KWAME NKRUMAH UNIVERSITY OF SCIENCE AND

TECHNOLOGY, KUMASI, GHANA

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

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QUALITY ASSESSMENT OF VETERINARY PHARMACEUTICALS: A CASE OF OXTETRACYCLINE AND PRAZIQUANTEL

BY

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MAY, 2016

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES KNUST, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN PHARMACEUTICAL CHEMISTRY

MAY, 2016

DECLARATION

I hereby declare that this submission is my own work and it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement to references has been made in the text.

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DEDICATION

I dedicate this dissertation to God

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ABSTRACT

Antimicrobial resistance is a global health problem with severe consequences. Efforts aimed at possible reduction must therefore be encouraged. Assurance of the quality of veterinary pharmaceuticals therefore has a direct link with improving disease control in humans. Resistant organisms can develop as a result of poor quality antibiotics. Resistant organisms from animals can be transferred through the food chain to humans. The food-borne route has been reported to be the dominant route in the spread of resistance from non – human sources to humans.

Assessment of the quality of veterinary pharmaceuticals containing Oxytetracycline and Praziquantel was studied. Veterinary Pharmaceuticals containing Oxytetracycline (OTC) and Praziquantel (PZQ) were sampled from a manufacturer, veterinary hospitals, farms and open market. In all, twenty- five (25) samples were analysed using RP-HPLC. Sensitive, fast, selective and precise RP-HPLC methods were developed and validated for Oxytetracycline and Praziquantel in their pure forms and in formulated products. Chromatographic conditions developed for Oxytetracycline was mobile phase composition of acetonitrile: water: trifluoroacetic acid (TFA) (85:14.5:0.5(1%v/v); v/v/v) respectively. The stationary phase employed was an Altima Cyano 100A 5µ, (250mm x 4.6mm) column at a wavelength of detection, 254nm. The retention time was 3.326 ± 0.05 minutes. A flow rate of 2ml/min was used.

Chromatographic conditions developed for Praziquantel was mobile phase composition of acetonitrile: water: TFA (45:54.95:0.05(1%v/v); v/v/v). The stationary phase employed was ReproSil 100 CN 5µ, (250mm x 4.6mm) and a

detection wavelength of 230 nm. The retention time was 6.80 ± 0.07 minutes. All determinations were made at ambient temperatures for Oxytetracycline and Praziquantel.

Calibration curves with good coefficient of correlation (r^2) values of 0.9994 and 0.9996 were obtained for Oxytetracycline and Praziquantel respectively. Highly sensitive LOD values of 0.01054mg/ml and 0.03156mg/ml were obtained for Oxytetracycline and Praziquantel respectively. LOQ values of 0.03196 mg/ml and 0.09564 mg/ml were obtained for Oxytetracycline and Praziquantel respectively. The analytical method developed was then applied to commercial veterinary products sampled. Fifteen (15) samples were of good quality whereas ten (10) samples were observed to be sub-standard.

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LIST OF ABBREVIATION

API	-	Active Pharmaceutical Ingredient
BP	-	British Pharmacopoeia
FDA	-	Food and Drugs Authority
HPLC	-	High Pressure Liquid Chromatography
ICH	-	International Conference on Harmonisation
IR	-	Infra Red
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
MRL	-	Maximum Residual Limits
N/A	-	Not Applicable
OTC	-	Oxytetracycline
PZQ	-	Praziquantel
RP-HPLC	-	Reverse Phase High Pressure Liquid Chromatography
RP-LC	-	Reverse Phase Liquid Chromatography
RSD	-	Relative Standard Deviation
SD	-	Standard Deviation
TEA	-	Triethylamine
TFA	-	Trifluoroacetic acid
USP	-	United States Pharmacopeia
UV	-	Ultra Violet
WHO	-	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 GENERAL BACKGROUND

Veterinary pharmaceuticals are substances employed in the diagnosis, prevention, control and treatment of animal diseases (Federal Office of Consumer Protection and Food Safety, 2016). Pharmaceuticals is a broad term that includes substances used for treatment as well as diagnosis and prevention of diseases (Federal Office of Consumer Protection and Food Safety, 2016). Veterinary medicines, veterinary drugs and veterinary pharmaceuticals are sometimes used interchangeably. They comprise antimicrobials, antiinflammatory agents and antineoplastic agents, among others.

Human health is linked directly to animal health and production. A number of communicable diseases are transmitted from animals to humans. It is reported by the World Health Organisation that many human diseases called zoonoses are caused by pathogens originating from an animal or from products of animal sources. These diseases like rabies continue to affect humans, especially the poor population living in developing countries (World Health Organization, 2016). The levels and patterns of antimicrobials employed in the feeding and treatment in poultry, piggery, etc. is the major determinant for the spread of resistant bacteria in animals and humans (Wegener, 2010). Human health is also directly affected by animals through the food chain. The consumption of animal products with residues of an antimicrobial agent could lead to possible residues of the agent in humans.

In an attempt to fight against the spread of communicable diseases that result in low production of animal products, antimicrobials are used as a means to combat these diseases. The quality and dosage of these drugs have consequences on the quality of veterinary products produced and hence its attendant consequences on human health.

Resistance is defined as decrease in the effectiveness of a drug in the treatment of a disease condition (National Library of Medicine, 2011). It refers to the ability of microbes such as bacteria, viruses, parasites, or fungi to grow in the presence of a chemical that would normally kill or limit their growth (National Institute of Allergy and Infectious Diseases, 2009). Antimicrobial resistance is a public health concern because a resistant infection can easily kill and also spread to others causing huge economic burdens to individuals and the society at large.

Certain trends are contributing to a global increase in antibiotic consumption. Prominent among these trends include an upsurge in access to antibiotics and the increasing demand for animal protein (CDDEP, 2016),(Novick, 1981). Antibiotics are used in poultry, piggery, etc. in sub-therapeutic doses to increase performance of production (National Research Council, 1999). The uncontrolled sale of antimicrobial agents in many low or middle income countries such as Ghana remains a concern as these agents can be obtained over the counter without prescription. The resultant effect is the use of antimicrobial agents when not indicated, thus, leading to the emergence of resistance.

The concept of One Medicine (One Health) postulates that there is no dividing line between animal and human medicine (Schwabe, 1984). Human and animal

science have a common body of knowledge on the origin of diseases in various species and in pathology, physiology, and anatomy (Schwabe, 1984).

One Health considers the fact that there is a relationship between humans and the ecosystem. This relationship presupposes that the activities of a member affects the others. This concept shows that the health of humans, animals and the environment are interrelated.

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Quality assessment of veterinary pharmaceuticals should therefore be a major concern in ensuring quality of health in humans.

1.2 PROBLEM STATEMENT

The quality of veterinary medicines has an effect on the health of human beings. Drug resistance may arise as a result of poor quality veterinary pharmaceuticals.

Animals form a significant part of the food chain and consuming animal products with levels of residues above the maximum residual levels specified for medicines could lead to accumulation of residues in human beings.

Dose imprecision as a result of unregulated and poor quality medicines could result in over administration or under administration, which will further lead to drug residues or treatment failure respectively. The resultant effect of all these is drug resistance.

Resistant organisms which arise as a result of exposure of organisms in animals to poor quality medicines could spread to humans through faecal matter of the animals and other sources.

Lack of simple analytical techniques and adequate regulation in the assessment of such products remain a challenge in developing countries such as Ghana.

1.3 JUSTIFICATION

There is lack of effective regulation on the quality and sale of veterinary medicines in developing countries such as Ghana. Reports have shown that the presence of residues of antibiotics in poultry, piggery, etc. differs among countries and are either rare or absent in areas with effective quality assurance systems (Abavelim, 2014).

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Furthermore, there is lack of information and data on the use of veterinary medicines in animals in Ghana. Little information can be obtained regarding the use of veterinary medicines especially those used on farms in Ghana.

The upsurge of antibiotic use in livestock among farmers is worth noticing. Farmers use these antibiotics as growth promoters. Many more use it to increase their yield of eggs in poultry, regardless of the consequences.

Animals serve as food, and also form part of the environment. Humans can be exposed to residues from animals. Also, resistant microbes developed in animals as a result of poor quality veterinary medicines can be transferred to humans through the food chain.

