identification of yellow spots to confirm the presence of chloramphenicol.

Liquid chromatography–electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) method was used for determining chloramphenicol residues in fish, shrimp, poultry, eggs, bovine and swine Silvia *et al*.,(2009). The samples were extracted with a phosphate extraction solution followed by liquid–liquid extraction with ethyl acetate. The liquid chromatography (LC) was performed on a C_{18} column at room temperature. The accuracy values laid between 85 and 120% and the precision was lower than 20%. The limit of quantification (LOQ) was 0.1 ng/g. The method was employed to analyze samples collected in Brazil by Silvia *et al*., (2009).

2.1.3 Levamisole residue in eggs

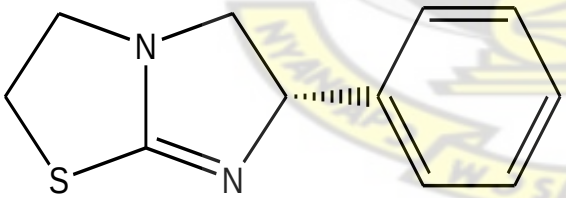


Fig 2.3: Chemical structure of Levamisole ((S)-6-Phenyl-2, 3, 5, 6-tetrahydroimidazo [2, 1-[1, 3] thiazole)

The pharmacokinetics of levamisole in 20 broiler breeder chicken (chicken that give eggs to breed broilers) has been studied by El-Kholy *et al*., (2006). A single dose of levamisole (40 mg/kg) was administered orally or intravenously to chicken before the onset of egg

production, prelay and repeated at the peak of egg production (A high-pressure liquid chromatographic with ultraviolet detection method (HPLC-UV) was used for quantification of levamisole in plasma. Using compartmental analysis, levamisole followed a three-compartmental open model with mean values of 0.4968 mg/kg and 0.01813 mg/kg at the prelay and at the peak of egg production periods, respectively. The mean values for volume of distribution at steady state determined by compartmental analysis were significantly different for prelay and peak of egg production (8.358 and 13.581 mg/kg, respectively).

Levamisole residues in chicken tissues, eggs and plasma were determined by (HPLC-UV) detection at 225 nm. The highest level of residue and longest withdrawal after oral administration of 40 mg/kg levamisole to chicken was in the liver. On day 3, the level of levamisole was undetectable in the plasma. On day 9, levamisole residue in eggs was 0.096 μg/g and on day 18, it was 0.06 μg/g or less in all the analyzed chicken tissues. Those levels were lower than the recommended MRL. The withdrawal time for levamisole in chickens was longer than for other species tested which was due in part to a larger dose of levamisole being recommended for chickens. The research concluded that levamisole in eggs was found to be less than the MRL and withdrawal periods are needed to be adhered to before medicated birds are slaughtered if their tissues are to be safe for human consumption. El-Kholy & Kemppainen, (2005).

2.1.4 Oxytetracycline residue in eggs

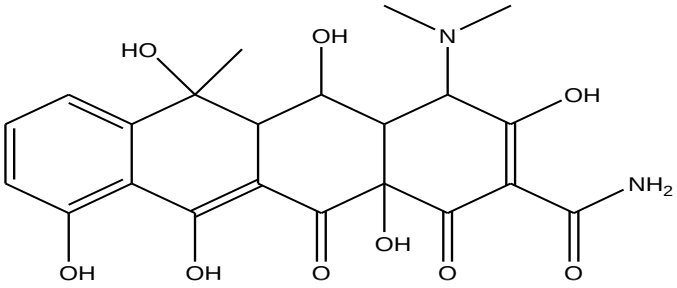


Fig 2.4: Chemical structure of Oxytetracycline (4-(dimethylamino)-3,5,6,10,11,12a-hexahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,5a,6,12,12 octahydrotetracene -2-carboxamide)

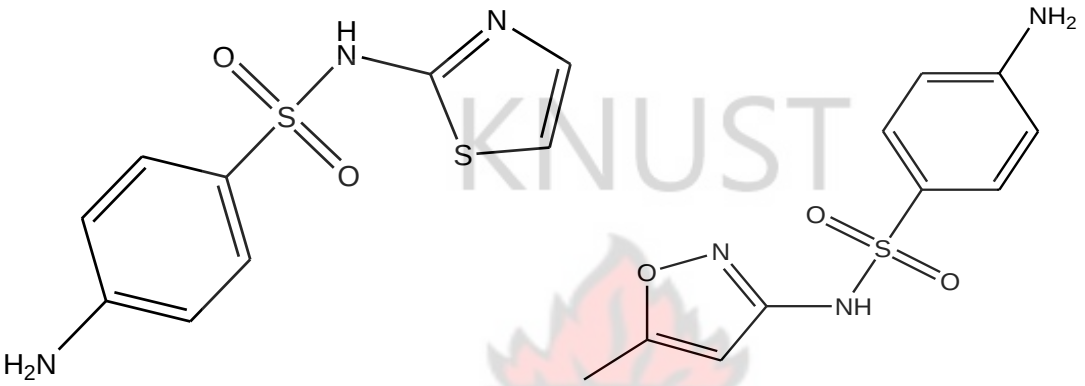
Tetracycline residues in table eggs collected from Qassim region was monitored from different regions located in Al-Qassim region from January 2006 to October 2006 using HPLC-UV. Al-wabel, (2011). Detection was done and compared with internationally acceptable MRLs and assessing proper implementation of the proper withdrawal times. HPLC analysis showed geographical and seasonal differences in Oxytetracycline levels. The number of positive samples found in all regions during October 2006 were (n=27) higher than that collected during January (n= 21), July (n= 19) and April (n= 17). Moreover, the percentages of total occurrence of the positive samples collected from Eion Al-Gawa (57.5) was higher than that in Al-Moznab (55), Buraidah (47.5), Al-Shamasia (27.5) and Oneiza (22.5) with 14, 12, 8, 5 and 5 samples exceeding MRLs, respectively. The results of the present investigation indicated that there was widespread misuse of tetracycline by egg layer chicken farms in Al-Qassim region reflecting a general lack of compliance with the recommended practices regarding the use of antimicrobial agents. These findings pointed to the need for a monitoring program for tetracycline residues in table eggs in Al-Qassim region at the national level to protect consumers' health.

Oxytetracycline residues in retail chicken eggs from five markets within Ibadan metropolis, Nigeria were determined by HPLC following solid-phase extraction (SPE). Idowu *et al*., (2010b). Recovery of oxytetracycline was 80.5%-87.8%, whereas 75.2% of the samples contained detectable oxytetracycline with an overall mean residue concentration of 479.0 $\mu g\,kg^{-1}$. Residue concentrations from Iwo Road, Apata, Challenge, Ojoo and Bodija markets were 421, 460, 468, 568 and 476 $\mu g\,kg^{-1}$, respectively. Eggs from Ojoo market had the highest levels, whereas 68.8% of the samples contained residues above the Codex Alimentarius Commission (CAC) MRL. The results were of public health interest as they indicated that a greater proportion of eggs being consumed in Ibadan could contain residues above the MRL for which unregulated access and indiscriminate use of antibiotics by poultry farmers could be responsible. National surveillance of eggs and other animal products for antibiotic residue and appropriate regulation of antibiotic was recommended to ensure food safety for consumers. Idowu *et al*., (2010b).

Oxytetracycline transfer into chicken egg yolk or albumen was determined to check whether the approved doses of oxytetracycline for breeder hens and meat-type poultry would produce drug residue transfer into egg components when fed to laying hens. Twenty hens were assigned to equal groups (n = 10) and fed either 50 or 200 mg/kg oxytetracycline for 5 days. Oxytetracycline concentrations in egg components were determined daily during a 2 day pretreatment control period, the 5 day dosing period, and following drug withdrawal. The stability and drug content of the medicated feed were determined the day dosing was started and the day of withdrawal. Residues of oxytetracycline were not detectable during the predosing, dosing, or withdrawal period in egg yolks. Oxytetracycline residues were detectable, however, in egg albumen during the 5th day of treatment and the 1st day of medicated feed withdrawal. These concentrations were close to the limit of the assay's sensitivity (117 ug/kg). These data indicated that illegal or unintentional dosing of laying

hens with feed medicated at the doses allowed for breeder hens or meat-type poultry should not produce consistently detectable levels of residues of oxytetracycline in eggs. Donoghue and Hairston, (1999).

2.1.5 Sulphonamides (Sulphamethoxazole and Sulphathiazole) residue in eggs.



(A) ***(B)***

Fig 2.5: Chemical structure of Sulphonamides

(A) Sulphathiazole (4-amino-N-(1,3-thiazol-2-yl)-benzenesulphonamide

(B) Sulphamethoxazole (4-amino-N-(5-methylisoxazol-3-yl)-benzenesulphonamide

The study was conducted (Mehtabuddin *et al*., (2012) to determine the residual level of sulphonamides in poultry meat and eggs. Sulphonamides are frequently used in poultry and suspected residues present in meat and eggs may be injurious to human health. A total of 30 egg samples, each consisting of 3 eggs, and 30 breast meat samples, collected randomly from sale points at different locations and poultry farms of Rawalpindi/Islamabad were used to detect the sulphonamide residues. These egg and meat samples were stored at 4°C and -20°C, respectively, until the time of analysis. Extraction of sulphonamides from eggs was performed using liquid-liquid extraction procedure with acetonitrile and n-hexane while acetonitrile was also used for meat samples followed by clean up with SPE columns (C_{18}). Detection of sulphonamide residues were made by HPLC-UV detector set at 265 nm using

C_{18} column (25 cm× 0.46, 5 μm) under isocratic conditions and using 0.01 M potassium di-hydrogen phosphate (KH_2PO_4) buffer and methanol (70:30 v/v) as a mobile phase with a flow rate of 1 ml/min. The limit of detection (LOD) was 0.02 μg/g and 0.025 μg/ml for meat and eggs, respectively. It was noted that 43% meat and 30% egg samples had detectable levels of sulfonamide residues whereas 23% meat and 10% egg samples exceeded recommended MRL and were unfit for human consumption. The study revealed the presence of sulfonamide residues in poultry meat and eggs because of indiscriminate use of sulfonamides in commercial broilers and layers without observing withdrawal period of this drug.

Zotou and Vasiliadou, (2010) used a new, rapid, sensitive and selective HPLC method with fluorescence detection to describe the simultaneous determination of 12 sulphonamides, in the presence of putrescine as internal standard, after pre-column derivatization with fluorescamine. The drugs were separated on a Chromolith Performance reverse phase (RP)-18 column (100 × 4.6 mm), using a gradient elution with a binary mobile phase of methanol 0.05M acetate buffer (pH 3.4). Linearity of derivatization was obtained for concentrations from 3.0 to 300 mg/L in standard solutions. The whole procedure was evaluated and fully validated, according to the EU Decision 2002/657/EC, for the determination of sulphonamides in turkey muscle and hen eggs following SPE. The LODs varied from 2 to 17 mg/kg in turkey and 2 to 15 mg/kg in egg samples. The average recoveries ranged between 96.9–108.6% in turkey muscle and 96.0–108.4% in egg samples, respectively.

The presence of sulphadimidine residues in eggs after a per oral administration of sulphadimidine. (120 mg/hen/day) to laying hens was studied in chicken eggs by Hussein *et al*., (2005). Premi®Test, four-plate microbiological method and HPLC were used for the detection of sulphadimidine residues. Positive findings of the Four-plate test (FPT) were

confirmed by the results of Premi®Test. Using the FPT, the absence of sulphadimidine residues was confirmed 72 hours after the last sulphadimidine administration. The presence of sulphadimidine residues had been detected by Premi®Test within 8 days and by the FPT within 3 days after the last administration. As compared with the results of Premi®Test, the FPT has reported false-negative results for five days (kappa < 0.6). Conformity of results obtained by both Premi®Test and HPLC was confirmed in this study (kappa = 0.6).

In Uganda, sulphonamide residues in commercial eggs were detected in layers in Kampala district. Whole eggs were homogenized in acetonitrile and centrifuged twice, extracts evaporated and residues dissolved in mobile phase (32:68, methanol: potassium dihydrogen phosphate). Fats were removed using hexane while anhydrous sodium chloride was added to break emulsions. Extracts were analyzed by reverse phase high performance liquid chromatography with photodiode array detector (RPHPLCPAD). Ninety-five percent of the farmers never observed withdrawal periods although 80% of them knew the importance of withdrawal periods. Most farmers attributed the non-observance of withdrawal periods to poverty and fear to lose their investments. Ninety-eight percent of the samples had detectable levels of the sulphonamides. Meanwhile, 98.3% of the samples that had detectable sulphonamide residues came from farmers who applied antimicrobials in feeds/ water. Consumers of hen eggs in Kampala district were at high risk of sulfonamide residue exposure due to poor farming practices (Sasanya *et al*., 2005).

A supercritical fluid extraction (SFE) method was described for the isolation of sulphonamides from chicken eggs Pensabene *et al*., (1997). Whole egg was mixed with hydromatrix and added to an extraction vessel containing neutral alumina. The sample was extracted at 40 °C with supercritical CO_2 at (680 bar and an expanded gas flow rate of 3.0

L/min to a total volume of 120 L. The sulphonamides were trapped in-line on an alumina sorbent bed. The sulphonamides were eluted post-SFE by using the HPLC mobile phase solvent system (phosphate buffer and methanol) followed by separation on an HPLC-UV at 265 nm. Recoveries from fortified liquid whole eggs (six replicates) at the 0.1-ppm level are 99.5% ± 2.2 for sulfamethazine, 87.8% ± 6.0 for sulfadimethoxine, and 97.6% ± 2.5 for sulfaquinoxaline. The LOD was 0.025 (mg/kg).

2.1.6 Piperazine residue in eggs.

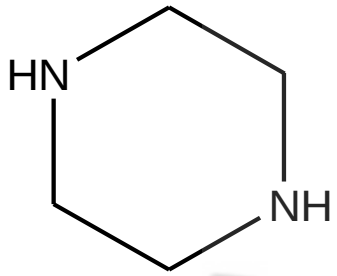


Fig 2.6: Chemical structure of piperazine (piperazine)

Aganga *et al*., (2003) did an experiment at Sebele to study the influence of feed type on egg production of Tswana laying chicken. Ten 23 weeks old laying Tswana chickens were bought from a local farmer and divided into two groups of five each. Group one was fed on layer mash and the other group was fed on composite local feed which was a mixture of sorghum, maize and sunflower traditionally used as a supplement feed under free range system in Botswana. All the layers were dewormed using piperazine to prevent diseases. The layers were individually caged to monitor daily feed and water intake. Collection and weighing of eggs were done every day for 90 days. Tswana layers fed on layers mash produced on average five eggs per week while those on composite local feed produced one egg per week that was 400% more production on layers compared to local supplementary feed.

2.1.7 Tiamulin residue in eggs

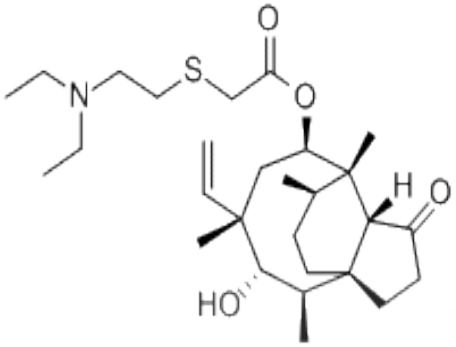


Fig 2.7: Chemical structure of Tiamulin (5-Hydroxy-4, 6, 9, 10-tetramethyl-1-oxo-6-vinyldecahydro-3a, 9-propanocyclopenta [8] annulen-8-yl, 2-(diethylamino) ethyl sulfanyl acetate)

This study was undertaken to evaluate the efficacy of some antimicrobials containing tiamulin and tilmicosin (as active principles) against *Mycoplasma gallisepticum* (infection both *in-vitro* and *in-vivo* for infected broiler chickens. For *in-vitro* investigation, the minimum inhibitory concentration of tiamulin and tilmicosin against the organism was done. However, the *in-vivo* evaluation of tiamulin and tilmicosin against field *Mycoplasma gallisepticum* infection was carried out on a commercial broiler chicken farm taken from *Mycoplasma gallisepticum* infected farm and proved to have such infection through bacteriological and serological examination at day old. Once the birds suffered from respiratory signs at 22 days of age, this flock was divided into three separate houses. Significant ($p < 0.05$) improvement in the mean body weights was observed in the treated chickens than the infected ones. Both tiamulin and tilmicosin were efficacious in the treatment of *Mycoplasma gallisepticum* infection in broiler chickens; nevertheless tiamulin medication was superior in controlling of such infection. It was recommended that testing the efficacy of the drugs *in-vitro* before application *in-vivo* to overcome the problem of drug

resistance, also tiamulin and tilmicosin were effective in eradication programmes of field. *Mycoplasma gallisepticum* in the broiler chickens. El-ghany, (2009).

A rapid, accurate, simple and reproducible HPLC method for determination of tiamulin in chicken's plasma was developed and validated by (Pomorska-mól and Kowalski, 2009). The drug and the standard were eluted from 5µm X-Terra RP_{18} (Waters) column at room temperature. The mobile phase was composed of 4M KH_2PO_4 – acetonitrile (65:35 v:v) (pH adjusted to 2.8) with a flow rate of 1.5 ml/ min. The effluent was monitored using a UV-VIS detector set at 208nm. The retention time of tiamulin was about 4.5 min. The suggested technique was characterized by superior performance parameters: linearity=0.9999, recovery=83.50%, repeatability relative standard deviation (RSD) $\leq$ 2.7%. These results demonstrated the validity of the HPLC method for the analysis of tiamulin. This could be a useful tool in the pharmacokinetics studies of tiamulin in animals.

A liquid chromatographic-atmospheric pressure ionization ion spray method was described for the determination of flunixin, tiamulin hydrogen fumarate in meat, and toltrazuril and the metabolite toltrazuril sulfon in meat and egg. Taylor *et al*., (2003). The method can also be used for the determination of flunixin in milk. Samples were extracted with acetone–tetrahydrofurane, after which the organic layer was separated from water with dichloromethane and evaporated to dryness. The dry residue was diluted in methanol-l-heptane sulfonic acid and fat was extracted with hexane and filtered. A low LOQ was reported for flunixin and toltrazuril (5 ng/g) for tiamulin and toltrazurilsulphon (2 ng/g) in meat. The LOD and LOQ for toltrazuril and toltrazuril sulfon in egg were similar to meat.

2.1.8 Multiresidue analysis of veterinary drugs in animal products

Chiaochan *et al.*, (2010) developed a simple and sensitive method for multiresidue analysis of 24 important veterinary drugs (including 3 aminoglycosides, 3-lactams, 2 lincosamides, 4 macrolides, 4 quinolones, 4 sulfon- amides, 3 tetracyclines, and amprolium) in chicken muscle. The method involved a simple extraction using (1:1, v/v) of 2% trichloroacetic acid in water–acetonitrile, followed by removing fat with hexane, dilution of sample extract, and filtration prior to liquid chromatography–tandem mass spectrometric (LC–MS/MS) analysis. Hydrophilic interaction LC proved to be very effective for separation of a wide range of polar and hydrophilic compounds (providing high sensitivity and good peak shape) compared to reversed phase and ion-pair separation. The method was successfully validated according to the EU Decision 2002/657/EC. Average recoveries were 53–99% at 0.5-MRL, MRL, and 1.5-MRL spiking levels, with satisfactory precision ≤15% RSD. The LOD values were lower than the maximum residue limits (MRLs) established by the EU. The evaluated method provides reliable screening, quantification, and identification of 24 veterinary drug residues in foods of animal origin. It was successfully tested in real samples (such as chicken muscle, shrimp, and egg).

Comparison of several extraction techniques for multiclass analysis of veterinary drugs in eggs using ultra-high pressure liquid chromatography–tandem mass spectrometry (UHPLCMSMS) was done as a determinative method for veterinary drugs (Frenich *et al*., (2010). This study compared four extraction methods for the simultaneous determination of tetracyclines, macrolides, quinolones, sulphonamides and anthelmintics (including benzimidazoles and avermectins) in eggs by UHPLCMSMS . Solvent extraction, solid-phase extraction matrix solid-phase dispersion and modified procedure were compared in terms of recovery and number of veterinary drugs extracted. The solvent extraction procedure with a

clean-up step provided better results than the other tested procedures. The solvent extraction procedure was validated, obtaining recoveries ranging from 60% (sulphaquinoxaline) to 119% (LM) with repeatability values (expressed in relative standard deviations (RSDs)) lower than 20% at two concentration levels except for erythromycin, emamectin and ivermectin. LOQs always equal or lower than $5gkg^{-1}$. Finally, the method was applied to egg samples, and erythromycin, enrofloxacin, difloxacin, thiabendazole, emamectin and fenbendazole were detected in four samples.

As a confirmatory procedure for determining veterinary drug residues, Chrusch *et al*., (2008) determined the performance characteristics of a new multi-residue method for non-steroidal anti-inflammatory drugs, corticosteroids and anabolic steroids in food animal tissues. A new LC–MS/MS method was developed for the analysis of 29 veterinary drug residues, spanning three different drug classes, in animal tissues. The procedure used measured the characteristic performance parameters of the method and the results obtained using fortified blank bovine muscle and kidney tissue are described. For a quantitative and confirmatory method, the characteristic performance parameters to be determined are the limits of quantification, trueness, recovery, precision, selectivity, ruggedness, and stability. The characteristic performance parameters defined for the method was verified during a validation study by an independent experienced analyst to determine whether the method was suitable for use in a regulatory monitoring and control program for residues of the 29 analytes.

Ultra-performance liquid chromatography combined with time-of-flight mass spectrometry(UPLC–ToF-MS) was used for screening and quantification of more than 100 veterinary drugs in milk. Nielen *et al*., (2008) The veterinary drugs represent different classes including benzimidazoles, macrolides, penicillins, quinolones, sulphonamides, pyrimidines, tetracylines, nitroimidazoles, tranquillizers, ionophores, amphenicols and non-steroidal anti-inflammatory agents (NSAIDs). After protein precipitation, centrifugation and solid-phase

extraction the extracts were analysed by UPLC–ToF-MS. From the acquired full scan data the drug-specific ions were extracted for construction of the chromatograms and evaluation of the results. The analytical method was validated according to the EU guidelines (2002/657/EC) for a quantitative screening method. At the concentration level of interest (MRL level) the results for repeatability (%RSD<20% for 86% of the compounds), reproducibility (%RSD<40% for 96% of the compounds) and the accuracy (80–120% for 88% of the compounds) were satisfactory. A set of 100 samples of raw milk were screened for residues. No suspected (positive) results were obtained except for the included blind reference sample containing sulphamethazine (88 μg/l) that tested positive for this compound. UPLC–ToF-MS was very powerful for the multi-compound analysis of veterinary drugs. The technique was powerful enough for the analysis of not only veterinary drugs but also organic contaminants like pesticides, mycotoxins and plant toxins in one single method.

Wang, *et al*., (2008) came up with a rapid analytical method for the determination of oxytetracycline, tetracycline and chloramphenicol antibiotics in animal feed based on subcritical water extraction without further sample clean-up followed by HPLC-UV detection. On extracting target antibiotics from spiked samples, the efficiency of the water extraction device was evaluated in terms of pH and volume of the extractant, temperature and time of the static extraction. The best extraction conditions were obtained by using 5.5 mL of water adjusted to pH 2 with hydrochloric acid as the extractant at 100 °C with 5-min static extraction. After filtration, the aqueous extract was directly injected into the HPLC column. Recoveries between 82.1% and 90.0% with RSDs ranging between 1.6% and 4.8% were achieved from spiked animal feed samples by using this method. Compared with the traditional ultrasonic extraction, this procedure was remarkably more efficient in extracting oxytetracycline, tetracycline and chloramphenicol, was simpler to perform and there was no use of toxic organic solvents.

HPLC method equipped with a photodiode array detector(HPLCPDA) was evaluated for the residual determination of 13 veterinary drugs, including clopidol, sulphadiazine, sulphathiazole, carbadox, sulphamerazine, ormethoprim, sulphamethazine, furazolidone, sulphamonomethoxine, sulphamethoxazole, ethopabate, sulphaquinoxaline, and sulphadimethoxine in chicken and swine muscles. Test samples were extracted with acetonitrile and filtered. The filtrate was partitioned with acetonitrile-saturated n-hexane for removing the interference. After evaporation to dryness, the residue was passed through a Sep-Pak C_{18} cartridge for sample cleanup prior to HPLC analysis. Veterinary drugs were determined by (HPLCPDA) using a Luna 5 μ C_{18} (2) 25 cm × 4.6 mm internal diameter of 5 μm) analytical column and a gradient elution of acetonitrile and 0.05M sodium dihydrogen phosphate. The average recoveries of 13 veterinary drugs from chicken and swine muscles at the levels of 0.1, 0.2 and 0.4 ppm were in the range of 71.9~96.9% and 71.1~99.6%, respectively, with coefficients of variation less than 8%. The LODs were 0.04 ppm for sulphathiazole and 0.02 ppm for other 12 drugs. Twenty-five samples each of chicken and swine muscles collected from local markets in Taipei were investigated for veterinary drug residues (Kao *et al*., 2001). One chicken muscle sample was found to contain 1.23 mg/kg sulphaquinoxaline, the level of which exceeded the regulated tolerance.

2.1.9 Antimicrobial and antibiotic residues in animal products

A study was conducted by Fagbamila *et al*., (2012) to evaluate a commercial test kit for the qualitative screening of eggs submitted to a laboratory as a first step for the testing of drug residues. Forty hens at the point of lay were kept for 4 weeks without administering any antibiotic. Eggs were then collected and tested for the absence of drug residues. Antibiotic-free birds were then divided into two groups. One group was administered tetracycline for two days and the other group left as control. Eggs were collected daily for two weeks from

both groups and tested for tetracycline residues using the disc diffusion method and a commercial test kit. Both methods detected the presence of drug residues in test eggs with the commercial test kit able to detect residues over a long period up to 10 days. This study indicated that the commercial test kit could be used for the detection of drug residues particularly when the aim was to screen large numbers of samples rapidly. However, it was not sensitive enough to detect drug residues at lower concentrations and not suitable for confirmatory testing.

Kehinde *et al*., (2012) detected antimicrobial drug residues in commercial eggs using premi® test. Nigeria is one of the African nations with known reports of drug residue occurrence in tissues and matrices. Although the actual prevalence is not certain, previous studies have shown an increasing trend over time in the occurrence of antimicrobial drug residues. This study was developed to further study the present status of occurrence of antimicrobial drug residues utilizing commercial eggs in the most centrally located state of the north central geopolitical zone, Nigeria. Other considerations investigated in the study include the possible roles of rapid expansion of unregistered small scale poultry and management system on the occurrence of antimicrobial drug residues. Out of the total of 105 commercial farms randomly selected from Kaduna State for the survey of antimicrobial usage, 92 (87.6%) used veterinary drugs frequently out of which 31.0% administered the drugs without adhering to veterinary recommendation. Further laboratory investigation showed a true occurrence of 7.6% residue out of the 1440 commercial eggs analyzed. Uncontrolled expansion of small scale/backyard farming and management system seemed to exert influence on the occurrence of antimicrobial drug residues.

A survey of antibiotic residues in table eggs was done in Khartoum State, Sudan by Sirdar *et al*., (2012). The risk to consumers of antibiotic residues in table eggs produced in Khartoum State, Sudan, was studied. All producing layer farms (n = 175) in the state were sampled in April, June and August 2008. A total of 933 eggs from 335 layer houses were screened for antibiotic residues by using the growth inhibition of *Geobacillus stearothermophilus* in-house test. A high proportion of layer farms (72% in April, 61% in June and 66% in August) and layer houses (63% April, 59% in June and 61% in August) were found to have antibiotic drug residues, with no significant difference in prevalence (p = 0.57) between study periods. The study showed that the consumer was at constant risk of exposure to antibiotic residues in table eggs. The paper discussed reasons for the high prevalence of antibiotic drug residues in Sudanese eggs and its implications, and made recommendations to address this important public health problem.

Thirty commercial layer farms were surveyed to obtain information on drug use and to screen eggs for antibiotic drug residues. Tetracycline residue was specifically tested using a commercial test kit. The study indicated that up to a third of farmers (33.3 %) were not adhering to the recommendation on drug use and thereby allowing drug residues in eggs. Of the 900 commercial eggs screened, 3.6 % tested positive for antibiotic drug residues but only 0.1% tested positive for tetracycline residue. The low level of tetracycline residue detected in this study was an indication of the declining use of this antibiotic in the poultry industry perhaps due to the increasing availability of cheaper alternatives. There is a need for strict regulation of veterinary drug in order to guarantee food safety and effective use. To ensure compliance with drug use in Nigeria, routine surveillance must be conducted using simple detection methods. This study was carried out by (Idowu *et al*., 2010).

Occurrence of antimicrobial residues in commercial chicken eggs was determined in Morogoro municipality between January and February by Nonga *et al*., (2010). Twenty smallholder farmers were interviewed on the types of antimicrobials, reasons of use and their awareness on antimicrobial withdrawal period. Seventy egg samples were collected for qualitative antimicrobial drug residues analysis by use of agar well diffusion and Delvotest assays. It was found that farmers used antimicrobial drugs as prophylaxis and treatment of common chicken diseases namely fowl typhoid (85%), infectious bursa disease (Gumboro) (65%) infectious coryza (65%), collibacilosis (55%), coccidiosis (54%), Newcastle disease (50%), helminthosis (20%) and fowl pox (15%). Antimicrobials accounted for 85% of the drugs commonly used. It was also found that 65% of the farmers treat their chicken themselves. The common drugs were oxytetracycline (75%), egg booster (50%), amprolium (35%), sulphamethoxypyridazine (35%), sulphanilamide (25%), chlortetracyclines (10%), chloramphenicol (10%), sulphadiazine–trimethoprim (20%), duoxycycline (20%), sulphadiazine (25%) and flumequine (10%). Eighty percent of the farmers who had knowledge on antimicrobial withdrawal period sold eggs before withdrawal period and almost 85% were unaware of possible effects of antimicrobial residues in humans. All 70 eggs samples tested were positive to antimicrobial residues by Delvotest kit, but 21.4% of samples tested positive with agar well diffusion test. It was concluded that the presence of antimicrobial residues in table eggs could be of public health significance to the egg consumers in Morogoro municipality.

Food safety is a term broadly applied to food quality that may adversely affect human health (Chowdhury *et al.*, 2009). There are two major areas of concern over the presence of residues of antibiotics in animal-derived foodstuffs with regard to human health. The first is allergic reaction and the second is development of antibiotic resistance in the gut bacteria of human.

Antibiotic resistance in human pathogens is now a major public health issue. Some of the resistance problem can be attributed to the transfer of resistant bacteria from animals to human and the transfer of resistance genes from animal pathogens and commensal bacteria to human pathogens. Control measures include improvements in food hygiene to reduce the spread of zoonotic bacteria to human via the food chain. However, to specifically address the issue, the livestock industries and their advisors must reduce and refine the use of antibiotics in animal production and replace antibiotics with alternative disease control measures as much as possible. In addition, the medical profession must control misuse and overuse of antibiotics in hospitals and community practice.

A total of 75 samples of stored poultry products; liver, breast and thigh muscle samples, were tested for the presence of four antibiotics residue; oxytetracycline, sulphadiazine, neomycin, and gentamycin using TLC (Shareef *et al*., 2009). The results revealed 39 (52%) positive samples. From 25 samples each of liver, breast and thigh muscle samples tested, 7 (28%) of liver and breast muscle were positive for sulphadiazine and oxytetracycline while 7 (28%) of thigh muscle were positive for oxytetracycline and 4 (16%) samples were positive for sulphadiazine. No neomycin or gentamycin residues were detected on TLC plates in all samples tested. Oxytetracycline was the most predominant antibiotic detected (28%), among the four studied antibiotics and followed by sulfadiazine (24%). Liver and breast muscle had the highest percentage of antibiotic detected (56%), followed by for thigh muscle (44%).

Studies were carried out on the presence of antimicrobial drug residues in chicken in edible tissues (breast muscles, liver and kidneys), slaughtered in two abattoirs in Bulgaria. A four-plate agar diffusion test using *Bacillus subtilis* and *Bacillus mycoides* as the test microorganisms was evaluated for the detection of antibiotic availability. From 75 samples

obtained from the first abattoir, two positive samples were found, while in the second there were no positive samples from breast muscles. The tissues with the highest number of samples containing antimicrobial residues was found in kidneys and livers of the chicken. It was stated that probably in some cases chicken meat producers do not respect regulations about withdrawal periods of the veterinary products Pavlov *et al*., (2008).

Tajick and Shohreh, (2006) through their research highlighted the importance and existence of antibiotics residue in meat. In this survey, 10 grams of chicken meat was crashed and squeezed in 10 ml ethanol. After centrifuging to clarify, the solvent evaporated totally, the chromatograms were observed on UV light after loading and running on silica F256 plates. The results showed that more than 50% of meat samples had noticeable antibiotics residue.

The occurrence of veterinary drug residues in poultry products in Kaduna state was determined. Information on drug use was obtained from ten layer flocks weekly for 10 weeks (Kabir *et al*., 2004). Two hundred commercial eggs and 378 slaughtered chicken faeces were examined for antibacterial drug residues using disc diffusion microbial inhibition test with *Bacillus cereus* and *Micrococcus luteus* respectively. All 10 farms used a drug at least once, nine used antibacterial drugs for either prophylaxis, therapy or both. None of the farms observed drug withdrawal period. Two eggs (1%) and 82 (21.8%) of the chicken faeces were positive. Broilers had a significantly higher incidence (33.1%) for antibacterial substances at slaughter than layers (23.6%) and local chicken (4.8%).

The use of antimicrobial agents in food-producing animals has become an important public health issue due to the spread of microbial resistance. This study was aimed at identifying the antimicrobial agents available for poultry use and highlighting their possible impact on public health. Twenty-three randomly selected poultry farms and all veterinary pharmacies in the

Eastern Province of Saudi Arabia were surveyed for the antibiotics used or dispensed. Further, a comprehensive literature survey was performed. Twenty-nine antimicrobial agents were identified as being available for poultry use, of which 22 (75.9%) were important for the treatment of human infections. Enrofloxacin, oxytetracycline, ampicillin, neomycin, sulphamethoxazole, colistin, doxycycline and erythromycin were the most frequently used drugs.

Food-borne hypersensitivity reactions and the emergence of microbial resistance, as well as cross-resistance to the various groups of antibiotics in animals and its transfer to human pathogens, are well documented. The misuse of antibiotics in the local poultry industry poses a serious health risk to the public and may complicate the treatment of human infections. The veterinary use of antimicrobial agents, especially those with dual animal and human applications, should therefore be restricted. The establishment of a government department concerned with food and drug safety is also highly recommended. This survey was carried out by use of antibiotics in the poultry industry in Saudi Arabia: implications for public health by Al-Mastafa and Al-Ghamdi, (2002).

2.2 Miscellaneous analytical methods for determining veterinary drug residues

A reliable, simple and sensitive (LC–ESI-MS/MS) confirmation method was developed for chloramphenicol determination in honey, fish and prawns (Barreto *et al*., 2012). For honey, samples were extracted with ethyl acetate an aliquot was evaporated to dryness and re-dissolved in mobile phase. For fish and prawns, tissues were extracted with acetonitrile and chloroform. The organic layer was evaporated to dryness and the residue was re-constituted with water: acetonitrile (90:10). LC separation was achieved on a C_{18} column with gradient elution using a mobile phase of acetonitrile and water. Analysis was carried out on a triple–

quadrupole tandem mass spectrometer in multiple reaction monitoring mode via electrospray interface operated in negative ionisation mode, with deuterated chloramphenicol-d5 (d5-CAP) as internal standard. Method validation was performed according to the criteria of Commission Decision 2002/657/EC. Four identification points were obtained for chloramphenicol with one precursor ion and two product ions. The limit of detection (LOD) was 0.02 µg/kg. Linear calibration curves were obtained over concentration ranges of 0.1–1.0 µg/kg in tissues. Mean recoveries ranged from 85.5% to 115.6%, with the corresponding intra- and inter-day variation ranging from 1.0% to 22.5%, depending on matrix type and level of concentration. The decision limit and detection capability of the method were obtained for all matrices: 0.04 and 0.06 µg/kg, respectively, for prawns and fish and 0.05 and 0.09 µg/kg for honey.