Monographs on Oxytetracycline in the British Pharmacopoeia (2013) and United States Pharmacopoeia (USP) 30 employ hard-to-come by stationary support materials and their corresponding reagents for the analysis of these compounds and their formulated products.

No monograph exists in the (British Pharmacopoeia Commission, 2013) and/or (USP 30) (veterinary sections) on the analysis of formulated products of

Praziquantel. Most veterinary formulations are multi-component formulations containing a number of active pharmaceutical ingredients (API).

This necessitated the development of the simple analytical methods that could be used to identify and quantify Oxytetracycline and Praziquantel in veterinary products.

1.4 GENERAL OBJECTIVE

To assess the quality of veterinary Oxytetracycline and Praziquantel products used in Ghana.

1.4.1 Specific Objectives

1. To conduct a survey on farms, veterinary hospitals, veterinary shops and open markets to investigate the various veterinary antimicrobial agents commonly purchased and used.
2. To develop and validate RP-LC methods for the determination and quantification of Oxytetracycline and Praziquantel. (HPLC with UV detection)
3. To assay veterinary formulated products containing Oxytetracycline and Praziquantel using the validated HPLC methods developed.

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CHAPTER TWO

LITERATURE REVIEW

2.1 VETERINARY PHARMACEUTICALS

Veterinary pharmaceuticals are substances used in the diagnosis, prevention, control and treatment of animal diseases (Federal Office of Consumer Protection and Food Safety, 2016). Pharmaceuticals comprise areas of prevention and diagnosis in addition to classical medication-based therapy (Federal Office of Consumer Protection and Food Safety, 2016). They are mainly medicines needed to keep animals healthy (Animal Health Institute, 2016).

2.1.1 Types of Veterinary Drugs

A large number of veterinary drugs are available for different purposes. The different types of veterinary drugs include:

Antibiotics used to destroy bacteria; antihelminthics used to destroy internal parasites; insecticides used to destroy ticks, lice, fleas and mites; hormones used for growth promotion and for fertility, drugs used in the treatment of conditions specific to certain parts of the body such as the heart, anaesthetics and anti-inflammatory medications (Animal Health Institute, 2016).

2.1.2 Veterinary Antibiotics

Four main broad categories of antibiotics have been identified. These categories are:

- Therapeutic (Treatment): These are used for curing infections in animals.

- Metaphylaxis (Control): These are sometimes used to control the spread of illness, usually when an infection is on the loose in an outbreak among farm animals.
- Prophylaxis (Prevention): Antibiotics may be given for prophylaxis to prevent infection because diseases can easily spread among livestock and poultry.
- Growth Promoters: These antibiotics may also cause the destruction of microbes in the gut. The growth of animals is thus enhanced because of the conversion of feed to muscle more quickly, consequently causing a more rapid growth. Antibiotics also cause the reduction of the activities of the immune system, reduction of nutrient wastage and the formation of toxins (American Meat Institute, 2014). In 2012, FDA in the U.S asked farmers to cease the use of antibiotics for reasons of growth (American Meat Institute, 2014).

It is important that the use of medicines in poultry, piggery, etc. is regulated to avoid contamination of animals and animal products consumed by humans.

2.1.3 Veterinary Antihelminthics

These are medicines used to kill worms. They are used to treat parasitic infestations. Most antihelminthics have a wide therapeutic index; they therefore show appreciable safety in their use against helminths (Merck Publishing Group, 2014).

2.2 LINK BETWEEN HUMAN AND VETERINARY PHARMACEUTICALS

The methodologies for manufacture of human medicines such as drug stability assessment are employed in the development of veterinary drugs. Certain human

medications may be used in animals. Examples of such medications are clotrimazole and methimazole (Kreckel, 2010). A number of veterinary drugs start off as human medications. Manufacturers usually start the production of the human medications and later reformulate for use in animals. The opinion that the production of human medicines is lucrative compared to that of veterinary drives this action.

However, certain manufacturers develop both veterinary and human drugs side by side. Changes in composition of formulation are made with respect to the amount of excipients and active pharmaceutical ingredient added (Palmer, 2011). A significant number of veterinary medicines contain the exact ingredients as their human analogues, though proportions may be different (Palmer, 2011).

2.2.1 Differences between Veterinary Medicines and Human Medicines

Veterinary medicines differ from human medicines in the dosage required by different animals, the dosage form and in the composition of the formulation. The dosage form is the most significant difference. The dosage forms for veterinary and human may differ for the same active pharmaceutical ingredient (Lukas, 2009).

Veterinary medicines are formulated with respect to the anatomy of the animal, bioavailability and other pharmacodynamic and pharmacokinetic parameters. The multiplicity of animal species has resulted in particular dosage forms, specific to a species as illustrated in Tables 2.1 and 2.2 (Klinik, Ferguson, & Magruder, 1998). No injectable or powder has been formulated for Oxytetracycline for humans (tablets and capsules), however, feed Premixes,

injectables, soluble powders and tablets have been formulated for animals as shown in Table 2.1.

The active ingredients may also differ in kind and amounts compared to human medicines (Palmer, 2011). Doses as low as 50mg of Oxytetracycline may be administered to animals. Praziquantel tablets formulated for human use contain 600mg Praziquantel while that for animals usually contain 50mg Praziquantel.

Changes in composition of formulation are made with respect to the amount of excipients and active pharmaceutical ingredient added (Palmer, 2011). Usually, more excipients like binding agents and flavours are added to the veterinary medicines.

The approval processes for veterinary drugs and human drugs are separate, though similar (Palmer, 2011).

Table 2.1: Dosage forms of Oxytetracycline and dosage in different animals

Oxytetracycline		
Other names: glomycin,hydroxytetracycline,riomitsin,terrafungine		
Use :	Broad Spectrum antibiotic	
Dose form:	Injectable	100mg/ml 200 mg/ml (LA-200)
	Oral tablets	250 mg/tablet
Dose	Bovine	5-10 mg/kg IM q24 hr or 20 mg/kg Q48-72hr (LA200)
	Dogs and Cats	20 mg/kg tid
	Swine, sheep and goats	6-11 mg/kg IV or IM 10-20 mg/kg PO qid
Note: Label withdrawal: meat 28 days, milk 120 hours, Use of tetracyclines in horses may cause intractable diarrhoea. Use only in the last half of pregnancy as use may affect foetal teeth and bones. Do not use in young animals.		

Source: (Veterinary drug formulary, 2014)

Table 2.2: Dosage forms of Praziquantel and the doses in different animals

Praziquantel		
Other names: Droncit		
Use:	Cestocide	
Dose form:	Injectable Oral	56.8 mg/ml, 10ml vial 23 mg tabs (feline) 34mg tabs (canine)
Dose:	Dog ,Cat	Dose according to label directions

Source: (Veterinary drug formulary, 2014)

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2.3 WITHDRAWAL PERIOD

Withdrawal period is the period necessary for an animal to break down a specified dose of administered medicine and the time needed for the concentration of the medicine to decrease to a safe, acceptable levels in the tissues (Jones, 2007). Strict adherence to withdrawal periods ensures that residues are within acceptable limits. Withdrawal period required for Oxytetracycline in meat and egg is shown in Table 2.1.

2.4 MAXIMUM RESIDUAL LIMITS

Maximum Residual Limits (MRLs) are the maximum concentrations of residues to be legally permitted in or on food (WHO, 2008). MRL is defined as the maximum concentration of residue in a food product obtained from an animal that has received a veterinary medicine. Generally, residues comprise the drug itself or other possible metabolic or degradation products of the medicine following administration (Eudrapharm, 2006). Table 2.3 illustrates the MRL of Oxytetracycline in various animals.

Table 2.3: Maximum Residual Limits of Tetracycline (Oxytetracycline, Tetracycline, and Chlortetracycline) In Animal Derived Foods.

TISSUE	**MRL (** µg/kg)

Milk	100
Liver	600
Eggs	400
Muscle	200
Kidney	1200

Source: (Abavelim, 2014)

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2.5 REGULATION OF VETERINARY PHARMACEUTICALS IN GHANA

The regulation of veterinary pharmaceuticals is the responsibility of the Food and Drugs Authority (FDA). The Public Health Act, 2012, Act 851, Part seven (7) governs the functions of the FDA in Ghana. The Act defines a drug as a substance or a mixture of substances prepared, sold or represented for use in the diagnosis, treatment, mitigation or prevention of disease, disorder of abnormal physical state or the symptoms of it in man or animal. Thus, veterinary pharmaceuticals are drugs and FDA is therefore responsible for ensuring the quality, efficacy and safety of drugs.