Mor *et al*., (2012) determined sulphonamide residues (sulphanilamide, sulphadiazine, sulphathiazole, sulphamerazine, sulphamethazine, sulphamethoxazole, and sulphadimethoxine) in cattle meat by the Charm II technique and the validation of sulphonamide levels by high performance liquid chromatography with fluorescence detector (HPLC-FLD). Of 157 meat samples, 9 samples (5.73%) were found positive by the Charm II method. To make quantitative confirmation of sulphonamide content of positive samples, HPLC-FLD was used and four samples were confirmed as positive. In HPLC analysis, the LOD was in the range 8–15 µg/kg and the LOQ was 13–25 µg/kg. Average recoveries of sulphonamides ranged from 44.6% to 81% with RSDs below 6% (n = 6). In conclusion it was considered that the results obtained in field screening by only using the Charm II system as is commonly practice in Turkey and worldwide was inadequate and thus the results should be confirmed by sensitive systems like HPLC.

Olusola *et al*., (2012) determined the levels of tetracycline and heavy metals (lead and cadmium) levels in frozen chicken. One hundred frozen chicken muscles samples were sourced from major markets in Lagos and Ibadan. The samples were analyzed using HPLC for tetracycline residue determination while Atomic Absorption Spectroscopy was used to determine the levels of lead and cadmium residues in the samples. Mean concentrations of tetracycline residue levels in the frozen chicken sampled ranged from 1.1589-1.0463 mg/kg which was higher than the maximum residue limit set by international food safety agencies. There were no significant differences in levels of tetracycline, lead and cadmium levels from the two markets. This study is of public health importance as the presence of these residues above the MRL in frozen chicken predisposed consumers to drug resistance, allergic reactions and poisoning as a result of toxicity.

Tolika *et al*., (2011) developed and validated an HPLC method for the determination of ten sulphonamide residues in milk. HPLCPDA at 265 nm, was developed and validated for the determination of ten sulphonamides sulphadiazine, sulphathiazine, sulphamethoxine, sulphamethaizole, sulphamethoxypyridazine, sulphamonomethoxine, sulphisoxazole, sulphadimethoxine and sulphaquinoxaline in milk. A mixture of ethyl acetate, n-hexane, and isopropanol was used for the extraction of target analytes from milk. The mobile phase, a mixture of 0.1% v/v formic acid, CH_3CN, and CH_3OH was delivered to the analytical column under a gradient program. The procedure was validated according to the EU regulation 2002/657/EC in terms of selectivity, stability, decision limit, detection capability, accuracy, and precision. Mean recoveries of sulphonamides from milk samples spiked at three concentration levels (0.5 MRL, 1 MRL, and 1.5 MRL). All RSDs were lower than 8.8%. The decision limits calculated by spiking 20 blank milk samples at MRL (100 mg/kg) ranged

from 101.61 to 106.84 mg/kg, whereas the detection capability ranged from 105.64 to 119.01 mg/kg.

Four common sulphonamides, sulphadiazine, sulphamethazine, sulphamethoxazole and sulphaquinoxaline were determined in chicken breast and liver samples using reverse phase HPLC-UV detector at 266 nm by Cheong *et al*., (2010). The concentration of sulphonamides detected in samples from 11 states in Peninsular Malaysia ranged from 0.006-0.062 µg/g in breast meat samples and 0.08-0.193 µg/g in liver samples. Except for sample from Johor, concentration of sulphonamides in all the samples were lower than MRLs established by Malaysia (0.1 µg/g). Exposure of sulphonamides in Malaysian consumers ranged from 0.002-0.088 µg/kg body weight. /day. The highest value of sulphonamides exposure was found in Johor with an estimated daily intake (EDA) of Sulphamethoxazole in Johor.

The use of chloramphenicol was prohibited in food producing animals due to its harmful and even potentially fatal side effects in human. In order to screen broiler carcasses for the drug residues, 31 broiler chickens from different farms were sampled. The samples from kidneys were homogenized, extracted using ethyl acetate and dried under N_2 flow. The samples were then assayed using enzyme-linked immunosorbent assay (ELISA). In the next phase, the concentration of chloramphenicol in the kidney, liver and thigh muscle of 13 positive chickens was compared following extraction and ELISA. More than half of the samples (54.8%) showed detectable concentrations of chloramphenicol. The highest concentrations of the drug were in the kidney and liver. According to research, there seems be a public health threat due to the illegal use of chloramphenicol in broiler farms and that kidney samples can be used for screening tests as proved by (Mehdizadeh *et al*., 2010).

Meat and other edible tissues from slaughtered cattle from Akure metropolitan abattoir from January to June 2007 were (HPLC) for oxytetracycline residue by Olatoye and Ehinmowo, (2010). The extraction was done using hydrochloric acid and acetonitrile for deproteinisation, while clean up was by liquid- liquid partitioning using dichloromethane and petroleum ether. Elution, detection and quantification were done on Lichrosorb RP–18 HPLC-UV detector. Out of a total of 180 beef samples analyzed during this study, 98 (54.44%) of the total samples had detectable levels of oxytetracycline residues from which 62 (34.44%) had oxytetracycline residues at violative levels above the (MRLs). The mean residues for positive samples were 51.8 µg/kg, 372.7 µg/kg and 1197.7 µg/kg for muscle, kidney and liver respectively. The standard deviations of residue in samples tested positive were 718.9 µg/kg, 366.8 µg/kg, and 90.53 µg/kg in liver, kidney and muscle respectively. These high level Oxytetracycline residues in greater proportion of meat destined for human consumption at violative levels could be as a result of the indiscriminate use and misuse of veterinary drugs as commonly practiced among livestock producers and marketers without observing withdrawal period prior to slaughter. These results indicate that consumers may be predisposed to health hazards and hinder international meat trade from Nigeria. Regulatory authorities should therefore ensure compliance with good agricultural practices including withdrawal period of drugs used for treatment of food animals, while livestock producers should also be educated on responsible use of drugs in food animals. Routine drug residues surveillance and monitoring programs in meat and other edible livestock products should be established in the country to ensure food safety.

At the Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bangalore-24, Shankar *et al*., (2010) as a means of confirming procedures for veterinary residues came up with rapid methods for detection of veterinary drug residues in meat. The use of

substances having hormonal or thyreostatic action as well as b-agonists is banned in many countries. However, sometimes forbidden drugs may be added to feeds for illegal administration to farm animals for promoting increased muscle development or increased water retention and thus obtain an economical benefit. The result is a fraudulent overweight of meat but, what is worse, residues of these substances may remain in meat and may pose a real threat to the consumer either through exposure to the residues, transfer of antibiotic resistance or allergy risk. This has exerted a great concern among the meat consumers. The control of the absence of these forbidden substances in animal foods and feeds is regulated in the EU by Directive 96/23/EC on measures to monitor certain substances and residues in live animals and animal products. Analytical methodology including criteria for identification and confirmation, for the monitoring of compliance was also given in Decisions 93/256/EEC and 93/257/EEC. More recently, Decision 2002/657/EC provided rules for the analytical methods to be used in testing of official samples. New substances with anabolic properties are being detected year by year increasing the list of forbidden compounds to be tested. Furthermore, the extended practice consisting in the use of "cocktails" (mixtures of low amounts of several substances that exert a synergistic effect) to have a similar growth promotion, reduces the margin for an effective analytical detection. Thus, the evolution of the "black market" is making really difficult to have an effective analytical control of the residues of these substances in foods of animal origin. Control laboratories must face an increasing demand of analysis like the growing number of residues to be analyzed in different types of samples, the strict guidelines for analytical methodologies according to the latest Directives, the increased costs of such new methodologies, the variety of residues to search per sample and the need to invest in sensitive new instruments for identification and confirmatory purposes. Rapid and versatile screening methodologies make its control easier and reduce the number of non-compliant samples to be confirmed through tedious and costly confirmatory analytical

methodologies. For instance, the multiresidue analysis can be performed better by using fast LC methods. Thus, the availability of new screening methodologies and the improvement of the existing ones will contribute to a better safety assurance of meat and other foods of animal origin.

Schneider *et al*., (2009) compared screening methods for antibiotics in beef, kidney juice and serum. Rapid screening tests can be used as part of an efficient program designed to monitor veterinary drug residues in cattle. In this work, three rapid tests were designed to screen samples for the presence of antibiotic residues, the Fast Antimicrobial Screen Test , Premi® and Kidney Inhibition Swab tests and were compared using beef kidney juice and serum samples. In order to provide a realistic assessment, potentially incurred samples of beef kidney juice and serum were obtained from 235 carcasses which had been retained by inspectors in a processing plant for further testing. In addition (LC–MS/MS) analysis was conducted on these samples to identify what antibiotics were present, if any, and their levels. The comparison of the three rapid screening test results with those from LC–MS/MS analysis allowed for a more complete comparison of the relative sensitivity of these analytical methods, as well as valuable information on false positive and negative response rates.

HPLC method for the determination of cyromazine and melamine residues in milk and pork is described by Wei *et al*., (2009). The method used NH_2 column and 97% acetonitrile eluate to determine the insecticide cyromazine and metabolite melamine residues in milk and pork. Samples were treated with NaOH and extracted with acetonitrile containing 20% NH_4OH. Target analytes of samples were cleaned up and concentrated by C_{18} column solid-phase extraction. A separation for cyromazine and melamine was achieved, and respective retention times were 8 and 12 min. The calibration curves for cyromazine and melamine were linear in

a concentration range of 0.01–1.0 µg/mL, with correlation coefficients of 0.9999 and 0.9997, respectively. Recoveries of cyromazine and melamine at fortified levels of 0.02, 0.05, and 0.1 mg/kg ranged from 84.5–90.8%, and 83.6–91.3%, respectively, with coefficient of variation of 3.1–7.8%.

A simple and rapid multiresidue method for the determination of seven sulphonamide residues (sulphadiazine, sulphapyridine, sulphamerazine, sulphamethazine, sulphamonomethoxine, sulphadimethoxine and sulphaquinoxaline) in milk samples was developed and validated. Gamba *et al*., (2008). The drugs were extracted with a mixture chloroform/acetone and simply cleaned up on a cation exchange solid phase extraction column. The analytes determination was carried out using LC with diode array detection. The procedure has validated as a quantitative confirmatory method according to the European Union (EU) Decision 2002/657/EC. The results of the validation process demonstrated that the method is suitable for application, as confirmatory method, in European Union statutory veterinary drug residue surveillance programmes. In addition, a hypothetical situation of sample judgement (compliance or not) in the case in which, at the same time, two different sulphonamides are found was discussed.

A simple, rapid, and sensitive method for the determination of traces of thirteen sulphonamide antibacterials in milk and eggs was presented. This method was based on the combination of polymer monolith micro extraction technique with hydrophilic interaction chromatography/mass spectrometry. The extraction was performed with a poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary column while the subsequent separation was carried out on a Luna NH_2 column. Acetonitrile (contain 0.05% formic acid, v/v) was used as the elution solution, which was well compatible with the mobile phase.

Good linearities were obtained for thirteen sulphonamides with the correlation coefficients above 0.997. The LODs of the method were found to be 0.4–5.7ng/mL of sulphonamides in whole milk and 0.9–9.8ng/g of sulphonamides in eggs. The recoveries of thirteen sulphonamides in two matrices ranged from 80.4 to 119.8%, with relative standard deviations less than 11.8% all carried out by (Zheng *et al*., 2008).

A new method for the simultaneous determination of three fluoroquinolones, enrofloxacin ciprofloxacin, and sarafloxacin in table eggs has been developed by Herranz and Marazuela, (2007) applying pressurized liquid extraction and liquid chromatography with fluorescence detection The influence of several extraction parameters (e.g. solvent mixture, temperature and extraction time) on extraction efficiency and coextracted matrix interferents was evaluated using fortified control eggs and matrix matched standard curves. The results showed that fluoroquinolones extraction efficiency depends mainly on solvent composition and the optimum extraction mixture was found to be phosphate 50 mM, pH 3.0/acetonitrile (50:50, v/v). The method was successfully applied to the determination been used and for confirmatory purposes.

In this study, HPLC method was used for the determination of quinolones in poultry products. These compounds were extracted with metaphosphoric acid: acetonitrile (3:7) followed by a bond elute resins and n-hexane to remove fats. The detection limits of quinolones were between 1 to 4 µg on a UV detector. Good linearity was observed from the calibration curve from the graph at concentrations from 2 to 400 µg. A comparison was made between the winter and the summer seasons. It was found that the amount of residual quinolones in the liver and kidney samples were more than those in muscles and eggs, Naeem *et al*., (2006).

A rapid, high-throughput antimicrobial screening assay has been developed that combined either a physical fluid extraction or a solvent extraction technique with the commercially available Premi®Test by Stead *et al*., (2005). In order to remove the subjectivity of the visual end-point measurement associated with this microbial inhibition assay, work was conducted to couple the Premi®Test to a scanner technology. The use of the solvent extraction provided an enhanced detection capability for a wide range of drugs at or below one-half the MRL concentrations in a variety of matrices, as demonstrated by dose response curve data. Secondary class-specific assays, for the identification of lactams and sulphonamides following the primary screen had been previously developed and validated using the scanner technology. Method validation using both fortified and incurred tissues had been undertaken to establish the ruggedness of the technique. The false positive and negative rates have been established at less than 5% for a range of drug/matrix combinations. This integrated screening strategy has provided a reliable tool for antimicrobial residue monitoring in surveillance programmes.

Schneider and Donoghue, (2003) developed an efficient multiresidue method for determination of fluoroquinolone antibiotics in eggs was developed in which quantitation and confirmation are achieved simultaneously using fluorescence and mass spectrometry. Eight fluoroquinolones were analyzed in fortified egg samples at levels of 10–100 ng/g. Recoveries for desethylene ciprofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin, orbifloxacin, sarafloxacin and difloxacin were generally in the range 60–100%, with excellent RSDs.

A determining technique of sulphonamides (sulphamonomethoxine, sulphadimethoxine and sulphaquinoxaline) in eggs, without use of organic solvents, was developed, Furusawa,

(2003a) utilizing a. HPLC method equipped with a photodiode array detector(HPLCPDA). The sample preparation was performed by homogenizing with perchloric acid solution using a handy ultrasonic-homogenizer followed by a centrifugal ultra-filtration unit. An analytical column and an isocratic mobile phase for HPLC are a reversed-phase C_4 column (150mm × 4.6mm internal diameter) and 0.18 mol^{-1} citric acid solutions respectively. Average recoveries of three sulphonamides (ranged from 80.3 to 88.4%, with RSDs between 3.4 and 5.8%. In all the processes, no organic solvents were used at all.

A confirmatory method for detection of a banned substance: The validation experience of a routine EU laboratory was done by Galarini *et al*., (2007). The Commission Decision 2002/657/EC is a fundamental reference document for the UE laboratories involved in residue analysis although its implementation has caused some difficulties in the requirements interpretation. In this work a pragmatic validation approach of a quantitative confirmatory method for the detection of 17-alpha and 17-beta-19-nortestosterone in bovine urine by gas chromatography mass spectrometry was proposed. The 19-nortestosterone is a banned anabolic steroid for which no minimum required performance limit has been laid down, therefore the limit reported in Italian Residue Monitoring Plan (2 g/L) was been considered as the reference level to evaluate the method performances. The decision limit and the detection capability were obtained by the calibration curve procedure. The minimum required performance level which represents the starting concentration of the calibration curves was preliminary fixed estimating the results dispersion of blank urine samples fortified at 2 g/L for each isomer. The found decision limit and the detection capability were 1.5 and 1.9 g/L for 17-alpha and 1.2 and 1.4 g/L for 17-beta-19-nortestosterone. The precision (repeatability and within-laboratory reproducibility) and recoveries were suitable for the investigated

concentration range (1–3 g/L). Finally, the method ruggedness (minor and major changes) was also been demonstrated.

Immunochemical screening assays using surface plasmon resonance was developed for chloramphenicol and chloramphenicol glucuronide residues in poultry muscle, honey, prawn and cows' milk using a sensor chip coated with a CM derivative and an antibody by (Ferguson *et al*., 2005). The antibody cross-reacted with chloramphenicol glucuronide 73.8% (poultry), 69.2% (honey), 75.7% (prawn) and 84.8% (milk). There was no cross-reaction with similar drugs or other commonly used antibiotics. The assay allowed the direct analysis of bovine milk (fat content ~3.5%). Poultry, honey and prawn samples were extracted with ethyl acetate followed by analysis on the biosensor. Between run precision (n=3) performed at the same levels yielded the following results: 3.0% (poultry), 4.7% (honey), 7.6% (milk) and 5.5% (prawn).

An antibody was generated that can bind metronidazole (a nitroimidazole drug used in veterinary medicine to treat poultry for coccidiosis and histomoniasis. A direct competitive (ELISA) was described. It was used to characterise binding of this antibody to a number of nitroimidazole drugs. It displayed cross-reactivity with dimetridazole, ronidazole, hydroxydimetridazole and ipronidazole. Egg and chicken muscle samples were extracted with acetonitrile and de-fatted by washing with hexane. Detection capabilities were determined: dimetridazole, <1 ppb (egg) and <2 ppb (muscle); metronidazole, <10 ppb; ronidazole and hydroxydimetridazole, <20 ppb; ipronidazole, <40 ppb. This study was conducted by (Huet *et al*., 2005).

A fast and cost effective method was developed to extract and quantify residues of veterinary antimicrobial agents (antibiotics) in animal manure by liquid–liquid extraction. The compounds investigated by (Haller *et al*., 2002) included six sulphonamides, one metabolite, and trimethoprim. The method was performed without sample clean up. Recoveries from spiked manure slurry samples (spike level 51 mg/kg) were as follows: sulphaguanidine (52%), 4 sulphadiazine (47%), STH (64%), sulphamethazine (89%), its metabolite N -acetyl-sulphamethazine (88%), SMTH (84%), sulphadimethoxine (51%), and trimethoprim (64%). RSDs of the recoveries were less than 5% within the same day and less than 20% between days. The LOQ was below 0.1 mg/kg liquid manure slurry for all compounds and calibration curves obtained from extracts of spiked samples were linear up to a level of 5 mg/kg liquid manure, except for trimethoprim (0.01–0.5 mg/kg). Analysis of six grab samples taken in Switzerland from manure pits on farms where medicinal feed had been applied revealed total sulfonamide concentrations of up to 20 mg/kg liquid manure.

2.2.1 Contamination processes that introduce veterinary drug residues in food

In the EU, animal feeding stuffs are subject to a comprehensive raft of legislation covering their composition, manufacture, storage, transport and usage. Contamination of feeding stuffs can and does occur during each of the above processes. Examples of contaminants include naturally occurring and synthetic toxic environmental compounds (e.g. mycotoxins and dioxins) which may contaminate raw feed materials. Zootechnical feed additives and veterinary medicines may also contaminate unmedicated feeding stuffs due to carry over during feed production. Contaminated feed can cause deleterious health effects in the animals and, through 'secondary exposure' of consumers to products deriving from these animals, may be harmful to people. The legislative framework controlling the use of veterinary medicines and zootechnical food additives in the EU was reviewed. From a contamination

perspective, 'problem' compounds include sulphonamides, tetracyclines, nitroimidazoles, nitrofurans, ionophore coccidiostats and nicarbazin. Examples of interventions to minimize contamination are given by through this study to find out about the contamination of animal feeding stuffs as a cause of residues in food: a review of regulatory aspects, incidence and control by (McEvoy, 2002)

An experiment was designed to establish the relationship between halofuginone contaminated feed and residues in eggs. Five groups of six-layer hens each were fed with halofuginone contaminated diets at concentrations ranging between 1 and 10% of the therapeutic dose for broilers (3 mg kg^{-1}) for 14 days. The group fed on the highest dose was then fed with a HFG-free diet for a further 14 days. Eggs were collected, homogenised, extracted and analysed. In general, the halofuginone concentration was much lower than those seen in similar studies on nicarbazin. However, comparison of the halufuginone concentrations measured in eggs and the (MRL) for halofuginone in bovine muscle suggested that feed contamination could give rise to potentially significant halufuginone residues in eggs. Depletion of halofuginone from eggs, following the feeding of a halofuginone free diet was 2.6 days, somewhat slower than the corresponding values for lasalocid and nicarbazin (1.1 and 1.6 days respectively), (Yakkundi *et al*.,2002).

2.3 Legislature behind the improper use of veterinary drugs

The impact of new legislation on the registration of veterinary drugs was studied by Clayton, (2005). The European legal framework covering medicinal products was reviewed by the European institutions during the period from November 2001 to February 2004. The new legislation for registration of medicinal products was published on 30 April 2004 in the Official Journal of the European Union. It sought to reach a balance between encouraging

innovation and facilitating the authorisation of generics. It also aimed to reduce product maintenance costs (renewals) and strengthen pharmacovigilance. However, the overall regulatory burden was not reduced, and this affected the global competitiveness of the European industry. There will be greater emphasis on good manufacturing practice (e.g. for starting materials of active ingredients) and scientific advice. The mutual recognition procedure was reorganised to drive harmonisation and reduce the rate of withdrawal from some member states during the procedure (e.g. automatic arbitration of any unresolved issues). New variations regulations with harmonised and clearer procedures were published in June 2003. They will reduce the workload more for competent authorities than for companies. The legislation covering maximum residue limits and residue monitoring was being reviewed; with the objective of developing a more balanced and coordinated approach.

2.4 Withdrawal period of veterinary drugs

Mulder *et al*., (2005) studied the deposition and depletion of the coccidiostats toltrazuril and halofuginone in eggs. Toltrazuril a triazinetrione derivative, and halofuginone, a quinazolinone derivative was licensed for the prevention and treatment of coccidiosis in broilers and turkeys was excluded for use in laying hens. Little is known regarding the deposition of residues of toltrazuril and halofuginone in eggs and their rate of depletion. In this study, laying hens were treated with therapeutic doses of toltrazuril and halofuginone. Eggs were collected before, during and after treatment. Residue concentrations of toltrazuril and its metabolite were determined in whole egg, as well as in the yolk and albumen. The residues were monitored daily in whole egg until 19 days post-treatment. Ponazuril was found the predominant residue formed. Toltrazuril concentrations in whole egg reached a maximum during treatment of 1500 g/kg while ponazuril concentrations increased to 11,000 g/kg, Halufuginone residues were monitored until 14 days post-treatment. Halofuginone was

detected in egg up to a concentration of 450 g/kg, during the medication period and declined fairly rapidly after the end of administration. After 12 days withdrawal, residue levels reached the LOD of 2 g/kg. Residue concentrations of halufuginone in yolk were approximately twice that in albumen.

CHAPTER THREE

3.0 MATERIALS AND METHODS

A comprehensive approach was adopted to determine the veterinary residues in poultry eggs. Egg samples were collected from five different poultry farms within Kentinkrono in the Kumasi Metropolis, Mamponteng in the Kwabre East and Offinso in the Offinso districts of the Ashanti Region and were transported to the laboratory on ice at 4°C.

3.1 SAMPLE COLLECTION

3.1.1 Eggs

A total of 200 egg samples comprising of 40 eggs each were collected from the 5 various poultry farms for veterinary residue analysis. The samples were stored in a refrigerator at 4°C until the time of analysis.

3.1.2 Study Sites/Areas

Samples were taken from 3 districts in the Ashanti Region. These are Mamponteng in the Kwabre East District in the central portion of the Ashanti region, Offinso in the Offinso South Municipal located in the extreme north-western part of the Ashanti Region and Kentinkrono which is about 20 km from the centre of Kumasi. Fig 3.1 shows the map of Ashanti Region showing the sampling areas.

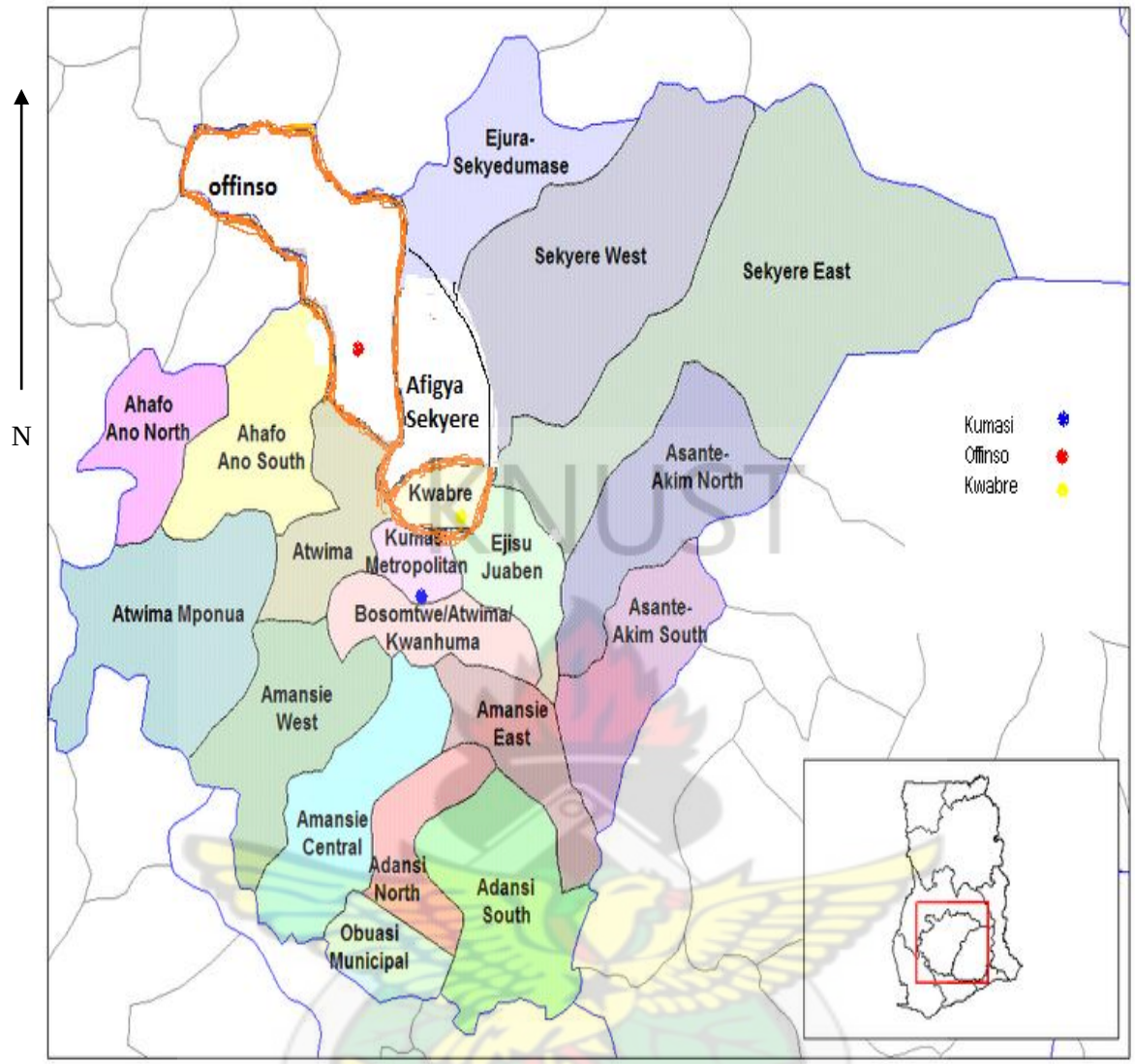


Fig 3.1: Map of Ashanti Region showing the sampling areas.

3.1.3 Sampling procedure

Eggs were collected from each of the ten poultry houses (housing units) from the farms in each district. Two egg samples were taken from each of the ten poultry houses in all the districts making a total of 20 eggs from the farms in one visit with the second batch of 20 eggs making a total of 40 eggs after the second visit from each farm. The sampling was done at random intervals. A final sample of 200 eggs was obtained from all the five poultry farms.

3.1.4 Sample handling and preservation

Each of the egg samples was marked with a permanent marker stating the date of collection, the farm and the number. The samples were put in an ice-chest and then stored in the refrigerator at 4 °C until the time of analysis.

3.2 Chemicals and Reagents

3.2.1 Chemicals

Anhydrous potassium dihydrogen phosphate (KH_2PO_4) HPLC grade (Fizmerk Chemicals, India), Methanol HPLC grade (Fisher Scientific, UK), Acetonitrile HPLC grade (Fisher Scientific, UK) and Hexane, HPLC grade, (Fisher Scientific, UK). All other chemical reagents were of analytical grade.

3.3 Equipment

Shimadzu LC-10 AT High Performance Liquid Chromatography (HPLC) with a photodiode array detector system with Shodex HPLC column oven L -7300, Detector L-7400, Auto sampler L–7100, Vacuum Degasser L-76610, Interface module D- 7000 and HSM software (Hitachi D–7000 series, Japan).Analytical Electrical Balance (Sartorius MC -21 S, Germany), Homogenizer (IKA, Ultra–Turrax T 25 basic, Germany), pH meter (Metrohm Co. Herisan, Switzerland) and rotary evaporator **(**Heidolph Instruments GmbH & *Co*., Germany).

3.4 Mobile Phase

Mobile phase comprises of Potassium dihydrogen phosphate (0.05 M) and Acetonitrile (HPLC grade) in the ratio of 7:3.

3.4.1 Preparation of Mobile Phase

A 0.05 M potassium dihydrogen phosphate (KH_2PO_4) was prepared by dissolving a mass of 6.80 g potassium dihydrogen phosphate (KH_2PO_4) in a 1 L volumetric flask and dissolved completely in redistilled water. It was diluted to volume with redistilled water and mixed well by manually shaking the flask horizontally and vertically several times. The solution was adjusted to pH 5.10 with 6 M HCL and 1M NaOH ,Kao et al.,(2001a).

A 700 ml of this potassium di-hydrogen phosphate solution was measured with 1000 ml graduated glass cylinder and poured into a 1000 ml media bottle. With the same cylinder, 300 ml acetonitrile (HPLC grade) was measured and poured into the same media bottle containing potassium di-hydrogen phosphate (KH_2PO_4) solution. A magnetic stir bar was added into the media bottle and the bottle was placed on the magnetic stirrer for thorough mixing.

3.4.2 Preparation of standards

An amount 50 mg of each of the standards (sulphanamide, tiamulin, oxy-tetracycline, albendazole, piperazine, chloramphenicol and levamisole) was dissolved in acetonitrile /methanol (3:7) and was topped to the 25 mL mark of a volumetric flask to make a stock solution of 2 mg/ml. Each stock was serially diluted to make concentrations of 10, 5, 1 and 0.5 µg/mL.

3.4.3 Standard Mix Solution

The above stock solutions were diluted with acetonitrile/0.05 M sodium dihydrogen phosphate (3:7, v/v) to a series of concentrations ranging from 0.2 to 1.0 µg/mL.

3.4.4 Chromatogram of the standard mix solution

HPLC with a photo diode array detector was chosen to allow the separation and identification of the multi-veterinary drugs by its retention time and spectrum in accordance with Kao *et al*., (2001); Zhao *et al*.,(2010). The various drugs were identified in the sample by their retention time and compared to a standard. The multiresidue spectrum obtained from the sample was identical with that of standard. Fig. 3.2 shows the HPLC chromatogram of the mixed standards.

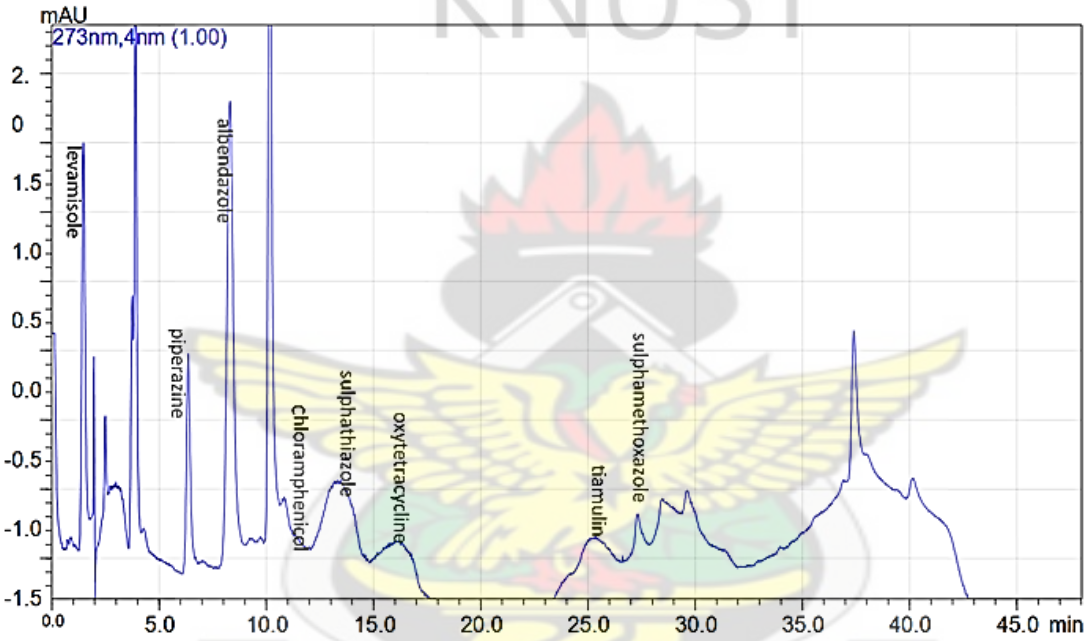


Fig 3.2: Chromatogram of the standard mix Solution of all the drug residues

3.5 Instrumental analysis and standard calibration

Concentrations of the mixed standard as well as the analysis of the sample were determined with High Performance liquid Chromatograph: Shimadzu LC-10AT equipped with a CBM-10A interface controller and an SPD-M6A photodiode array detector at excitation and emission wavelengths of 200~400 nm. 20 µl of the sample was injected into the HPLC Mightysil column (150 ml, 4.6mm-15µm) heated to 40 ^{o}C. The mobile phase was a mixture of potassium di-hydrogen phosphate/ acetonitrile (70:30, v/v) and the stationary phase was

Sep-Pak C_{18} cartridge. AOC 20I injector and a photodiode array detector was used, For the calibration curve, Five concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 μg/mL) of mixed standard were prepared as described and 20 μL of each was injected. Standard curves were plotted according to the peak areas versus concentrations (Kao et al., 2001a).

The peaks of the veterinary drugs were identified by comparison with standards.

3.5.1 Quantification

Sample and standard solutions were individually injected to the HPLC. Peak identification was made by comparing the retention times of samples with those of standards. The following formula was used to calculate the amounts of veterinary drugs in test samples (Kao *et al.*, 2001):

$$Amount\ of\ residues\ (ug/g) = C\ x\ \frac{V}{W}$$

Where

C is the drug concentration (μg/mL) calculated by standard curve,

V is the volume of sample solution (mL), and

W is the weight of sample (g).

3.6 PREPARATION OF EGG SAMPLES, EXTRACTION TECHNIQUES AND CLEAN-UP PROCEDURES

3.6.1 Extraction of Egg Samples

Whole eggs were homogenized with an Ultra-Turrax T25 basic homogenizer for 20 seconds at 7000/minute. A 2 ml portion of homogenized egg sample and 20 ml of acetonitrile were homogenized together at high speed with an Ultra-Turrax T25 basic homogenizer for 5 minutes and filtered with Whatman 0.45 µm filter paper. The residue after filtration was mixed with another 50 mL of acetonitrile. The mixing and filtration procedures were repeated for three times. The combined filtrate was transferred into a separating funnel containing 30 mL of acetonitrile-saturated n-hexane and shaken for 5 min. The acetonitrile layer was collected into a concentration bottle and evaporated to dryness at 40 ^{o}C using a rotary evaporator.