However, there seems to be a wide gap between the regulation of veterinary pharmaceuticals and human medicine. More stringent regulations seem to apply to the registration and sale of human medicines compared to veterinary pharmaceuticals in Ghana.

2.6 RELATIONSHIP BETWEEN VETERINARY ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE0 (AMR) IN HUMANS

Resistance refers to the loss of susceptibility of a microbe to an antimicrobial which the microbe was previously susceptible to. The WHO defines antimicrobial resistance as the capability of a microorganism to resist treatment

to an antimicrobial agent that was previously used for the treatment of a disease caused by that microorganism (World Health Organization, 2015).

Antibacterial resistance predates medical use of antibiotics by humans. The utilization of antimicrobials in animal husbandry practices and its use in feed is a cause of resistance in microorganisms (Lee et al., 2000).The treatment of all animals, even when few animals are affected, by many livestock farmers has played a role in the development of resistance. Furthermore, the use of sub-therapeutic doses and over dosage in animals are not uncommon practices among livestock producers. Viral infections are even sometimes treated with antibiotics.

Practices like these needlessly expose healthy individuals to antibiotics. All these and more contribute to antibiotic resistance, a situation which calls for serious attention (Abavelim, 2014).

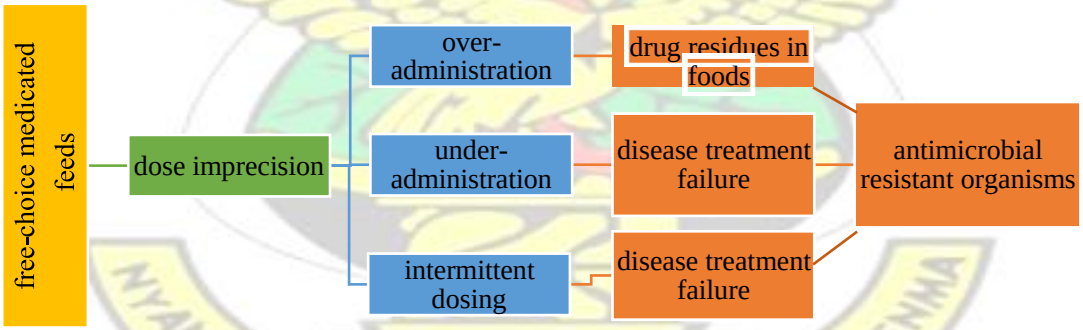
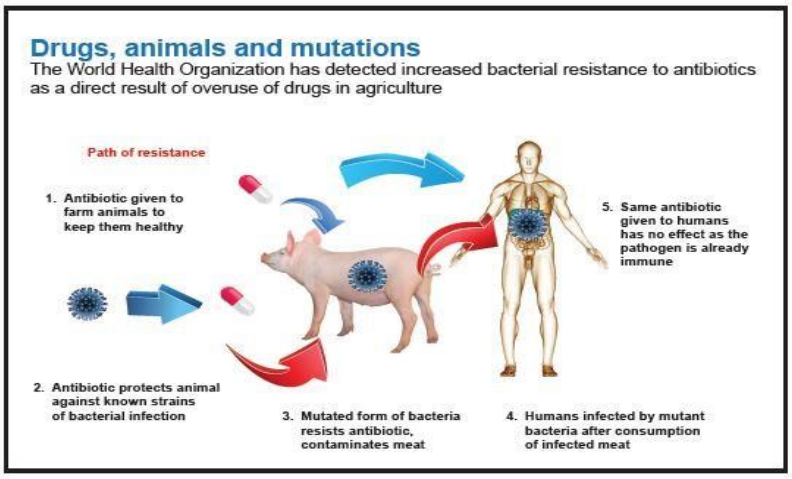


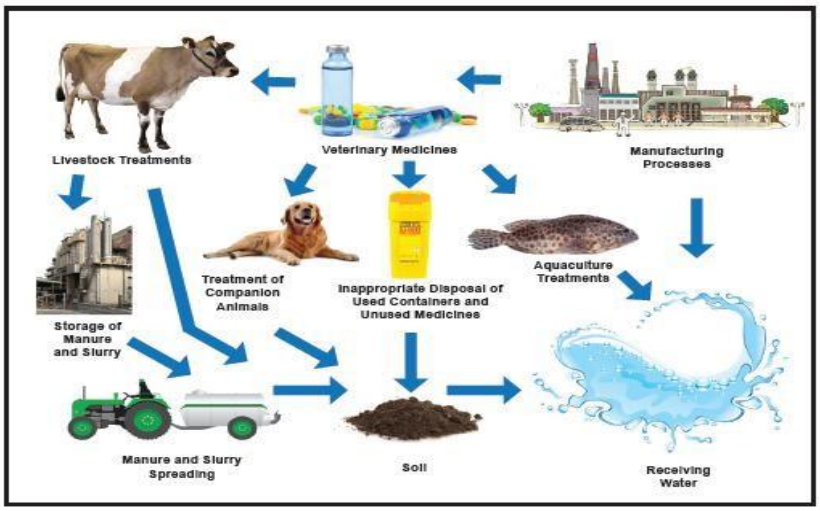
Figure 2.1: Link between veterinary pharmaceuticals and the development of antimicrobial resistant organisms



Antibiotic Resistance
Sourced from World Health Organization

Source: World Health Organization
Figure 2.2: Antimicrobial resistance from animals to humans

Antimicrobial resistance can be spread from non-human sources like animals to humans as shown in Figure 2.3. Veterinary pharmaceuticals play a pivotal role in the transfer of resistant microbes or antimicrobial residues that could lead to development of resistance (Figure 2.2). Animals form part of the food chain. Medicines given to animals could therefore affect directly or indirectly members of the food chain which include humans (Figures 2.2 and 2.4).



Source: Veterinary Medicines in the Environment
http://toxics.usgs.gov/highlights/vet_meds.html

Figure 2.3: Relationship between veterinary medicine and the environment

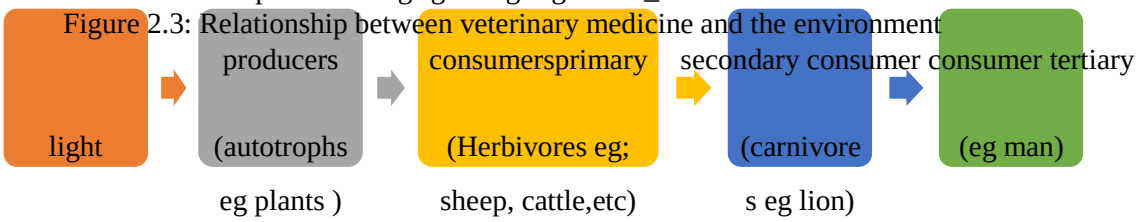


Figure 2. 4: Food Chain

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The presence of antibiotics in human food is connected with some untoward effects including hypersensitivity, gastrointestinal and neurological disorders (Abavelim, 2014).

2.7 PROFILE OF OXYTETRACYCLINE

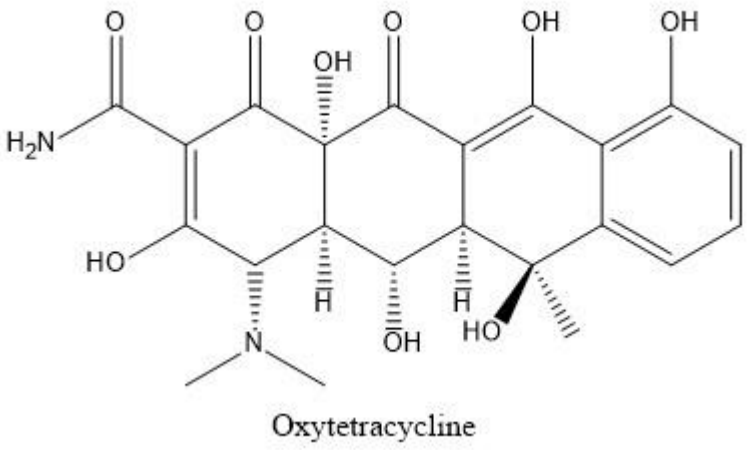


Figure 2.5: Structure of Oxytetracycline

2.7.1 Description of Oxytetracycline

Oxytetracycline is a tetracycline antibiotic. Oxytetracycline HCl has the IUPAC name(4S,4aR,5S,5aR,6S,12aS-4-(Dimethylamino)-3,5,6,10,12,12ahexahydro-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-tetracene-2carboxamide hydrochloride.