3.6.2 Sample Cleanup

The dry residue obtained after the evaporation was mixed with 0.05 M sodium dihydrogen phosphate and applied onto a Supelco C_{18} (1g × 6 ml) cartridge at a flow rate of 1-2 ml/min .The cartridge was pre-conditioned with 10 ml of methanol and 10 ml of 0.05 M sodium dihydrogen phosphate solution. The concentration bottle was washed twice with 5 ml of sodium dihydrogen phosphate solution which was applied on the same cartridge. The eluent was discarded. The same concentration bottle was washed twice with 5 ml aliquot of methanol and the resulting solution was passed through the same cartridge. The eluate was collected and evaporated to dryness and was mixed with 1 ml of acetonitrile. The above solution was collected and filtered through a membrane and transferred to a HPLC vial (National Scientific Company, Japan) for HPLC analysis Kao et al.,(2001a). Fig 3.3 is schematic of the extraction procedure adopted from Kao et al.,(2001a).

3.6.3 Extraction procedure

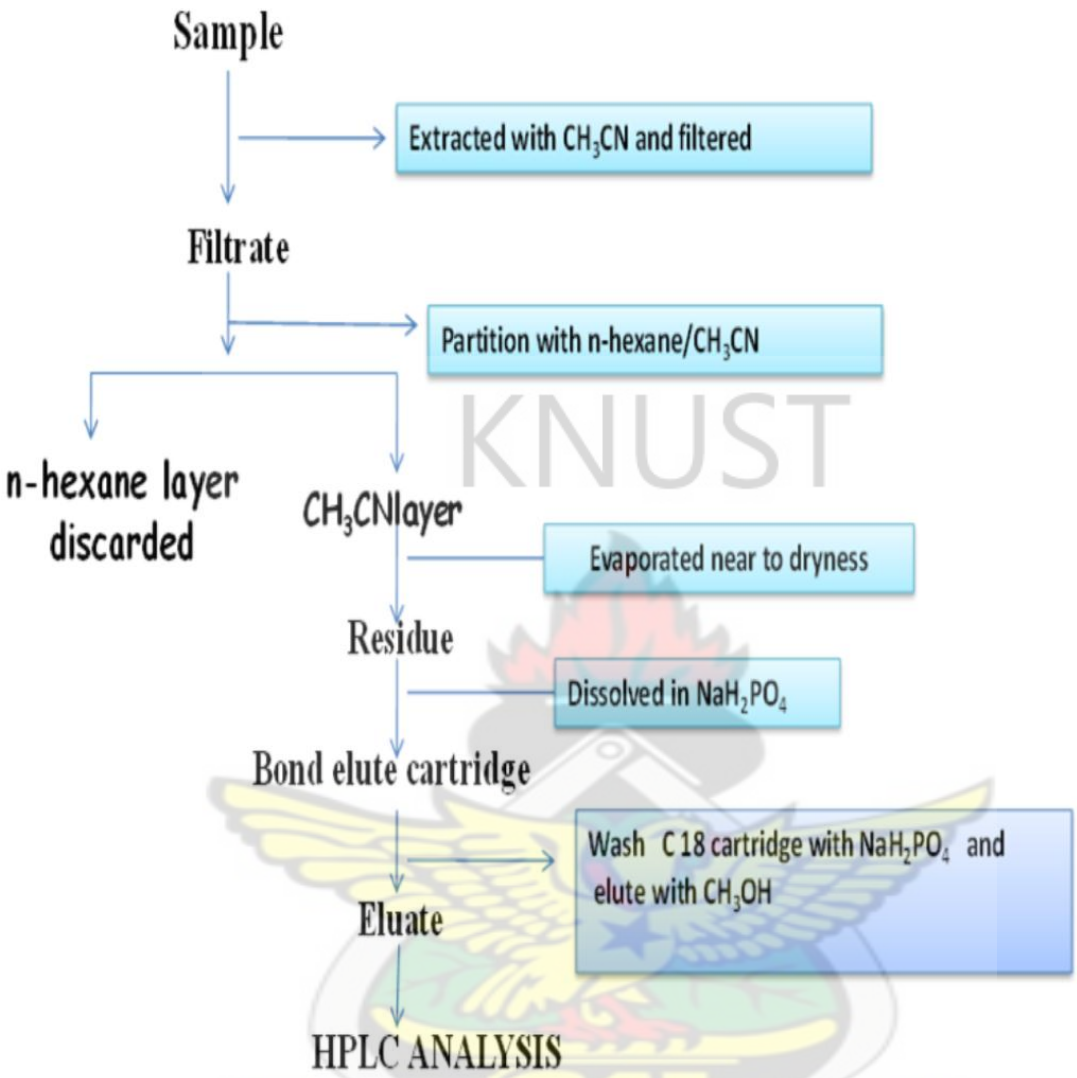


Fig 3.3: Extraction procedure of liver, kidney and muscle samples (Kao, et al., 2001)

3.7 RECOVERY TEST

Recovery test was performed in triplicate by spiking standards at 3 levels (0.1, 0.2, and 0.4 ug/g) into egg samples. 2 ml of the homogenized egg samples was spiked with 1 ml of the mixed standard. The spiked samples and blank sample without standard were then analyzed by HPLC. Recovery was calculated by comparing the analyzed concentrations with spiked concentrations by the formula

Recovery = Amount of residue obtained after spiking sample × 100%

Spiked concentration

The average recoveries of 8 veterinary drugs in chicken eggs were in the range of 76.0% - 98.8% and the correlation coefficient which was ≥ 0.9991. Of the 8 veterinary drugs, Sulphamethoxazole and Tiamulin showed the least recovery with average recoveries of 76.0% and 78.0% respectively, while Albendazole obtained the highest average recovery of 98.8%. Appendices K-M gives the average recoveries of 8 veterinary residues for concentrations of 0.1 ug/g, 0.2 ug/g and 0.4 ug/g respectively.

3.8 DATA ANALYSES

The software used for the analysis of the data retrieved from the HPLC 2010 was Graph Pad Prism.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 PREPARATION OF CALIBRATION CURVE

To determine the amount of the eight veterinary drug residues in the egg samples, a standard calibration curve for all the eight drugs was obtained by running a mixed standard solution on HPLC and then plotting peak areas against concentrations in µg /ml later converted to µg/kg.

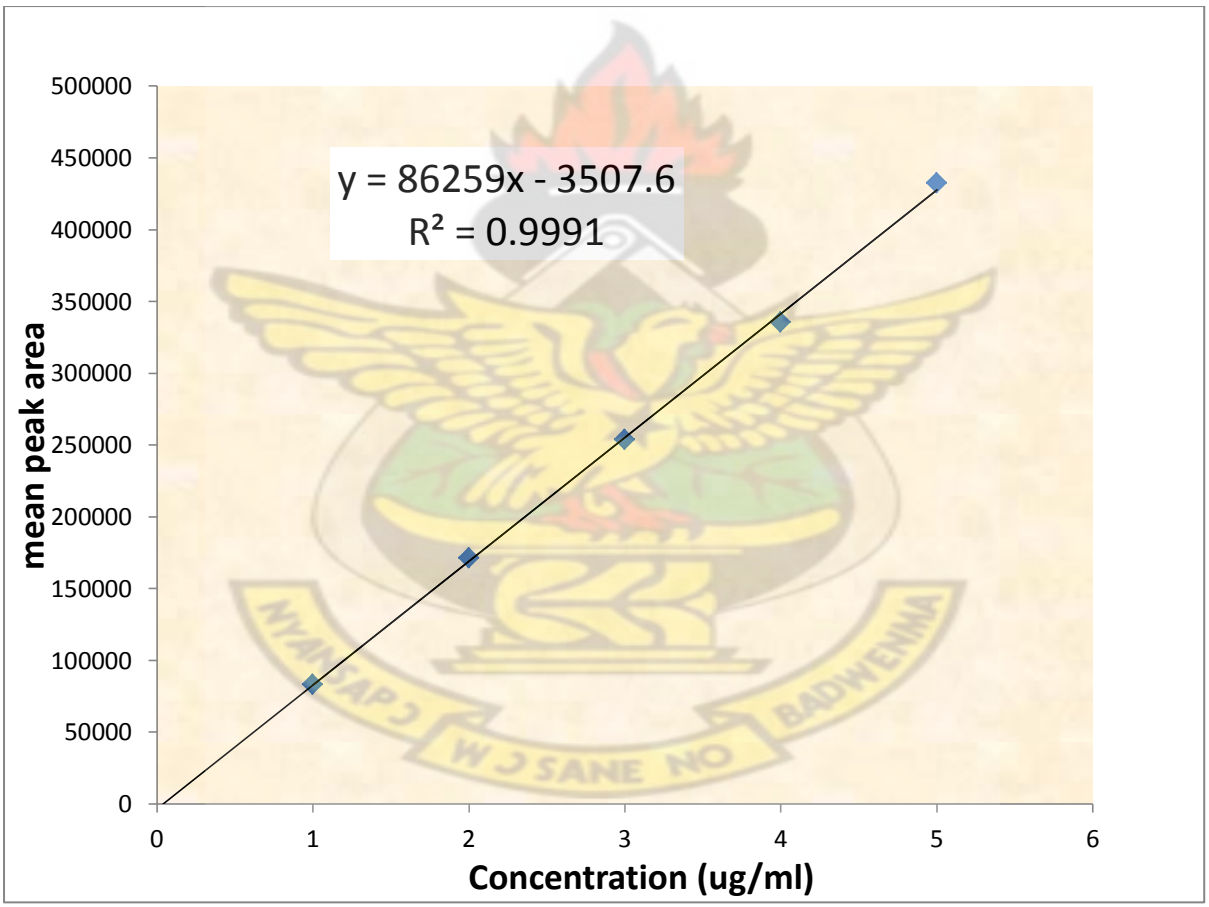


Fig 4.1: The standard calibration curve for all the eight veterinary drugs

For the curve, the best fit of the line was calculated by equation of line which was y= 86259 x -3507.6. Linearity was evaluated through the correlation coefficient which was 0.9991. The correlation coefficient, intercept and slope of calibration curve were calculated.

The best fit of data was determined by linear regression using the following equation:

$Y = mx + c$

Where,

Y = Peak area

m = Slope

x = Concentration

c = Intercept

4.1.1 The Limit of Detection (LOD)

The limit of detection was 0.01 µg/kg for eggs which was calculated from the correlation coefficient, intercept and slope of calibration curve.

4.1.2 The Limit of Quantification (LOQ)

The limit of quantification was 0.03 µg/kg for eggs which was calculated from the correlation coefficient, intercept and slope of calibration curve

4.2 RETENTION TIMES OF THE DRUG RESIDUES

The retention times for the drugs in order of increasing retention times are shown in table 4.1:

Table 4.1: Retention times for the veterinary drug residues

Name of residue	Retention time/min
Levamisole	3.751
Piperazine	5.580
Albendazole	7.080
Chloramphenicol	12.430
Sulphathiazole	14.065
Oxytetracycline	18.745
Tiamulin	25.382
Sulphamethoxazole	28.509

4.3. THE WAVELENGTH OF DETECTION

HPLC with photodiode array (PDA) detection was used for multiresidue analysis of veterinary drug in the egg samples. In this study, a PDA detector was therefore used as a tool to optimize the wavelength for the detection of the 8 veterinary drugs. The UV spectra ranged at 200~400 nm for the drugs as shown in a similar case by (Kao *et al*., 2001) .

4.4 DETERMINATION OF THE LEVELS OF DRUG RESIDUES IN EGG SAMPLES USING HPLC

Two hundred egg samples (200) from 3 different districts in the Ashanti Region of Ghana were analyzed. Nine veterinary drug residues including albendazole, piperazine, tiamulin, chloramphenicol, levamisole, sulphathiazole, sulphamethoxazole and o xytetracycline were analyzed for. The concentrations of the various residues in each sample were calculated in µg/kg sample. The mean concentration, standard deviation, minimum and maximum levels of the residues were also recorded. These values were then compared to the JECFA Maximum Residue Limits (MRLs). The raw data are found in the Appendices A-J.

4.5 RESULTS FROM THE LEVELS OF THE VETERINARY DRUG RESIDUES IN CHICKEN EGGS

4.5.1 Results from Farm 1

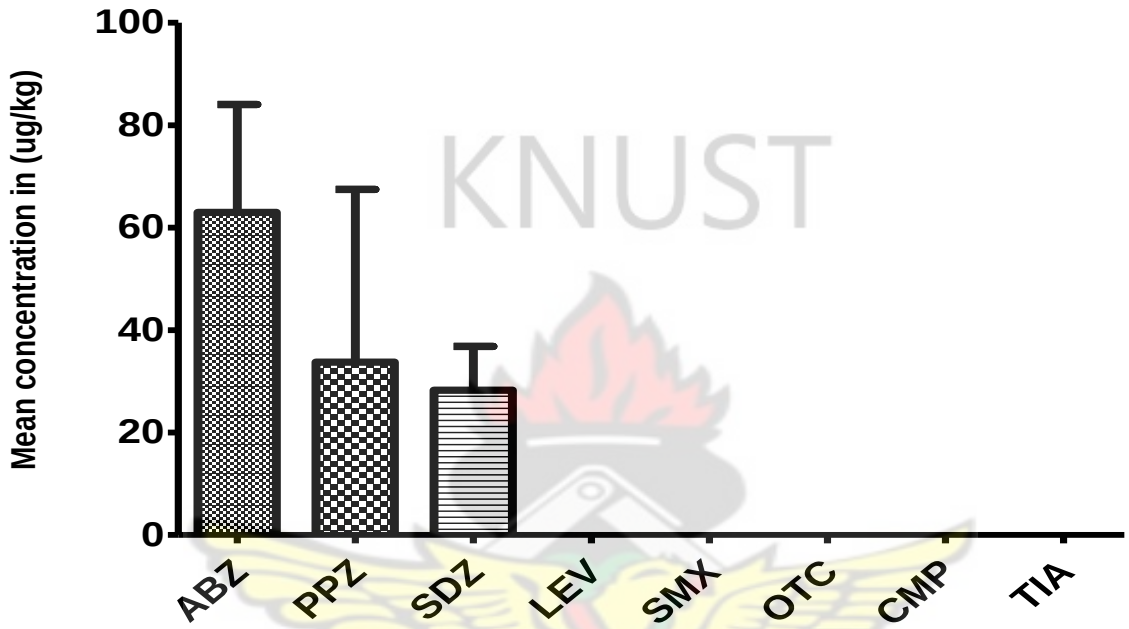


Fig 4.2 Levels of veterinary drug residues in egg samples from Farm 1

Farm 1 is found in the Offinso District and was labeled as AS. The average concentration of veterinary drug residues in egg samples of chicken from Farm 1 from Offinso-South Municipality is found in Fig 4.2

Albendazole occurred at an average concentration of 63 ± 0.42 µg/kg. Out of the 40 samples analyzed, 30 (75 %) had detectable concentrations of albendazole. The JECFA MRL was not stated clearly in literature for albendazole in poultry eggs. Sulphathiazole was the second most frequently detected drug residue in Farm 1. It was detected at a mean concentration of 28.25 ± 0.17 µg/kg. The detectable concentration of sulphathiazole was 100% meaning sulphathiazole residue was detected in all the 40 egg samples. This detectable level was 3 times below the JECFA MRL given by the Codex Alimentarius Commission of 100 µg/kg as

stated in Mehtabuddin *et al*., (2012). Piperazine had an average concentration of 33.75 ± 0.68 µg/kg. Out of the 40 samples analyzed from the Farm1 in the Offinso district, 10 (25%) had detectable concentrations of piperazine. This level is about 60 times lower than the maximum residue limit of 2000 µg/kg given in the Annex III of Council Regulation (EEC) No 2377190 in the Committee for Veterinary Medicinal Products Summary Report for piperazine (Inspections, 2002). There were no detectable concentrations of residues of chloramphenicol (CMP), sulphamethoxazole (SMX), levamisole (LEV), Oxytetracycline (OTC) and tiamulin (TIA) residues in the egg samples from Farm 1 in the Offinso-South municipality.

4.5.2 Results from Farm 2

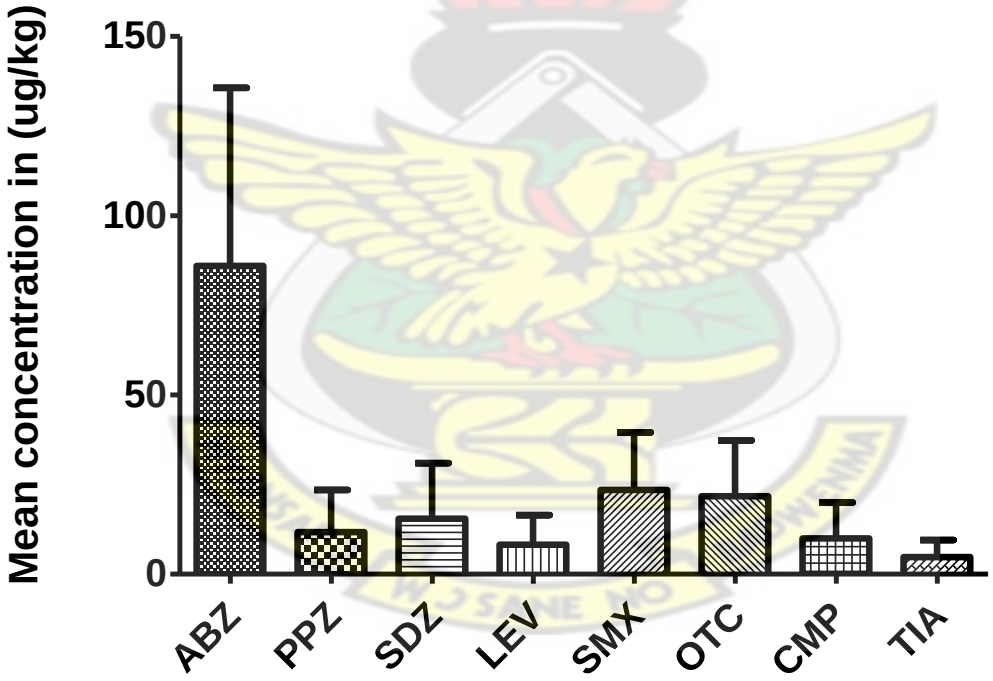


Fig 4.3 Levels of veterinary drug residues in egg samples from Farm 2

Farm 2 is also located in the Offinso District and was labeled as BF. The average concentration of veterinary drug residues from the egg samples from Farm 2 in the Offinso Districts were as follows; Albendazole was the most frequently detected chemical followed

by sulphamethoxazole and oxytetracycline. Albendazole occurred at an average concentration of 86 ± 0.99 µg/kg. Out of the 40 samples analyzed, 20 (50 %) had detectable concentrations of albendazole. The JECFA MRL was not stated in literature for albendazole in poultry eggs. Chloramphenicol recorded an average concentration of 10.00 ± 0.02 µg/kg which was (25%) detectable concentration. The JECFA MRL was not stated in literature for chloramphenicol in poultry eggs. Levamisole showed detectable concentration at an average of 8.25± 0.016 µg/kg. The MRL was not stated clearly in literature for levamisole in poultry eggs. Out of a total of 40 samples analyzed, there were 20 egg samples detected for oxytetracycline. In all the egg samples, oxytetracycline residues had an average concentration of 21.75± 0.03 µg/kg which was about 20 times below the JECFA MRL given by the Codex Alimentarius Commission (CAC) of 400 µg/kg (Commission, 2011). The level of Piperazine was detected at an average concentration of 11.75± 0.0 µg/kg which was about 180 times far below the maximum residue limit of 2000 µg/kg given by in the Annex III of Council Regulation (EEC) No 2377190 in the Committee for Veterinary Medicinal Products Summary Report for piperazine (Inspections, 2002). Out of a total of 40 samples analyzed, 20 (50%) had detectable concentrations of sulphamethoxazole at an average concentration of 23.5 ± 0.032 µg/kg which was about 4 times lower than the JECFA MRL given by the CAC of 100 µg/kg for sulphonamide residues determination in commercial poultry meat and eggs, Mehtabuddin *et al*., (2012). Sulphathiazole was detected at a mean concentration of 15.50 ± 0.03 µg/kg which was about 6 times below the JECFA MRL given by the CAC of 100 µg/kg Mehtabuddin *et al* ., (2012). Tiamulin had the least mean detectable concentration of 4.75± 0.00 µg/kg. Tiamulin was detected in 25 % of the 40 egg samples analyzed in Farm 2. This level was about 250 times far below the maximum residue limit of 1000 µg/kg given by the Annex I of Council Regulation (EEC) No 2377/90 in the Committee for Veterinary Medicinal Products Summary Report for tiamulin (Unit, 1999).

4.5.3 Results from Farm 3

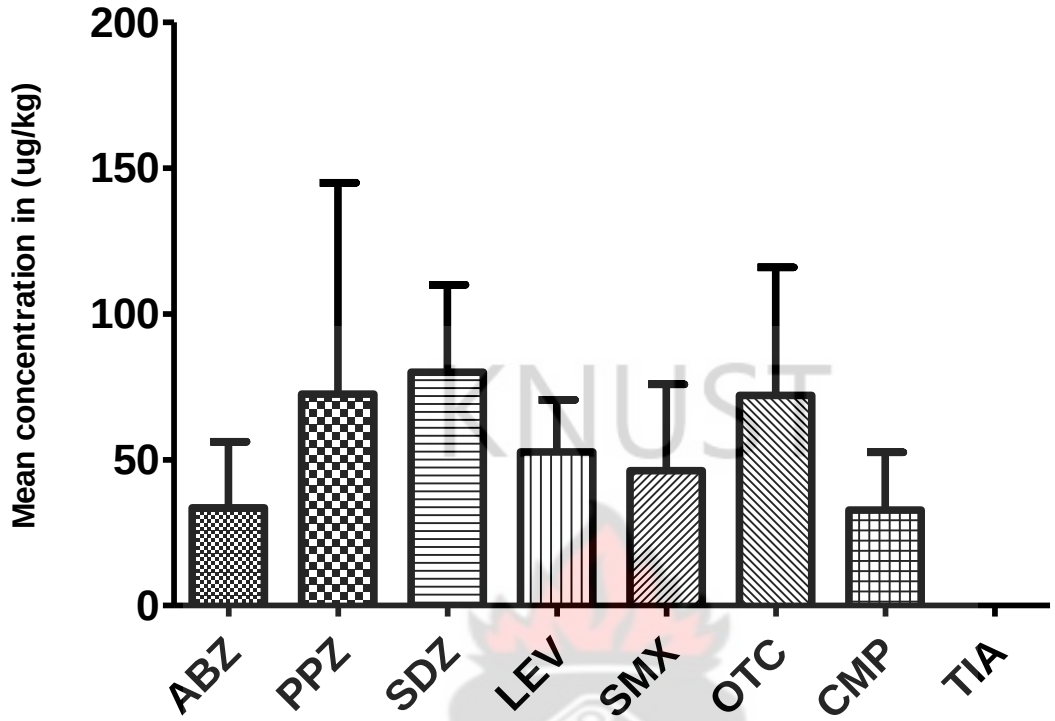


Fig 4.4 Levels of veterinary drug residues in egg samples from Farm 3

Farm 3 is found in the Kwabre East District and egg samples were labeled as TG. A total of 40 egg samples of eggs were taken from Farm 3.

Results from Farm 3 indicated that Sulphathiazole had the highest mean concentration of 80.0 ± 0.59 µg/kg and were just the same as the JECFA maximum residue limit given by The Codex Alimentarius Commission of 100 µg/kg. Piperazine was detected at an average concentration of 72.5 ± 0.15 µg/kg with that of oxytetracycline residue almost around the same mean concentration of 72.08 ± 0.88 µg/kg. This level of Piperazine was about 27 times below the maximum residue limit of 2000 µg/kg given by in the Annex III of Council Regulation (EEC) No 2377190 in the Committee for Veterinary Medicinal Products Summary Report for piperazine (Inspections, 2002) whilst that of the Oxytetracycline level was about 5 times lower than the JECFA maximum residue limit given by The Codex Alimentarius Commission of 400 µg/kg (Commission, 2011). Albendazole occurred at an

average concentration of 33.5 ± 0.05 µg/kg. Chloramphenicol was detected at an average concentration of 32.75 ± 0.04 µg/kg. Levamisole was also detected at an average concentration of 52.75 ± 0.03 µg/kg from the samples taken. The maximum residue limit was not stated clearly in literature for levamisole in poultry eggs. Sulphamethoxazole had an average concentration 2 times lower than that of the JECFA maximum residue limit given by The Codex Alimentarius Commission of 100 µg/kg which was published by (Mehtabuddin *et al*., 2012) for sulphonamide residues determination in commercial poultry meat and eggs also had concentration of 46.25 ± 5.93 µg/kg. No traces of tiamulin residues were detected in the egg.

4.5.4 Results from Farm 4

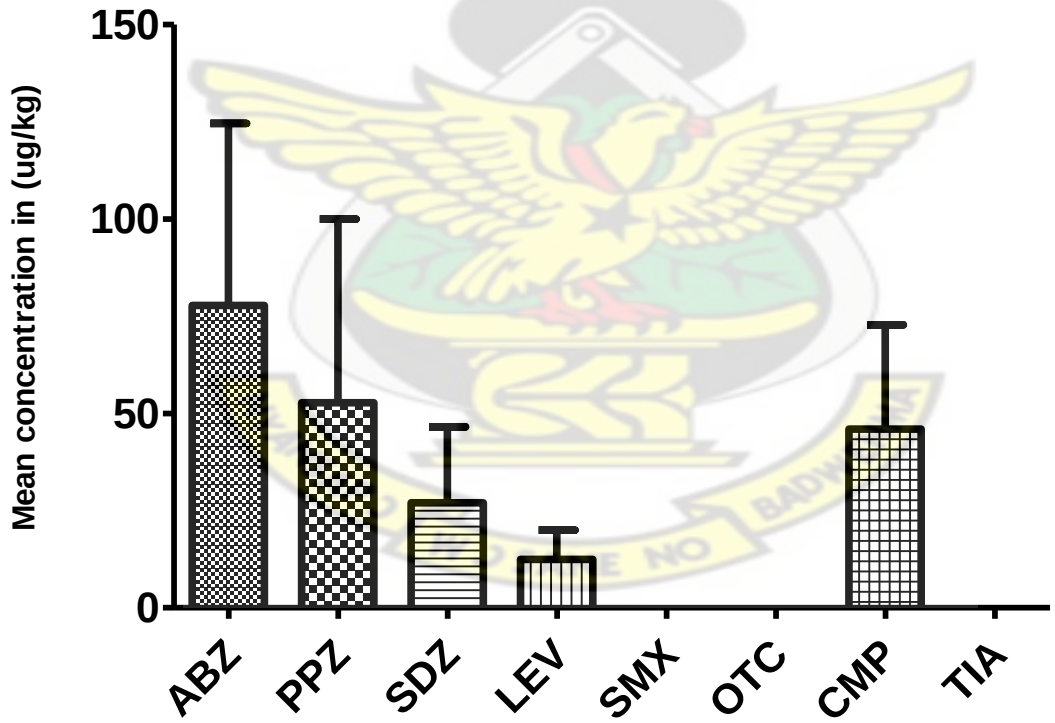


Fig 4.5 Levels of veterinary drug residues in egg samples from Farm 4

Farm 4 is found in the Kumasi Metropolis District and egg samples were labeled AG. A total of 40 samples of eggs were taken from Farm 4. No traces of Sulphamethoxazole, Oxytetracycline and Tiamulin residues were detected in the egg samples. Albendazole

occurred at an average concentration of 77.75 ± 0.94 µg/kg. Out of the 40 samples analyzed, 30 (75 %) had detectable concentrations of albendazole. The JECFA maximum residue limit was not stated clearly in literature for albendazole in poultry eggs. Chloramphenicol was also detected at an average concentration of 46 ± 0.54 µg/kg from the samples taken from the farm 4.. A total of 40 egg samples were collected from Farm 4 for the detection of levamisole residues. Levamisole was also detected at an average concentration of 12.5 ± 0.015 µg/kg. Piperazine was detected at an average concentration of 52.75 ± 0.94 µg/kg with 20(50%) detectable concentrations of piperazine out of the 40 samples analyzed. This level was about 38 times below the maximum residue limit of 2000 µg/kg given by in the Annex III of Council Regulation (EEC) No 2377190 in the Committee for Veterinary Medicinal Products Summary Report for piperazine (Inspections, 2002). Sulphathiazole was detected at a mean concentration of 27 ± 0.039 µg/kg level was about 4 times lower than the JECFA maximum residue limit given by The Codex Alimentarius Commission of 100 µg/kg, Mehtabuddin *et al*., (2012).

4.5.5 Results from Farm 5

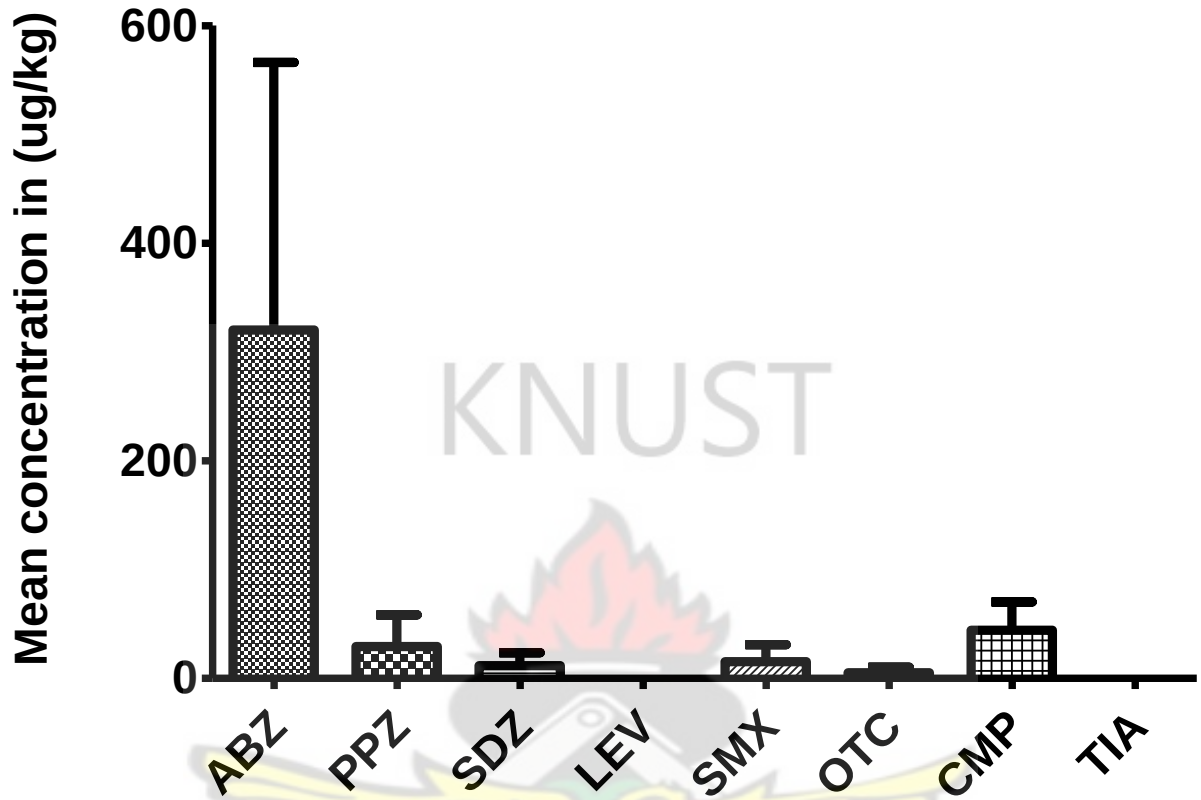


Fig 4.6 Levels of veterinary drug residues in egg samples from Farm 5

Farm 5 was also found in the Kumasi Metropolis and was labeled as ML. A total of 40 egg samples were taken from Farm 5. Per the results obtained from farm 5, there were no traces of levamisole and tiamulin residues. The two Sulphonamides (Sulphathiazole and Sulphamethoxazole, both detected at mean concentrations of 11.75 ± 0.02 µg/kg and 15.5 ± 0.03 µg/kg level were both around 8 times and 6 times respectively below the JECFA maximum residue limit given by the Codex Alimentarius Commission of 100 µg/kg which was published by Mehtabuddin *et al*., (2012) for sulphonamide residues in commercial poultry meat and eggs. Chloramphenicol registered 44.25 ± 0.52 µg/kg mean concentration whilst Oxytetracycline also registered an average concentration of 5.167 ± 0.01 µg/kg which was about 80 times below the JECFA maximum residue limit given by the Codex

Alimentarius Commission of 400 µg/kg (Commission, 2011). Piperazine was detected at an average concentration of 29.25 ± 0.06 µg/kg which was about 68 times below the maximum residue limit of 2000 µg/kg given by in the Annex III of Council Regulation (EEC) No 2377190 in the Committee for Veterinary Medicinal Products summary report for piperazine (Inspections, 2002). Albendazole had the highest average concentration of 320.3 ± 4.92 µg/kg.

4.6 RESULTS FROM THE THREE DISTRICTS

The subsequent discussion uses the same data as previous farms and further states any general patterns that developed.

4.6.1 Results from the Offinso District

Results from the two Farms (Farm 1 and 2) in the Offinso District showed that Albendazole occurred at the highest average concentration of 74.5 ± 0.72 µg/kg whilst Tiamulin recorded the lowest average concentration of 2.375 ± 0.006 µg/kg which was 400 times below the maximum residue limit of 1000 µg/kg given in the Annex I of Council Regulation (EEC) No 2377/90 in the Committee for Veterinary Medicinal Products Summary Report for Tiamulin (Unit, 1999).

4.6.2 Results from the Kwabre East Districts

Results the farm in the Kwabre East District showed that Sulphathiazole had the highest mean concentration of 80.0 ± 0.59 µg/kg and was just below the JECFA maximum residue limit given by The Codex Alimentarius Commission of 100 µg/kg as stated in (Mehtabuddin *et al*., 2012). Piperazine was detected at an average concentration of 72.5 ± 0.15 µg/kg with that of oxytetracycline residue almost around the same mean concentration of 72.08 ± 0.88 µg/kg.

4.6.3 Results from the Kumasi Metropolis Districts

The Kumasi Metropolis district had Farm 4 and 5. No traces of tiamulin were present in the two farms in the Kumasi Metropolis. Levamisole, Oxytetracycline and Sulphamethoxazole recorded very low concentrations of 6.25 ± 0.01 µg/kg, 2.58 ± 0.07 µg/kg and 7.75 ±0.02 µg/kg respectively with Oxytetracycline recording the lowest mean concentration.

4.7 Possible Explanations to Observations

From the interview with the poultry farmers, Albendazole was readily accessible to the poultry farmer and cheap so it was mostly used as a dewormer for the poultry birds on farms mostly in the Ashanti Region. The eggs were collected from the farm at the point of sale thus the eggs were sent for sale thus accounting for the high levels of residues. Tiamulin was the least detected.

Despite a rapid rate of elimination, detectable residues of Flubendazole and Albendazole can be found in eggs laid up to a week or more after treatment, Goetting *et al.*, (2011). Levamisole is used for treating nematode infections, but it is ineffective against cestodes or trematodes .There are no data available on the absorption or primary excretion pathways of levamisole in birds, but the drug concentrates in the liver following oral dosing in chickens and residues are found in the eggs of laying hens for up to 2 weeks after treatment (El-Kholy & Kemppainen, 2005).

The differences could be attributed to the geographical differences resulting in the different detectable concentration. Albendazole in the Kumasi Metropolis was the highest followed by that in the Offinso District and the Kwabre East districts respectively. Piperazine was highly detected in the Kwabre East district followed by the Kumasi Metropolis and then the Offinso district. Sulphathiazole was detected mostly in the farm in the Kwabre East District followed

by the two farms in the Kumasi Metropolis and the Offinso district. Tiamulin which was mostly not detected had its highest concentration in the Kumasi Metropolis.