The production of Oxytetracycline is by the growth of certain strains of *Streptomyces rimosus* or by alternative means (British Pharmacopoeia Commission, 2013).

Oxytetracycline HCl has a chemical formula $C_{22}H_{24}N_2O_9$, HCl and a molecular weight of 496.9g/mol.

It is a yellow, crystalline powder and it is hygroscopic. Oxytetracycline HCl dissolves freely in water (1 in 2 of water), but sparingly soluble in ethanol (96%). It is soluble in 1 in 45 of methanol, less soluble in dehydrated alcohol, practically insoluble in chloroform and ether. It darkens on exposure to sunlight or moist air above 90˚C. It decomposes above 180˚C. Precipitation of Oxytetracycline occurs in water solutions of Oxytetracycline which causes the solution to become turbid on standing.(British Pharmacopoeia Commission, 2013), (Mofatt , Jackson , Moss , & Widdop , 2005).

The pKas are 3.3, 7.3, 9.1 at 25 ˚C (Mofatt , Jackson , Moss , & Widdop , 2005).

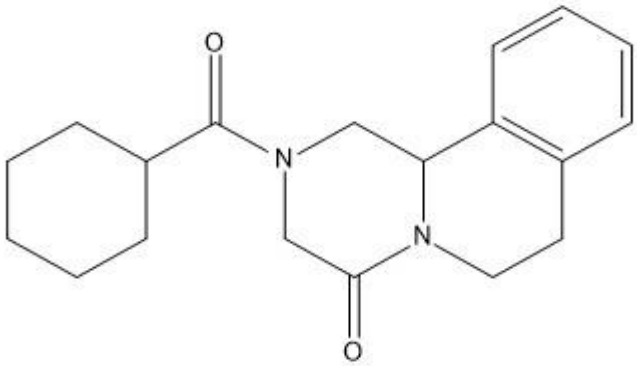
2.7.2 Uses of Oxytetracycline

It is a broad-spectrum antibiotic. It is used as cure in both humans as well as animals. Oxytetracycline could be used to correct breathing disorder in livestock. Oxytetracycline is prevalently used in animals to treat bacterial infections. Among these infections are respiratory infections of the sinuses, wound infections, pneumonia, infections of blood cells and oral cavity. It is used largely in farm animals namely chicken, calves, turkey, beef cattle, dairy cattle, swine and sheep.

2.7.3 Pharmacokinetics

Substantial amounts of Oxytetracycline are also found in faeces of animals that had parenteral administration of the antibiotic, which concentrates quickly in the liver and is eliminated with bile to the intestine (unipharm). Excretion is mostly through the kidneys, approximately 25 to 30% of a dose can be found in urine, and 10 to 20% is found in faeces due to the bile concentration and the passing of the antibiotic from bile to the intestine. Elimination of Oxytetracycline can also be through the milk of lactating females that are in general therapy (unipharm).

2.8 PROFILE OF PRAZIQUANTEL



Praziquantel

Figure 2.6: Structure of Praziquantel

2.8.1 Chemistry of Praziquantel

The IUPAC name of Praziquantel is (11bRS)-2-(cyclohexylcarbonyl)-1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino [2, 1a] isoquinolin-4-one.

It exists as an enantiomer. The (-) isomer is responsible for most of its activity. It is a white or almost white, crystalline powder. Praziquantel is very slightly soluble in water, freely soluble in ethanol (96%) and in methylene chloride. It

has a molecular weight of 312.40g/mol (Mofatt , Jackson , Moss , & Widdop , 2005).

2.8.2 Uses of Praziquantel

Praziquantel is an antihelminthic for the treatment of tapeworms in pets. (Mayo Clinic, 2016). It is safe to use in puppies over four (4) weeks of age and kittens over six (6) weeks of age.

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2.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD DEVELOPMENT

HPLC is an analytical technique used for both qualitative and quantitative analysis. It is a separation technique that possesses the ability to analyse, with relative ease, small quantities of substances ranging from nanogram to pictogram. There are various mechanisms for separation in HPLC. These include adsorption, partition, gel permeation or filtration, chiral interactions and ion exchange.

The components of HPLC include the mobile phase stored in reservoirs, a stationary phase, a pump and a detector. This technique involves the separation of a sample by pumping the dissolved sample under a very high pressure through the column to ensure fast and high resolution separation of the components (Harris, 2010).

The chromatographic conditions used for any analytical work are necessary because certain variations could cause changes in the results. Chromatographic conditions such as the mobile phase chemical character and composition, the stationary phase, column length, flow rate, detector used, etc. are among the conditions to be specified in each analysis with HPLC.

2.9.1 Components of HPLC Chromatograph

- **Mobile Phase**

The mobile phase employed in HPLC is essential as its characteristics such as composition and pH have enormous effect on the separation of sample components. Generally, the choice of mobile phase to be used is dependent on the properties of the analyte. A combination of solvents is usually employed as mobile phase. This is because the use of a single solvent could prevent adequate retention required for separation as certain components could run along with the mobile phase. The characteristics and choice of mobile phase is essential in the analysis of compounds. The mobile phase used could influence the efficiency of the separation and integrity of the stationary phase.

The ionic strength and pH of the aqueous portion of mobile phases are key in the development of robust methods for a reversed phase liquid chromatography (Agilent Technologies, 2015).

The pH of the mobile phase can affect selectivity, retention and peak shape. Mobile phase modifiers like TFA, triethylamine (TEA) and buffers are used to obtain a pH suitable for analysis (Agilent Technologies, 2015).

- **Stationary Phase**

Various mechanisms for separation are exhibited in the stationary phase depending on the type used. Based on the physicochemical properties of the compounds in the sample, varied interactions occur with the stationary phase. The most widely used stationary phase are the silica based stationary phases.

These can be chemically modified to act as normal phase or reverse phase stationary phase. The choice of stationary phase depends on the compounds to be resolved and also on the mobile phase employed.

- **Detectors**

The detector is the component that detects the resolved compounds. The detector produces an electrical signal proportional to the amount of component eluting the column. There are various detectors available for HPLC analysis such as the UV/Visible, refractive index, photodiode array, fluorescence, conductivity, etc. detectors (Harvey, 2000).

- **Pump**

The pump pushes the mobile phase through the column at high pressure and at a constant controlled flow rate. There are two types of pumps, namely, constant pressure pump and the constant flow pump.

The constant pressure pump exerts a constant pressure to the mobile phase with the flow resistance of the column determining the flow rate through the column.

The constant flow pump sustains a given flow of liquid. The pressure generated is based completely on the flow resistance. The constant flow pump is usually preferred in analysis as a change in pressure compensates for the variation in flow resistance (Kar, 2005).

2.10 METHOD VALIDATION

Method validation is the systematic procedure in confirming the suitability of an analytical procedure for the intended purpose for a specific test. It forms a significant part of any good analytical practice. The requirement for validation

of analytical methods is encountered very frequently in the pharmaceutical industry because it forms the basis for the approval of all regulatory filings.

Methods are validated according to the International Conference on Harmonisation (ICH) protocol. Validation parameters include specificity, precision, robustness, accuracy, etc. (ICH Expect Working Group, 2005)

2.11 REVIEW OF ANALYTICAL METHODS USED TO ASSAY OXYTETRACYCLINE AND PRAZIQUANTEL

2.11.1 Overview of Methods Developed for Oxytetracycline Analysis

Various methods have been developed for the analysis of Oxytetracycline and Praziquantel using UV- Visible spectrophometry, HPLC and Mass Spectrometry.

An isocratic method developed for the analysis of Oxytetracycline in preparations like powders, ointment, capsules, vaginal ointments and tablets involved the use of Silasorb C8 column, 10 µ, (250mm x 4mm) and a mobile phase composition of methanol: 0.01M oxalic acid (30:70), pH 3.0. The flow rate was 0.95ml/min was and the wavelength of detection was 250nm (Papadoyannis , Samanidou, & Kovatsi LA, 2000).