Acetonitrile and hexane were used as solvents in the extraction process just like (Sasanya et al., (2005), Consuelo *et al.*, (2010), Taylor et al., (2003), Mehtabuddin *et al.*, (2004), Schneider and Donoghue, (2003), Omeiza *et al.*, (2012), Tolika *et al.*, (2011), Kao *et al.*, (2001b)).

Acetonitrile was found to be the best solvent for sample extraction because it is easy to evaporate and the lipid/oil interference could readily be removed from the extract solution by introducing n-hexane(Kao *et al.*, 2001b). A buffer, 0.01 M KH_2PO_4 (pH=5.10) Sasanya *et al.*, (2005), Kao *et al.*, (2001b) was used for preparation of the mobile phase. Across all the farms in the districts on the whole and in the individual farms, the average concentrations of all the drug residues of eggs were below the MRL given by JECFA just like the levamisole concentration of eggs was lower than that of the MRL, El-Kholy and Kemppainen, (2005) The level of residues in chicken liver was significantly higher compared to breast meat samples.. Liver plays a major role in body metabolism and has a number of functions in the body including glycogen storage, plasma protein synthesis and drug detoxification. Therefore, the levels of compound in liver sample are usually higher than other parts of chicken such as the egg, Cheong *et al.*, (2010). Unintentionally cross-contamination of veterinary drugs into feeding stuffs may generate a violative residue problem in animal products, Mehtabuddin *et al.*, (2012).

Kentinkrono in the Kumasi Metropolitan registered the highest mean concentration of 122.3 ± 2.21 µg/kg and closely followed by the Kwabre-East District which recorded 97.52 ± 6.64 µg/kg. Offinso-South Municipality recorded the lowest mean concentration of 64.53 ± 4.87 µg/kg drug residues.

4.7 DIETARY EXPOSURE ASSESSMENT

Dietary exposure assessment combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate may then be compared with the relevant health-based guidance value for the food chemical of concern, if available, as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures, where acute exposure covers a period of up to 24 hours and chronic or long-term exposure covers average daily exposure over the entire lifetime (WHO/FAO, 2011).

The general equation for both acute and chronic dietary exposure is:

$$Dietary\ exposure\ = \frac{\sum\ concentration\ \ of\ chemicals\ in\ food\ \times\ food\ consumption}{Body\ \ weight}$$

for all foods containing the residue.

4.7.1 Acceptable Daily Intake (ADI)

This is an estimate of the amount of a substance in food expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable risk (Standard human = 60 kg International Programme on Chemical Safety (IPCS), 2010; Common Wealth of Australia, 2013). The ADI is listed in units of µg per kg of body weight.

Table 4.2: General considerations for dietary exposure estimates for veterinary drug residues (µg/kg)

Drug Residues	Concentration of Chemical in Food (µg /kg)	Food Consumption ADI (µg /kg)	GEADE (µg /kg)
Albendazole	0.581	0.05	0.004
Piperazine	0.200	0.25	0.0008
Sulphathiazole	0.163	0.02	0.00005
Sulphamethoxazole	0.074	0.05	0.00001
Levamisole	0.085	0.003	0.000004
Oxytetracycline	0.099	0.03	0.00005
Chloramphenicol	0.133	N/A	-
Tiamulin	0.004	0.03	0.000002

4.7.2 GEADE - Global Estimated Acute Dietary Exposure

The Committee for Veterinary Medicinal Product (Summary report, 2009) concluded that, no ADI could be estimated for Chloramphenicol, because of the inability to identify a 'threshold' level for induction of aplastic anaemia in humans, lack of an adequate carcinogenicity study are a lack of an adequate reproductive toxicity study.

Table 4.2 shows the general considerations for dietary exposure estimates for veterinary drug residues. Albendazole residues recorded the highest concentration of 0.581 µg/kg in the egg samples, followed by Piperazine (0.200 µg/kg) and Sulphathiazole (0.163 µg/kg) respectively.

Tiamulin recorded the lowest concentration of (0.004 µg/kg) drug residues in all the egg samples.

Although the global estimated acute dietary exposure for the veterinary drug residues under study have not yet been established, the GEADE of each drug residue was compared to its ADI. For albendazole, the highest global estimated acute dietary exposure for the drug residues under study recorded 0.004 µg/kg compared to Tiamulin and Levamisole which recorded the lowest global estimated acute dietary exposure of 0.000002 µg/kg and 0.000004 µg/kg respectively. All the global estimated acute dietary exposure of the drug residues was lower than their ADI's. However, the GEADE of sulphamethoxazole was quite what close to its ADI.

CHAPTER FIVE

5.1 CONCLUSION

The results from the study indicated that, of the 200 samples analyzed for the eight veterinary drugs; albendazole had 70% detectable concentration, chloramphenicol had 35% detectable concentration, levamisole had 30% detectable concentration, piperazine had 30% detectable concentration, sulphamethoxazole had 25% detectable concentration, sulphathiazole had 55% detectable concentration, oxytetracycline had 30% detectable concentration and tiamulin had 5% detectable concentration

The mean levels of all the drug residues detected were below the JECFA MRLs. However detectable albendazole residues may be injurious to human health because the residues may accumulate to higher levels in human beings.

After several interactions with the farmers, it was a known fact that all the farmers did not observe the withdrawal period of the veterinary drugs before they are administered to the birds and finally sold out to the consumers. Some of the poultry farmers did not prefer over-the-counter drugs.

Chloramphenicol which is frequently used in poultry farms in Ghana may be injurious to human health since it accumulates in meat and eggs. In view of all these circumstances, foods of animal origin must be monitored for the presence of veterinary drug residues. These results even though lower than their respective ADI'S indicate that consumers are predisposed to health hazards due to bioaccumulation.

5.2 RECOMMENDATIONS FOR FURTHER DEVELOPMENT

Efforts are, needed on the part of the national authorities, Non-Governmental Organizations and industries to manage the problem of residual drugs in chicken eggs in Ghana.

To this end the following steps are recommended.

1. Poultry farmers should be made to strictly avoid the use of over-the-counter drugs.
2. Veterinary drugs used for the control and treatment of diseases must also be issued by the appropriate authorities to avoid the indiscriminate use of drugs.
3. The poultry farmer must be educated on the withdrawal period of different drugs and their health effects on human if they are not adhered to.
4. The media be it electronic or by print means should be used for the education of the drug residues to the general public in Ghana.
5. Monitoring laboratories must be set up throughout Ghana to control drugs residues in eggs, meat and other food products which were studied in this study.
6. The government of Ghana should develop an implementation for the above suggestions.

REFERENCES

Aganga, A. A., Tshwenyane, S and Molefhe, L. (2003). Influence of Feed Type on Egg Production of Tswana Laying Chicken. *International Journal of Poultry Science*, *2*(4), 256–258.

Al-Mastafa, Z. H and Al-Ghamdi, M. S. (2002). Use Of Antibiotics In The Poultry Industry In Saudi Arabia : Implications For Public Health. *Ann Saudi Medicine*, *22*(1,2), 4–7.

Al-wabel, N. A. (2011). Monitoring of Tetracycline Residues in Table Eggs Collected From Qassim Region, KSA. *Journal of Agricultural and Veterinary Sciences*, *4*(2), 109–123.

Barreto, F., Ribeiro, C., Hoff, R. B and Costa, T. D. (2012). Food Additives & Contaminants : Part A Determination and confirmation of chloramphenicol in honey , fish and prawns by liquid chromatography – tandem mass spectrometry with minimum sample preparation : validation according to 2002 / 657 / EC Directive. *Food Additives & Contaminants*, *29*(3), 550–558.

Bistoletti, M., Alvarez, L., Lanusse, C and Moreno, L. (2012). Disposition kinetics of albendazole and metabolites in laying hens. *Veterinary Pharmacology and Therapeutics*, *36*(2), 161–168.

Cheong, C. K., Hajeb, P., Jinap, S and Ismail-Fitry, M. R. (2010). Sulfonamides determination in chicken meat products from Malaysia. *International Food Research Journal*, *17*(4), 885–892.

Chiaochan, C., Koesukwiwat, U., Yudthavorasit, S and Leepipatpiboon, N. (2010). Analytica Chimica Acta Efficient hydrophilic interaction liquid chromatography – tandem mass spectrometry for the multiclass analysis of veterinary drugs in chicken muscle. *Analytica Chimica Acta*, *682*(1-2), 117–129.

Chowdhury, R., Haque, M. N., Islam, K. M. S and Khaleduzzaman, A. B. M. (2009). A review on antibiotics in an animal feed. *Bang. Journal of Animal Science ., 38*(1,2), 22–32.

Chrusch, J., Lee, S., Fedeniuk, R and Boison, J. O. (2008). Determination of the performance characteristics of a new multi-residue method for non-steroidal anti- inflammatory drugs, corticosteroids and anabolic steroids in food animal tissues. *Food Additives & Contaminants*, *25*(12), 1482–1496.

Clayton, R. (2005). Impact of new legislation on the registration of veterinary drugs. *Analytica Chimica Acta*, *529*(1), 3–6.

Commission, C. A. (2011). *Maximum residue limits for veterinary drugs in foods Codex Alimentarius Commission Maximum Residue Limits for Veterinary Drugs in Foods* (pp. 1–36).

Consuelo Chafer-Perica, Maquieira, A and Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *Trends in Analytical Chemistry*, *29*(9), 1039–1049.

Darko, G and Akoto, O. (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food and Chemical Toxicology*, *46*(12), 3703–3706.

Donoghue, D J and Hairston, H. (1999). Oxytetracycline transfer into chicken egg yolk or albumen. *Poultry Science*, *78*(3), 343–5.

Donoghue, Dan J. (2003). Antibiotic Residues In Poultry Tissues and Eggs : Human Health Concerns ? *Poultry Science*, *82*(1), 618–621.

El-ghany, W. A. A. (2009). The In-vitro and In-vivo evaluation of tiamulin and tilmicosin for the treatment of mycoplasma gallisepticum infected broiler chickens. *International Journal of Poultry Science*, *8*(12), 1189–1198.

El-Kholy, H., Kemppainen, B., Ravis, W and Hoerr, F. (2006). Pharmacokinetics of levamisole in broiler breeder chickens. *Journal of veterinary pharmacology and therapeutics*, *29*(1), 49–53.

El-Kholy, H., & Kemppainen, B. W. (2005). Levamisole residues in chicken tissues and eggs. *Poultry Science*, *84*(1), 9–13.

Fagbamila, I. O., Ngulukun, S., Ardzard, S., Sati, N., Ajayi, O., Ankeli, P. I and Muhammed, M. (2012). microbials by commercial kit. *Research journal of veterinary sciences*, *5*(3), 75–80.

Ferguson, J., Baxter, A., Young, P., Kennedy, G., Elliott, C., Weigel, S and Sharman, M. (2005). Detection of chloramphenicol and chloramphenicol glucuronide residues in poultry muscle , honey , prawn and milk using a surface plasmon resonance biosensor and Qflex ® kit chloramphenicol. *Analytica Chimica Acta*, *529*(1,2), 109–113

Frenich, A. G., Aguilera-luiz, M. M., Luis, J., Vidal, M and Romero-González, R. (2010). Analytica Chimica Acta Comparison of several extraction techniques for multiclass analysis of veterinary drugs in eggs using ultra-high pressure liquid chromatography – tandem mass spectrometry. *Analytica Chimica Acta*, *661*(2), 150–160.

Furusawa, N. (2003a). Rapid high-performance liquid chromatographic determining technique of sulfamonomethoxine, sulfadimethoxine and sulfaquinoxaline in eggs without use of organic solvents. *Analytica Chimica Acta*, *481*(2), 255–259.

Furusawa, N. (2003b). An Organic Solvent – Free Method for Determining Oxytetracycline in Cow's Milk The author developed an organic solvent – free method for the. *Lcgc North America*, *21*(4), 4–6.

Galarini, R., Piersanti, A., Falasca, S., Salamida, S and Fioroni, L. (2007). A confirmatory method for detection of a banned substance : The validation experience of a routine EU laboratory. *Analytica Chimica Acta*, *586* (1,2), 130–136.

Gamba, V., Terzano, C., Fioroni, L., Moretti, S., Dusi, G and Galarini, R. (2008). Development and validation of a confirmatory method for the determination of sulphonamides in milk by liquid chromatography with diode array detection. *Analytica Chimica Acta*, *637*(1,2), 18–23.

Goetting, V., Lee, K. A and Tell, L. A. (2011). Pharmacokinetics of veterinary drugs in laying hens and residues in eggs : a review of the literature. *Veterinary Pharmacology and Therapeutics*, *34*(6), 521–624.

Haller, M. Y., Muller, S. R., McArdell, C. S., Alder, A. C and Suter, M. J. F. (2002). Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography – mass spectrometry. *Journal of Chromatography A*, *952*(1), 111–120.

Herranz, S and Marazuela, M. D. (2007). Development of a new sample pretreatment procedure based on pressurized liquid extraction for the determination of fluoroquinolone residues in table eggs. *Journal of Chromatography A*, *1140*(1), 63–70.

Huet, A., Mortier, L., Daeseleire, E., Fodey, T., Elliott, C and Delahaut, P. (2005). Development of an ELISA screening test for nitroimidazoles in egg and chicken muscle. *Analytica Chimica Acta*, *534*(1), 157–162.

Hussein K, Marcin^Ák S, Máté D, KO Árová Ivona , S. J. and Z. N. (2005). *Acta Veterinaria (Beograd)*, *55*(5,6), 493–500.

Idowu, F., Junaid, K., Paul, A., Gabriel, O., Paul, A., Sati, N and Jarlath, U. (2010a). Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *International Journal of Poultry Science*, *9*(10), 959–962.

Idowu, F., Junaid, K., Paul, A., Gabriel, O., Paul, A., Sati, N and Jarlath, U. (2010b). Antimicrobial screening of commercial eggs and determination of tetracycline residue

using two microbiological methods. *International Journal of Poultry Science*, *9*(10), 959–962.

Inspections, T. E. A. for the E. of M. P. V. M. and I. (2002). *Committee for veterinary medicinal products* (1–6).

Kabir, J, Umoh, V, J, Audu-okoh, E, Umoh, J, U, Kwaga, J, K, P. (2004). Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State , Nigeria. *Food Control*, *15*(2), 99–105.

Kao, Y., Chang, M., Cheng, C and Chou, S. (2001a). Multiresidue Determination of Veterinary Drugs in Chicken and Swine Muscles by High Performance Liquid Chromatography. *Journal of Food and Drug Analysis*, *9*(2), 84–95.

Kao, Y., Chang, M., Cheng, C and Chou, S. (2001b). Multiresidue Determination of Veterinary Drugs in Chicken and Swine Muscles by High Performance Liquid Chromatography. *Journal of Food and Drug Analysis*, *9*(2), 84–95.

Kehinde, O. G., Junaidu, K., Mohammed, M., Abdulrahman, A. M and State, K. (2012). Detection of antimicrobial drug residues in commercial eggs using Premi ® Test. *International Journal of Poultry Science*, *11*(1), 50–54.

Khattab, W. O., Elderea, H. B., Salem, E. G and Gomaa, N. F. (2010). Transmission of administered amoxicillin drug residues from laying chicken to their commercial eggs. *J Egypt Public Health Assoc*, *85*(5,6), 297–316.

McEvoy, J. D. . (2002). Contamination of animal feedingstuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. *Analytica Chimica Acta*, *473*(1), 3–26.

Mehdizadeh, S., Kazerani, H. R and Jamshidi, A. (2010a). *Iranian Journal of Veterinary Science and Technology*, *2*(1), 25–32.

Mehdizadeh, S., Kazerani, H. R and Jamshidi, A. (2010b). Screening of Chloramphenicol Residues in Broiler Chickens Slaughtered in an Industrial Poultry Abattoir in Mashhad , Iran. *Iranian Journal of Veterinary Science and Technology*, *2*(1), 25–32.

Mehtabuddin, A. A. Mian, T. Ahmad, S. Nadeem , Z. I. T. and J. A. (2012). Sulfonamide Residues Determination In Commercial Poultry Meat. *The Journal of Animal & Plant Sciences*, *22*(2), 473–478.

Mor, F., Kocasari, F. S., Ozdemir, G and Oz, B. (2012). Determination of sulphonamide residues in cattle meats by the Charm-II system and validation with high performance liquid chromatography with fluorescence detection. *Food Chemistry*, *134*(3), 1645–1649.

Mulder, P. P. J., Brinke, E. M., Bolck, Y. J. C., Berendsen, B. J. A and Gerc, H. (2005). Deposition and depletion of the coccidiostats toltrazuril and halofuginone in eggs. *Analytica Chimica Acta*, *529*(1), 331–337.

Mushtaq, A., Khurshid, K and Pal, S. S. (2010). Comparison of efficacy and safety of oral ivermectin with topical permethrin in treatment of scabies. *Journal of Pakistan Association of Dermatologists*, *20*(3), 227–231.

Naeem, M., Khan, K and Rafiq, S. (2006). Determination of residues of quinolones in poultry products by High Pressure Liquid Chromatography. *Journal of Applied Sciences*, *6*(2), 373–379.

Nielen, A. A. M. Stolker , P. Rutgers , E. Oosterink , J. J. P. Lasaroms , R. J. B. Peters , J. A. van Rhijn, M. W. F. (2008). Comprehensive screening and quantification of veterinary drugs in milk using UPLC – ToF-MS. *Analytical Bioanalytical Chemistry*, *391*(6), 2309–2322.

Nonga, H. E., Simon, C., Karimuribo, E. D and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro Municipality , Tanzania. *Zoonoses and public health*, *57*(5), 339–344.

Olatoye, I and Ehinmowo, A. . (2010). Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. *Nigerian Veterinary Journal*, *31*(2), 93–102.

Olusola, A. V., Diana, B. E and Ayoade, O. I. (2012). Assessment of Tetracycline, lead and cadmium residues in frozen chicken Vended in Lagos and Ibadan, Nigeria. *Parkistan Journal oF Biological Sciences*, *15*(17), 839–844.