Another method for the analysis of OTC in tissues of cows has been developed. The method involved a polymeric reversed phase (PLRP-S) column and a mixture of 0.5% (v/v) of formic acid, 0.001M of oxalic acid, and
3% (v/v) of tetrahydrofuran in water (mobile phase A) and tetrahydrofuran (mobile phase B). The column temperature was 60 °C. Identification and quantification of Oxytetracycline and its epimer were done using MS-MS detection technique (Cherlet, De Baere, & De Backer, 2003).

Furthermore, analysis of OTC residues in infant formulary using a C-18 column (250mm x4.6mm) at a wavelength of 355nm using a solution of methyl alcohol(Khosrokhavar , Jamal, Shahram , & Jannat, 2011) has been developed.

Another method used for analysis of Oxytetracycline residues in honey involved the use of Kromosil C18 analytical column, 5u, (250mm x4.6mm I.D) and a mixture of acetonitrile: water (85:15 v/v) as mobile phase.1.5ml/min was the flow rate and the wavelength of detection was 360nm.The injection volume was 20 µl (Rao, Kumar, & Sekharan, 2015).

Most of these methods developed were used for the analysis of residues in animals and other products from plant, animal and other sources. None of these methods was developed in Africa and none has been developed for the quality assessment of veterinary formulations of Oxytetracycline available on the Ghanaian or African market.

2.11.2Overview of Methods for Praziquantel Analysis

Analysis of Praziquantel has been done using Luna C18 column, 5 µ, (250mm × 4.6 mm I.D) using 50% ACN and 50% phosphate buffer solution (pH 2.9) as mobile phase. The flow rate used was 1.3 ml/min with 210 nm as the wavelength of detection. The temperature used was 30 °C (Vignaduzzo, Operto, & Castellano, 2015).

Praziquantel has been analysed using HPLC on a reverse phase C-18 column (250mm x 4 mm). An isocratic gradient of acetonitrile (70%) was used for ten (10) minutes. A flow rate of 1 ml /min and wavelength of detection 217nm were employed. PZQ was determined by comparison of its spectra and retention time

in extract samples with commercial standard using a PDA detector Jasco MD 1510.

Another method involved the use of a C18 column, 5 µ, (250 mm x 4.6 mm I.D.) and a mobile phase composition of acetonitrile: distilled water (60:40 v/ v). The column temperature was 25°C. The flow rate was 1 ml / min and the wavelength of detection was 210 nm.(Oltean, 2011).

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Separation and quantification of Praziquantel has been achieved using C18 column, 5 µ, (150 mm x4.6 mm) and acetonitrile: methanol: 20 mM phosphate buffer (0.2 % TEA, pH 4.5) (50:10:40, v/v/v) as mobile phase. The detection was done at 210 nm.(Tatar, Ates, & Kucukguzel, 2015).

Another method for the analysis of Praziquantel involved a gradient elution method. The conditions were a C18 column, 5 µ, (250mm x 4.6 mm). A gradient elution involving a mobile phase A containing water adjusted to pH 2.5 with 85% phosphoric acid, mobile phase B containing acetonitrile. The column temperature was 40˚C. A flow rate of 1.2 mL/min was employed and varying wavelengths were used at different times (Bialecka & Kulik, 2010).

Praziquantel has been determined by HPLC using a C18 column (250 mm x 4 mm). An isocratic gradient of acetonitrile (70 %) was applied for 10 minutes at a flow rate 1 ml /min. The quantitation was carried out at 217 nm (Marsik, Podlipna, & Vanek, 2015).

None of these methods was developed in Africa.

In general, a number of these methods developed for Oxytetracycline and Praziquantel are comparatively expensive considering the economic status of

developing countries like Ghana. This may not enhance quality control of veterinary pharmaceuticals produced and the regulation of such products as the cost and complexity may deter both manufacturers and regulators.

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CHAPTER THREE

METHODOLOGY

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Instrumentation and Equipment

Adam – analytical weighing balance, WA 210; 210/ 0.0001g , Agilent 1200 infinity, Shimadzu LC 20AB instrument, Altima Cyano 100A 5µ, (250mm×4.6mm), ReproSil 100 CN 5µ,(250mm×4.6mm), Fourier Transform Infrared Spectrometer Module 200-X Serial – 200043, Beakers (10ml, 25ml, 250ml, 1000ml) , Conical flasks, delivery pipettes (1ml, 2ml,5ml, 10ml, 20ml and 25ml) , Fisher Scientific (UK), Sonicator, Graduated pipettes (1ml , 2ml, 5ml, 20ml,25ml), Measuring cylinders (100ml, 500ml), Metler Toledo Scale, AB104-S, No. 1 whatman filter papers , Plastic funnel , Spatula, Syringe, Volumetric flasks (10ml, 25ml, 50ml, 100ml, 500ml and 1000ml).

3.1.2 Reagents and Samples

Oxytetracycline pure powder (assay: 99.24%), Praziquantel Pure powder (assay: 99.40%), Acetonitrile (HPLC grade from Fisher Scientific, UK), Trifluoroacetic acid (HPLC grade) from Fisher Scientific, UK, Different brands of veterinary Oxytetracycline injection from different companies, Veterinary Oxytetracycline soluble powders from different companies, Tablet brands of veterinary Praziquantel obtained from different companies and Distilled water.

3.2 METHODS

3.2.1 Sampling

Commercial brands of Oxytetracycline HCl and Praziquantel were obtained from farms, veterinary hospitals and open markets. Dosage form of samples included injections, powders and tablets.

Sampling sites were all in Ghana but mainly in Kumasi and its environs. Few samples were obtained from a veterinary hospital in Tema because of proximity to the site of analysis.

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Veterinary Hospitals visited were located in Amakom and Asokwa. Few samples were obtained from a veterinary hospital in Tema.

The open market sources included veterinary shops in Adum, Kumasi, Ohwimase-Kwadaso, Amakom Roundabout (6.6872,-1.6073), Asokwa and Bantama.

Different farms located in Onwe, Ayeduase, Appiadu (6.68576,-1.60549) and KNUST were selected as sampling sites.

A sample of formulated product containing Oxytetracycline was obtained from a manufacturing company in Accra.

For Oxytetracycline, the dominant formulation was powders while that of Praziquantel was tablets. The injectables were obtained from the open markets (shops) and veterinary hospitals. The samples (mainly injections) taken from the veterinary hospitals were analyzed immediately after they were obtained.

All samples were kept in a cool, dry place, below 25˚C as prescribed for storage. Samples containing Oxytetracycline were prepared and stored in amber coloured bottles prior to analysis because of the photosensitive nature of the

tetracyclines. All samples were analyzed soon after their collection. Prior to analysis, storage conditions required for the samples were strictly adhered to.

Table 3.1: Profile of Drug Samples Used

Code of drug	Batch Number	Manufacturing Date	Expiry Date	FDA Registration number
A	1410802(FDB No: 04-8024)	10/2014	10/2017	A
B	1410801(FDB No: 04-8024)	10/2014	10/2017	A
C	151005	10/2015	04/2017	N/A
D	0142452	10/2014	10/2016	N/A
E	150318	03/2015	09/2016	N/A
F	150902	09/2015	09/2017	N/A
G	1412801(FDB No: 04-8024)	12/2014	12/2017	A
H	1410802(FDB No: 04-8024)	10/2104	10/2017	A
I	150903	12/2015	11/2017	N/A
J	0143398	12/2014	12/2016	N/A
K	151107	11/2015	11/2017	N/A
L	150320	12/2015	12/2017	N/A
M	151007	10/2015	04/2017	N/A
N	SS1502	01/2015	12/2017	N/A
O	551405	06/2014	07/2017	N/A
P	F151017	10/2015	10/2018	N/A
Q	22328.10(Lot No.)	05/2015	05/2018	N/A
R	23532.11	06/2015	06/2017	N/A
S	1410802 (FDB No: 04-8024)	10/2014	10/2017	A
T	23817.10	09/2015	09/2017	N/A

A=Available N/A = Not Available

Table 3.2: Table Showing the Profile of Drug Samples Used

CODE	Batch Number	Manufacturing Date	Expiry Date	FDA Registration number
Sample PA	DG4119	06/2014	05/2018	N/A
Sample PB	DG4119	06/2014	005/2018	N/A
Sample PC	DG4119	06/2014	05/2018	N/A
Sample SA	SBT 1504	07/2015	07/2018	N/A
Sample SB	SBT 1502	03/2015	03/2017	N/A

3.2.2 Preparation of Solutions

- **Preparation of 0.1M HCl**

0.86ml of concentrated hydrochloric acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed to cool, after which it was quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

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- **Preparation of Praziquantel Diluent**

Using a graduated cylinder, 450 ml of HPLC grade acetonitrile and 550ml of distilled water were measured to produce a 1000ml of mobile phase and transferred into the solvent reservoir bottle. The solution was sonicated to ensure proper mixing. The solution was then labelled appropriately.

- **Mobile phase Preparation for Oxytetracycline**

The solvents used were individually poured into reservoirs A, C and D corresponding to water, acetonitrile and TFA respectively. The pump was set to deliver the solvents in a ratio of 14.5:85:0.5% v/v/v for water acetonitrile and TFA respectively.

- **Preparation of Oxytetracycline Diluent**

Using a graduated cylinder, 850 ml of HPLC grade acetonitrile and 148ml of distilled water were measured and transferred into the beaker. 2 ml of a 1%v/v TFA solution was pipetted and added to the contents of the beaker. A magnetic stirrer was placed into the beaker and the resulting solution was stirred for about 15 minutes. The solution was then labelled appropriately.

- **Mobile Phase Preparation for Praziquantel**

Using a graduated measuring cylinder, 450 ml of HPLC grade acetonitrile and 549.5ml of distilled water were measured to produce a 1000ml of mobile phase and transferred into the solvent reservoir bottle. 0.5 ml of a 1%v/v TFA solution was pipetted and added to the contents of the solvent reservoir bottle. The solution was sonicated to ensure proper mixing. The solution was then labelled appropriately.

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- **Preparation of Stock Solution of Pure Powder and Working Concentration Solution of Oxytetracycline**

An analytical balance was used to weigh 500mg of pure Oxytetracycline powder. The weighed powder was transferred into a 100ml volumetric flask half filled with 50 ml of the diluent. The solution was sonicated for fifteen minutes to ensure complete dissolution. The solution was made up to the 100ml mark on the volumetric flask with the diluent. The solution was well shaken to ensure homogeneity. 5ml of the resulting solution was pipetted into a 50ml volumetric flask. It was made up to 50 ml using the diluent to produce a solution with concentration, 0.5mg/ml which was the working standard or concentration. The solution was shaken and appropriately labelled.

- **Preparation of Stock Solution of Pure Powder and Working Concentration solution of Praziquantel**

An analytical balance was used to weigh 400mg of pure Praziquantel powder. The weighed powder was transferred into a 100ml volumetric flask half filled with 50 ml of the diluent. The solution was sonicated for fifteen minutes to ensure complete dissolution. The solution was made up to the 100ml mark on

the volumetric flask with the diluent. The solution was well shaken to ensure homogeneity. 5ml of the resulting solution was pipetted into a 50ml volumetric flask. It was made up to 50 ml using the diluent to produce a solution with concentration, 0.4mg/ml which was the working standard or concentration. The solution was shaken and appropriately labelled.

Preparation of Sample Solution of Oxytetracycline Formulations

A 100ml volumetric flask was half- filled with diluent. An amount of powder or injection sample equivalent to 500mg Oxytetracycline was weighed and drawn with syringe respectively into the volumetric flask. The solution was sonicated for fifteen minutes to ensure complete dissolution. More diluent was added to make up to the 100ml mark. The resulting solution was sonicated to ensure homogeneity after which it was filtered through a whatman No. 1 filter paper. The initial five (5) ml of filtrate was discarded. 5ml of the resulting filtrate was pipetted into a 50ml volumetric flask. It was made up to 50 ml with the diluent to produce a solution with concentration, 0.5mg/ml. The solution was shaken and appropriately labelled.

All solutions were prepared in amber coloured glassware since Oxytetracycline is unstable and often changes from yellow to brown with exposure to light.

Preparation of Sample Solution of Praziquantel Formulations

A 100ml volumetric flask was half- filled with diluent. An amount of the formulated product (powdered tablets) equivalent to 400mg Oxytetracycline was weighed into the volumetric flask. The solution was sonicated for fifteen minutes to ensure complete dissolution. More diluent was added to make up to

the 100ml mark. The resulting solution was sonicated to ensure homogeneity after which it was filtered through a whatman No. 1 filter paper. The initial 5ml of filtrate was discarded. 5ml of the resulting filtrate was pipetted into a 50ml volumetric flask. It was made up to 50 ml with the diluent to produce a solution with concentration, 0.4mg/ml. The solution was shaken and appropriately labelled.

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3.2.3 Identification Tests

- **Identification of Pure Samples Using UV/ Vis Spectrophotometry**
  - **Identification of Oxytetracycline Pure Powder**

0.20g of Oxytetracycline was appropriately weighed into a 100ml volumetric flask. 50 ml of 0.1M HCl was added and shaken for 15 minutes. Sufficient amount of 0.1M HCl was added to produce 100ml. The solution was shaken and well mixed. 1ml of the solution was taken using a pipette into a 100ml volumetric flask and sufficient amount of 0.1M HCl was added to produce 100ml. The solution was shaken and was appropriately labelled. The solution was placed in the cuvette using 0.1M HCl as blank and the spectrum of Oxytetracycline was obtained (United States Pharmacopoeial Convention, 2006).

  - **Identification of Praziquantel Pure Powder**

0.040g of Praziquantel pure powder was weighed into a 100ml volumetric flask. 50ml of 96% ethanol was added and shaken for 15 minutes. Sufficient 96% ethanol was added to produce 100ml. The solution was shaken and labelled appropriately. This solution was put in the cuvette and the spectrum of Praziquantel obtained.

- **Identification of Oxytetracycline and Praziquantel Pure Powders Using Infra- Red Spectrophotometry**

Oxytetracycline pure powder (2mg) was triturated with 300mg of finely powdered and dried potassium bromide using an agate mortar and pestle. The resulting mixture was spread uniformly in a die and compressed using a hydraulic press. The disc obtained was then placed in the sample holder and run to obtain a spectrum which was compared to that of the chemical reference standard in the Pharmacopoeia. The same was done for Praziquantel.

- **Identification of Pure Powder of Oxytetracycline Using Chemical Reaction**

2ml of sulfuric acid was added to 1mg of pure sample of Oxytetracycline (USP 30). A light red colour was produced.

- **Identification of Samples of Oxytetracycline and Praziquantel**
  - **Identification of Samples of Oxytetracycline Using HPLC**

Using the chromatographic conditions specified in this study, the retention times of the chromatograms of a 0.5mg/ml concentration of

Oxytetracycline in both the pure sample and the various formulations (samples) respectively of Oxytetracycline were found to be identical and consistent.

- **Identification of Samples of Praziquantel Using HPLC**

Using the chromatographic conditions specified in this study, the retention times of the chromatograms of a 0.4mg/ml concentration of Praziquantel in both the

pure sample and the various formulations (samples) of Praziquantel respectively were found to be identical and consistent.

- **Identification of Samples of Oxytetracycline Using a Chemical Reaction**

2ml of sulfuric acid was added to weight of samples equivalent to 1mg Oxytetracycline. A light red colour was produced (USP 30) as was observed for the pure Oxytetracycline powder.

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3.2.4 Pharmacopoeial Tests

- **Uniformity of Weight**

Praziquantel Tablet

The uniformity of weight test was done according to the British Pharmacopoeia 2013 specifications for the uniformity of weight of tablets.

Twenty (20) Praziquantel tablets were individually weighed. The total weight of the twenty tablets was also obtained by weighing all the tablets together. Calculation of the respective deviations in mass of individual tablets was done. The percentage deviations with reference to the average weight of the tablet was also computed for. The tablets were triturated using a porcelain mortar and pestle for weighing and for preparation of samples.

- **Assay of Oxytetracycline Pure Powder**

Oxytetracycline pure powder was assayed using the method described in the UV identification of Oxytetracycline.

The purity of the pure sample was calculated (Mofatt , Jackson , Moss , & Widdop , 2005)

- **Assay of Praziquantel Pure Powder**

Praziquantel was assayed as per the method for the UV identification of Praziquantel described above. The purity was then computed.