Omeiza, G. K., Kabir, J., Mamman, M., Ibrahim, H and Fagbamila, I. O. (2012). Response of Nigerian farmers to a questionnaire on chloramphenicol application in commercial layers. *Veterinaria italiana*, *48*(1), 87–93.

Pavlov, A., Lashev, L., Vachin, I and Rusev, V. (2008). Residues Of antimicrobial drugs In chicken meat And offals. *Trakia Journal of Sciences*, *6*(1), 23–25.

Pensabene, J. W., Fiddler, W and Parks, O. W. (1997). Isolation of Sulfonamides from whole egg by Supercritical Fluid Extraction. *Journal of Chromatographic Science*, *35*(6), 270–274.

Pomorska-mól, M and Kowalski, C. (2009). Determination of tiamulin in chickens ' plasma by HPLC with UV-VIS Detection. *Journal of Liquid Chromatography & Related Technologies*, *32*(7), 1023–1031.

Reig, M., and Toldra, F. (2008). Meat veterinary drug residues in meat : Concerns and rapid methods for detection. *Meat Science*, *78*(1,2), 60–67.

RUYCK, ir. H. DE. (2003). *Development Of Confirmatory Methods And Disposition Of Some Veterinary Drugs In Food*.

Sasanya, J. J., Okeng, J. W. O., Ejobi, F and Muganwa, M. (2005). Use of sulfonamides in layers in Kampala district, Uganda and sulfonamide residues in commercial eggs. *African health sciences*, *5*(1), 33–9.

Schneider, M. J and Donoghue, D. J. (2003). Multiresidue determination of fluoroquinolone antibiotics in eggs using liquid chromatography – fluorescence – mass spectrometry n ଌ. *Analytica Chimica Acta*, *483*(1), 39–49.

Schneider, M. J., Mastovska, K., Lehotay, S. J., Lightfield, A. R., Kinsella, B and Shultz, C. E. (2009). Comparison of screening methods for antibiotics in beef kidney juice and serum ଌ. *Analytica Chimica Acta*, *637*(1,2), 290–297.

Shankar, B. P., Prabhu, M. B. H., Chandan, S., Ranjith, D and Shivakumar, V. (2010). Rapid methods for detection of veterinary drug residues in meat. *Veterinary World*, *3*(5), 241–246.

Shareef, A. M., Jamel, Z. T and Yonis, K. M. (2009). Detection of antibiotic residues in stored poultry products. *Iraqi Journal of Veterinary Sciences*, *23*(3), 45–48.

Silvia, Regina Rocha Siqueira, Jose´, L. D., Nucci, G. de and Reyes, F. G. R. (2009). A high-throughput method for determining chloramphenicol residues in poultry, egg, shrimp, fish, swine and bovine using LC-ESI- MS/MS. *Journal of separation science*, *32*(23,24), 4012–4019.

Sirdar, M. M., Picard, J., Bisschop, S and Alexander R. Jambalang, B. G. (2012). A survey of antimicrobial residues in table eggs in Khartoum State , Sudan , 2007 – 2008. *Journal of Veterinary Research*, *79*(1), 1–9.

Stead, S., Richmond, S., Sharman, M., Stark, J and Geijp, E. (2005). A new approach for detection of antimicrobial drugs in food Premi ® Test coupled to scanner technology. *Analytica Chimica Acta*, *529*(1,2), 83–88.

Stolker, A. A. M and Brinkman, U. A. T. (2005). Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals — a review. *Journal of Chromatography A*, *1067*(1,2), 15–53.

Stolker, A. A. M., Zuidema, T and Nielen, M. W. F. (2007). Residue analysis of veterinary drugs and growth-promoting agents. *Trends in Analytical Chemistry*, *26*(10), 967–979.

Tajick, M. A and Shohreh, B. (2006). Detection of antibiotics residue in chicken meat using TLC. *International Journal of Poultry Science*, *5*(7), 611–612.

Taylor, P., Hormazábal, V., Yndestad, M and Ostensvik, O. (2003). Determination of flunixin and tiamulin hydrogen fumarate in meat and toltrazuril and the metabolite toltrazurilsulfon in meat and eggs Using LC/MS. *Journal of Liquid Chromatography and related technologies*, *26*(5), 791–801.

Tolika, E. P., Samanidou, V. F and Papadoyannis, I. N. (2011). Development and validation of an HPLC method for the determination of ten sulfonamide residues in milk according to 2002 / 657 / EC. *Journal of Separation. Science.*, *34*(14), 1627–1635.

Unit, T. E. A. for the E. of M. P. V. M. and I. T. (1999). *Committee For Veterinary Medicinal Products,Tiamulin Smmary Report*.

Wang, L., Yang, H., Zhang, C., Mo, Y and Lu, X. (2008). Determination of oxytetracycline , tetracycline and chloramphenicol antibiotics in animal feeds using subcritical water extraction and high performance liquid chromatography. *Analytica Chimica Acta*, *9*, 54–58.

Wei, R., Wang, R., Zeng, Q., Chen, M and Liu, T. (2009). High-performance liquid chromatographic method for the determination of cyromazine and melamine residues in milk and pork. *Journal of Chromatographic Science*, *47*(7), 581–584.

Yakkundi, S., Cannavan, A., Young, P. ., Elliott, C and Kennedy, D. . (2002). Halofuginone contamination in feeds as a cause of residues in eggs. *Analytica Chimica Acta*, *473*(1), 177–182.

Zhao, L., Dong, Y. H and Wang, H. (2010). Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment*, *408*(5), 1069–1075.

Zheng, M., Zhang, M., Peng, G and Feng, Y. (2008). Monitoring of sulfonamide antibacterial residues in milk and egg by polymer monolith microextraction coupled to hydrophilic interaction chromatography / mass spectrometry. *Analytica Chimica Acta*, *625*(2), 160–172.

Zotou, A and Vasiliadou, C. (2010). LC of sulfonamide residues in poultry muscle and eggs extracts using fluorescence pre-column derivatization and monolithic silica column. *Journal of separation science*, *33*(1), 11–22.

APPENDICE

Appendix A: Column Statistics of levels of veterinary drug residues in eggs from Farm 1, Offinso Districts

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0	0.0	19.00	0.0	0.0	0.0	0.0	0.0
25% Percentile	20.25	0.0	19.25	0.0	0.0	0.0	0.0	0.0
Median	82.00	0.0	20.00	0.0	0.0	0.0	0.0	0.0
75% Percentile	86.75	101.3	45.50	0.0	0.0	0.0	0.0	0.0
Maximum	88.00	135.0	54.00	0.0	0.0	0.0	0.0	0.0
Mean	63.00	33.75	28.25	0.0	0.0	0.0	0.0	0.0
Std. Deviation	42.10	67.50	17.17	0.0	0.0	0.0	0.0	0.0
Std. Error	21.05	33.75	8.587	0.0	0.0	0.0	0.0	0.0
Lower 95% CI of mean	-3.996	-73.66	0.9235	0.0000e+000	0.0000e+000	0.0000e+000	0.0000e+000	0.0000e+000
Upper 95% CI of mean	130.0	141.2	55.58	0.0000e+000	0.0000e+000	0.0000e+000	0.0000e+000	0.0000e+000
Sum	252.0	135.0	113.0	0.0	0.0	0.0	0.0	0.0

Appendix B: Column Statistics of levels of veterinary drug residues in eggs from Farm 2, Offinso Districts

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median	0.0860	0.0	0.0	0.0	0.0130	0.0105	0.0	0.0
75% Percentile	0.1720	0.03525	0.0465	0.02475	0.0575	0.05475	0.0300	0.01425
Maximum	0.1720	0.0470	0.0620	0.0330	0.0680	0.0660	0.0400	0.0190
Mean	0.0860	0.01175	0.0155	0.00825	0.0235	0.02175	0.0100	0.00475
Std. Deviation	0.09930	0.0235	0.0310	0.0165	0.03210	0.03112	0.0200	0.0095
Std. Error	0.04965	0.01175	0.0155	0.00825	0.01605	0.01556	0.0100	0.00475
Lower 95% CI of mean	-0.07202	-0.02564	-0.03383	-0.01801	-0.02758	-0.02776	-0.02182	-0.01037
Upper 95% CI of mean	0.2440	0.04914	0.06483	0.03451	0.07458	0.07126	0.04182	0.01987
Sum	0.3440	0.0470	0.0620	0.0330	0.0940	0.0870	0.0400	0.0190

Appendix C: Column Statistics of levels of veterinary drug residues in eggs from Farm 3, Kwabre-East Districts

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0	0.0	0.01875	0.01575	0.0	0.005083	0.0	0.0
Median	0.0190	0.0	0.0890	0.0670	0.0305	0.04617	0.0255	0.0
75% Percentile	0.0815	0.2175	0.1323	0.0755	0.1083	0.1650	0.07275	0.0
Maximum	0.0960	0.2900	0.1420	0.0770	0.1240	0.1960	0.0800	0.0
Mean	0.0335	0.0725	0.0800	0.05275	0.04625	0.07208	0.03275	0.0
Std. Deviation	0.04535	0.1450	0.05999	0.03563	0.05928	0.08800	0.03963	0.0
Std. Error	0.02268	0.0725	0.03000	0.01782	0.02964	0.04400	0.01981	0.0
Lower 95% CI of mean	-0.03867	-0.1582	-0.01547	-0.003948	-0.04807	-0.06794	-0.03030	0.0000e+000
Upper 95% CI of mean	0.1057	0.3032	0.1755	0.1094	0.1406	0.2121	0.09580	0.0000e+000
Sum	0.1340	0.2900	0.3200	0.2110	0.1850	0.2883	0.1310	0.0

Appendix D: Column Statistics of levels of veterinary drug residues in eggs from Farm 4, Kumasi Metropolis

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0050	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median	0.0515	0.0085	0.0125	0.0100	0.0	0.0	0.0415	0.0
75% Percentile	0.1768	0.1498	0.0685	0.0275	0.0	0.0	0.0965	0.0
Maximum	0.2080	0.1940	0.0830	0.0300	0.0	0.0	0.1010	0.0
Mean	0.07775	0.05275	0.0270	0.0125	0.0	0.0	0.0460	0.0
Std. Deviation	0.09376	0.09451	0.03915	0.0150	0.0	0.0	0.05362	0.0
Std. Error	0.04688	0.04725	0.01957	0.0075	0.0	0.0	0.02681	0.0
Lower 95% CI of mean	-0.07144	-0.09763	-0.03530	-0.01137	0.0000e+000	0.0000e+000	-0.03933	0.0000e+000
Upper 95% CI of mean	0.2269	0.2031	0.08930	0.03637	0.0000e+000	0.0000e+000	0.1313	0.0000e+000
Sum	0.3110	0.2110	0.1080	0.0500	0.0	0.0	0.1840	0.0

Appendix E: Column Statistics of levels of veterinary drug residues in eggs from Farm 5, Kumasi Metropolis

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0560	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.06275	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median	0.0835	0.0	0.0	0.0	0.0	0.0	0.0380	0.0
75% Percentile	0.8145	0.08775	0.03525	0.0	0.0465	0.0155	0.09475	0.0
Maximum	1.058	0.1170	0.0470	0.0	0.0620	0.02067	0.1010	0.0
Mean	0.3203	0.02925	0.01175	0.0	0.0155	0.005167	0.04425	0.0
Std. Deviation	0.4920	0.0585	0.0235	0.0	0.0310	0.01033	0.05210	0.0
Std. Error	0.2460	0.02925	0.01175	0.0	0.0155	0.005167	0.02605	0.0
Lower 95% CI of mean	-0.4626	-0.06384	-0.02564	0.0000e+000	-0.03383	-0.01128	-0.03866	0.0000e+000
Upper 95% CI of mean	1.103	0.1223	0.04914	0.0000e+000	0.06483	0.02161	0.1272	0.0000e+000
Sum	1.281	0.1170	0.0470	0.0	0.0620	0.02067	0.1770	0.0

Appendix F: Column Statistics Of Data From Offinso District

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	80	80	80	80	80	80	80	80
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median	0.0820	0.0	0.0195	0.0	0.0	0.0	0.0	0.0
75% Percentile	0.1510	0.03525	0.0455	0.0	0.0195	0.01575	0.0	0.0
Maximum	0.1720	0.1350	0.0620	0.0330	0.0680	0.0660	0.0400	0.0190
Mean	0.0745	0.02275	0.02188	0.004125	0.01175	0.01088	0.0050	0.002375
Std. Deviation	0.07167	0.04825	0.02418	0.01167	0.02448	0.02345	0.01414	0.006718
Std. Error	0.02534	0.01706	0.008549	0.004125	0.008656	0.008293	0.0050	0.002375
Lower 95% CI of mean	0.01458	-0.01758	0.001659	-0.005629	-0.008718	-0.008734	-0.006823	-0.003241
Upper 95% CI of mean	0.1344	0.06308	0.04209	0.01388	0.03222	0.03048	0.01682	0.007991
Sum	0.5960	0.1820	0.1750	0.0330	0.0940	0.0870	0.0400	0.0190

Appendix G: Column Statistics Of Data From Kwabre East District

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	40	40	40	40	40	40	40	40
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0	0.0	0.01875	0.01575	0.0	0.005083	0.0	0.0
Median	0.0190	0.0	0.0890	0.0670	0.0305	0.04617	0.0255	0.0
75% Percentile	0.0815	0.2175	0.1323	0.0755	0.1083	0.1650	0.07275	0.0
Maximum	0.0960	0.2900	0.1420	0.0770	0.1240	0.1960	0.0800	0.0
Mean	0.0335	0.0725	0.0800	0.05275	0.04625	0.07208	0.03275	0.0
Std. Deviation	0.04535	0.1450	0.05999	0.03563	0.05928	0.08800	0.03963	0.0
Std. Error	0.02268	0.0725	0.03000	0.01782	0.02964	0.04400	0.01981	0.0
Lower 95% CI of mean	-0.03867	-0.1582	-0.01547	-0.003948	-0.04807	-0.06794	-0.03030	0.0000e+000
Upper 95% CI of mean	0.1057	0.3032	0.1755	0.1094	0.1406	0.2121	0.09580	0.0000e+000
Sum	0.1340	0.2900	0.3200	0.2110	0.1850	0.2883	0.1310	0.0

Appendix H: Column Statistics Of Data From Kumasi Metropolis

	ABZ	PPZ	SDZ	LEV	SMX	OTC	CMP	TIA
Number of values	80	80	80	80	80	80	80	80
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25% Percentile	0.0290	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median	0.0830	0.0	0.0	0.0	0.0	0.0	0.0380	0.0
75% Percentile	0.1770	0.0920	0.0415	0.0150	0.0	0.0	0.0965	0.0
Maximum	1.058	0.1940	0.0830	0.0300	0.0620	0.02067	0.1010	0.0
Mean	0.1990	0.0410	0.01938	0.00625	0.00775	0.002583	0.04513	0.0
Std. Deviation	0.3526	0.07384	0.03098	0.01188	0.02192	0.007307	0.04896	0.0
Std. Error	0.1247	0.02611	0.01095	0.004199	0.00775	0.002583	0.01731	0.0
Lower 95% CI of mean	-0.09577	-0.02073	-0.006528	-0.003680	-0.01058	-0.003525	0.004196	0.0000e+000
Upper 95% CI of mean	0.4938	0.1027	0.04528	0.01618	0.02608	0.008692	0.08605	0.0000e+000
Sum	1.592	0.3280	0.1550	0.0500	0.0620	0.02067	0.3610	0.0

Appendix J: One Way Anova Statistics Of Comparison Among The Three Districts

Parameter				
Table Analyzed	DATA COMPARISON THREE DISTRICTS			
One-way analysis of variance				
P value	0.0346			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	24			
F	1.691			
R squared	0.2223			
ANOVA Table	SS	df	MS	
Treatment (between columns)	0.3219	23	0.01400	
Residual (within columns)	1.126	136	0.008279	
Total	1.448	159		
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary
TM CON 2 vs AB CON 3	-0.1990	5.051	No	ns
TM CON 2 vs STH CON 2	-0.0800	---	No	ns
TM CON 2 vs AB CON	-0.0745	---	No	ns
TM CON 2 vs PZ CON 2	-0.0725	---	No	ns
TM CON 2 vs OT CON 2	-0.07208	---	No	ns
TM CON 2 vs LM CON 2	-0.05275	---	No	ns
TM CON 2 vs SMTH CON 2	-0.04625	---	No	ns
TM CON 2 vs CLPH CON 3	-0.04513	---	No	ns

Appendix K: Recovery of 8 veterinary drugs spiked into chicken eggs with 0.1ppm

Drug	**Peak area**	**Concentration**	**% recovery**
Albendazole	4947	0.098	98.0
Piperazine	4665	0.095	95.0
Sulphathiozole	4691	0.095	95.8
Levamisole	4448	0.092	92.0
Sulphamethoxazole	2865	0.076	76.0
Oxy-tetracycline	3714	0.084	84.0
Chloramphenicol	4691	0.095	95.0
Tiamulin	2914	0.078	78.0

Appendix L : Recovery of 8 veterinary drugs spiked into chicken eggs with 0.2 ppm

Drug	Peak area	Concentration	% recovery
Albendazole	13512	0.197	98.5
Piperazine	13505	0.197	98.5
Sulphathiozole	13028	0.192	96.0
Levamisole	13021	0.192	96.0
Sulphamethoxazole	10147	0.158	79.0
Oxy-tetracycline	11335	0.172	86.0
Chloramphenicol	13029	0.192	96.0
Tiamulin	10934	0.167	83.5

Appendix M: Recovery of 8 veterinary drugs spiked into chicken eggs with 0.4 ppm

Drug	Peak area	Concentration	% recovery
Albendazole	31048	0.395	98.8
Piperazine	31028	0.392	98.0
Sulphathiozole	29484	0.385	96.3
Levamisole	27652	0.376	94.0
Sulphamethoxazole	22522	0.319	79.8
Oxy-tetracycline	25974	0.342	85.5
Chloramphenicol	27652	0.376	94.0
Tiamulin	25994	0.342	85.5