3.2.5 HPLC Method Development

Table 3.3: Conditions for the HPLC Development

Condition	Oxytetracycline	Praziquantel
Column	Altima Cyano 100A 5µ, 250mm x 4.6mm	ReproSil 100 CN 5µ, 250mm×4.6mm
Wavelength of detection (nm)	254	230
Mobile phase composition	acetonitrile:water:trifluoroacetic acid(TFA) (85:14.5:0.5(1%v/v); v/v/v)	Acetonitrile: water:TFA (45:54.95:0.05(1%v/v); v/v/v).
Injection volume(µl)	10	20
Flow rate(ml/min)	2.0	1.0
Temperature(°C)	Ambient	Ambient
Elution mode	Isocratic	Isocratic
Diluent	Acetonitrile:water:TFA (85:14.5:0.2%(1%v/v) v/v/v)	Acetonitrile : water (45: 55 v/v)

3.2.6 Validation of the HPLC Method

The developed methods were validated with respect to ICH protocol.

- **Linearity**
  - **Linearity of Oxytetracycline**

A stock solution of concentration 5mg/ml of Oxytetracycline was prepared. From this concentration, eight different concentrations; 0.30mg/ml, 0.35 mg/ml, 0.40 mg/ml, 0.45mg/l, 0.50mg/ml, 0.55 mg/ml, 0.60mg/ml were prepared by serial dilution. 10µl of each of the concentrations was injected and loaded three times onto the column. The calibration curve was obtained by plotting the mean peak areas against their respective concentrations using

Microsoft Excel. From the graph, the coefficient of correlation was obtained and the limits of detection and quantification were calculated.

➢ **Linearity of Praziquantel**

A stock solution of concentration 4mg/ml of Oxytetracycline was prepared. From this concentration, eight different concentrations; 0.15mg/ml, 0.2 mg/ml, 0.4mg/ml, 0.6mg/l, 0.8mg/ml, 1.0 mg/ml, 1.2 mg/ml and 1.4mg/ml were prepared by serial dilution. 20µl of each of the concentrations was loaded and injected three times onto the column. The calibration curve was obtained by plotting the mean peak areas against their respective concentrations using Microsoft Excel. From the graph, the coefficient of correlation was obtained and the limits of detection and quantification were calculated.

- **Specificity**

(a) Blank or diluent was injected and the chromatogram obtained observed for any blank interference.

(b) The working standard solution of Oxytetracycline (0.5mg/ml) was then injected and response (peak area) obtained.

(c) Blank or diluent was injected again into the chromatograph and analyzed.

The same as described was done for Praziquantel using the working concentration of Praziquantel (0.4mg/ml).

- **Precision**

➢ **Precision of Oxytetracycline**

❖ **Intra- day precision (Repeatability)**

The working standard solution of Oxytetracycline with concentration 0.5mg/ml was injected and loaded onto the column. Three different determinations were carried out at different times within the day.

❖ **Inter-day precision (Intermediate Precision)**

The working standard (0.5mg/ml) solution of Oxytetracycline was injected and loaded three (3) times on three consecutive days. The peak areas were recorded and the relative standard deviations calculated.

➢ **Precision of Praziquantel**

The same method for precision for Oxytetracycline was carried out for Praziquantel using the working concentration of 0.4mg/ml.

- **Accuracy**

➢ **Accuracy Test for Oxytetracycline**

Three different weights of Oxytetracycline pure powder corresponding to 80%, 100% and 120% of the working concentration for Oxytetracycline were weighed into separate volumetric flasks. Known amounts of sample R equivalent to 80%, 100% and 120% of the working concentration were added to the corresponding volumetric flask containing the same amount of the pure powder. The solution was prepared by adding the diluent to achieve concentrations of 0.9mg/ml (80% level), 1.0mg/ml (100% level) and 1.10mg/ml (120% level). The solutions were sonicated and allowed to cool. Solutions were injected in triplicate and percentage recoveries estimated.

➢ **Accuracy Test for Praziquantel**

Three different weights of Praziquantel pure powder corresponding to 80%, 100% and 120% of the working concentration for Praziquantel were weighed into separate volumetric flasks. Known amounts of sample PA equivalent to 80%, 100% and 120% of the working concentration were added to the corresponding volumetric flask containing the same amount of the pure powder as the sample. The solution was prepared by adding the diluent to achieve concentrations of 0.72mg/ml (80% level), 0.8mg/ml (100% level) and 0.88mg/ml (120% level) respectively. The solutions were sonicated and allowed to cool. Solutions were injected in triplicate and percentage recoveries estimated.

- **Robustness**
  - **Robustness for Oxytetracycline**

The working standard solution (0.5mg/ml) was injected using a different column, ReproSil 100 CN 5µ, 250mm×4.6mm and the responses recorded. Relative Standard deviations (RSD), the t-test and F-test using GraphPad Prism 6, which are statistical tools were used to determine the robustness of the method by comparing responses obtained using the different columns.

  - **Robustness for Praziquantel**

The working standard solution (0.4mg/ml) was injected using flow rate of 1.2ml/minute and the responses recorded. Relative Standard deviations (RSD), the t-test and F-test, which are statistical tools were used to determine the robustness of the method by comparing responses obtained using the different flow rates.

- **Stability of Solution**
  - **Stability of solution for Oxytetracycline**

Freshly prepared solutions of the working concentration of Oxytetracycline was injected and loaded at different time intervals (0, 2, 4, 6, 8, 10 and 12 hours). Three injections were made at the respective time intervals and the mean peak areas at the various times were calculated. A graph of mean peak area against time was plotted using GraphPad Prism 6.

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  - **Stability of Solution for Praziquantel**

The method for the stability of Oxytetracycline as described above was employed using the working concentration of Praziquantel.

3.2.7 Assay of Veterinary Formulated Products of Oxytetracycline and Praziquantel Sampled

All samples were prepared as described in the method for preparation of samples of Oxytetracycline and Praziquantel. The solutions were injected three (3) times and the corresponding mean peak areas computed. The mean percentage content of Oxytetracycline and Praziquantel in the various formulated products (samples) were calculated using the response factor.

CHAPTER FOUR

4.0 RESULTS AND CALCULATIONS

4.1 SAMPLING

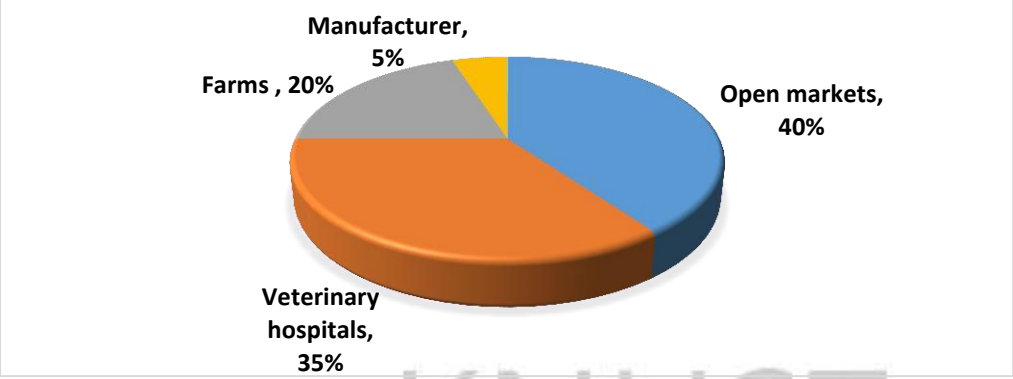
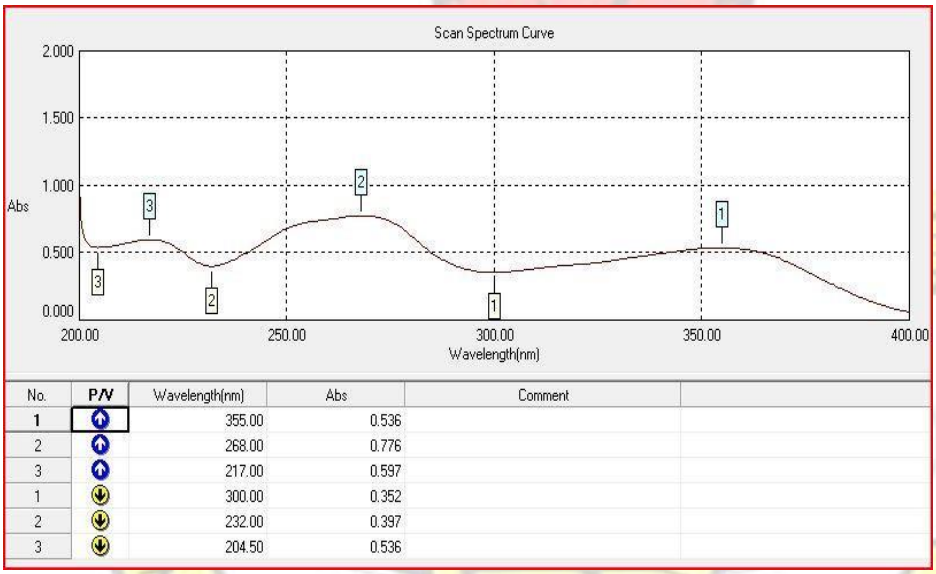


Figure 4.1: Sources of Samples for analysis

4.2 IDENTIFICATION TESTS

4.2.1 Identification of Pure Powder Using UV/Vis Spectrophotometry



No.	P/V	Wavelength(nm)	Abs	Comment
1	↑	355.00	0.536	
2	↑	268.00	0.776	
3	↑	217.00	0.597	
1	↓	300.00	0.352	
2	↓	232.00	0.397	
3	↓	204.50	0.536	

Figure 4.2:U.V Spectrum of Oxytetracycline

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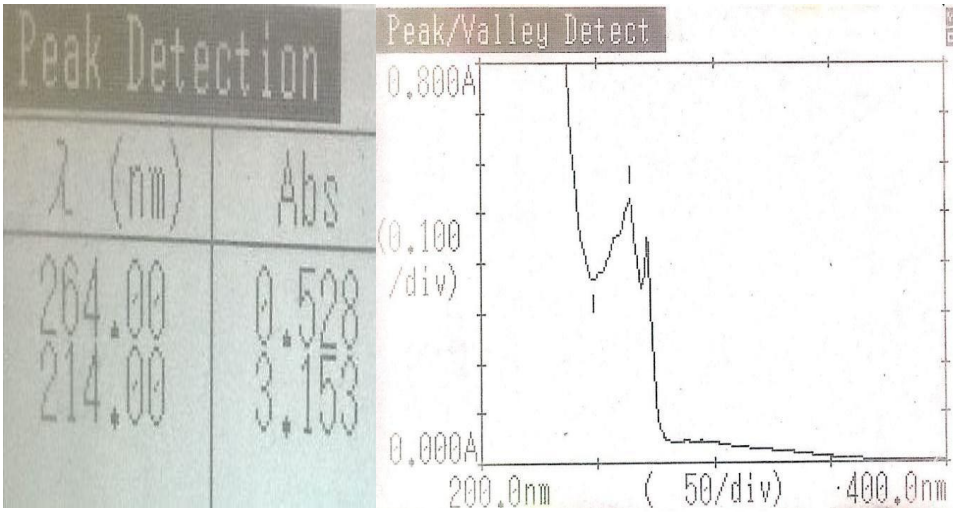

Figure 4.3: U.V Spectrum of Praziquantel

4.2.2 Identification of Pure Powders Using IR Spectroscopy

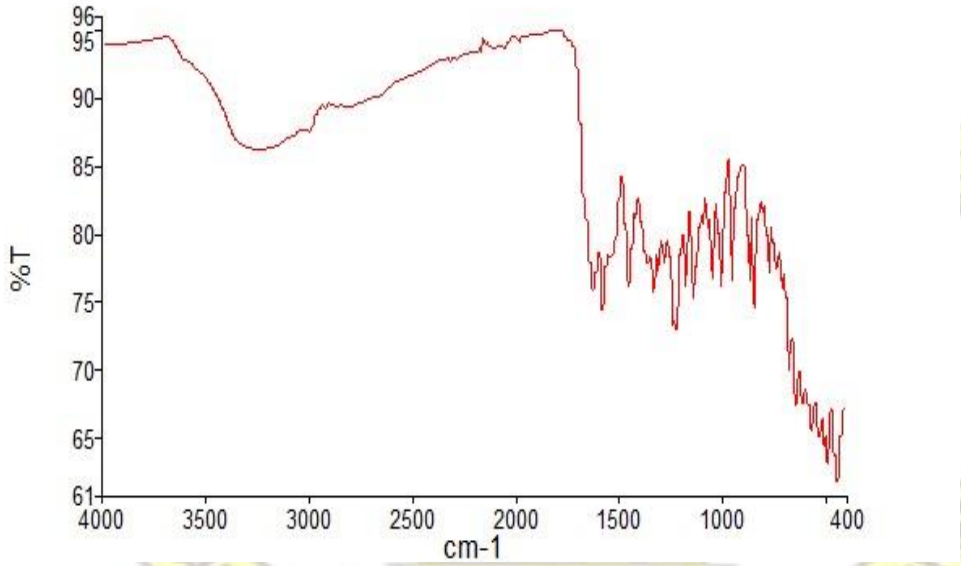

Figure 4.4: IR Spectrum Oxytetracycline

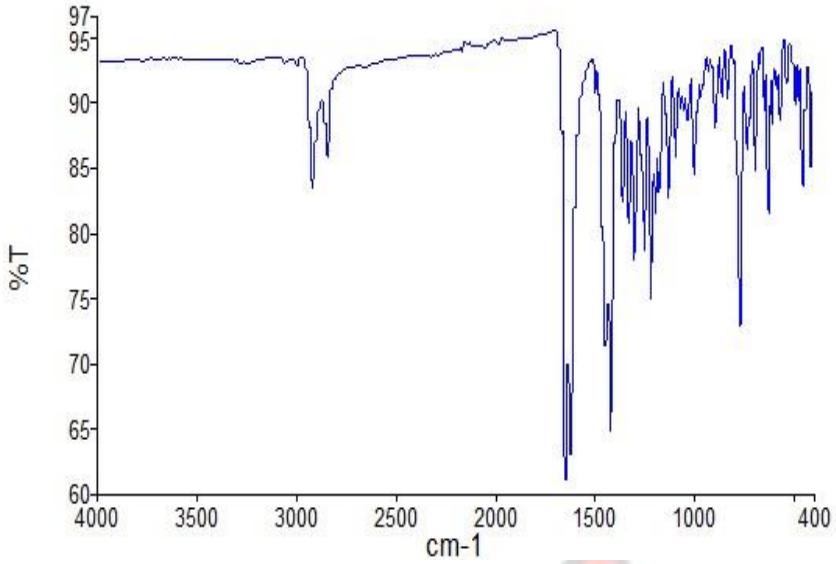

Figure 4.5: IR Spectrum Praziquantel

4.2.3 Identification of Samples of Oxytetracycline and Praziquantel Using HPLC

- **Identification of Samples of Oxytetracycline Using HPLC**

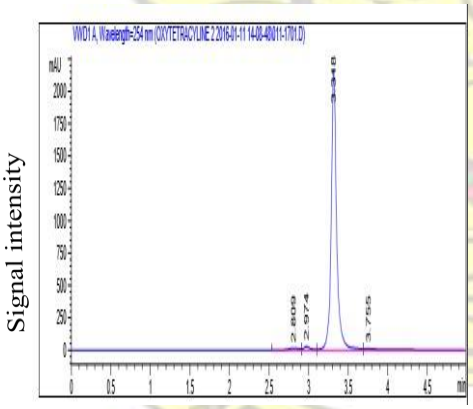

Figure 4.6: Chromatogram of pure Oxytetracycline (0.5mg/ml)

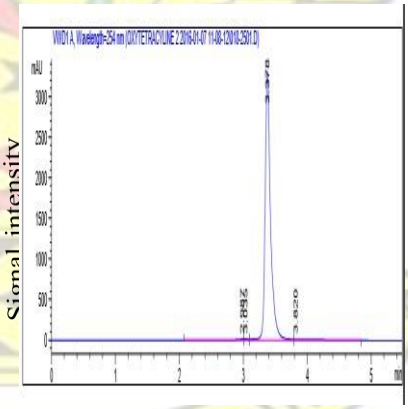

Figure 4.7: Chromatogram of a sample containing Oxytetracycline

- **Identification of Samples of Praziquantel Using HPLC**

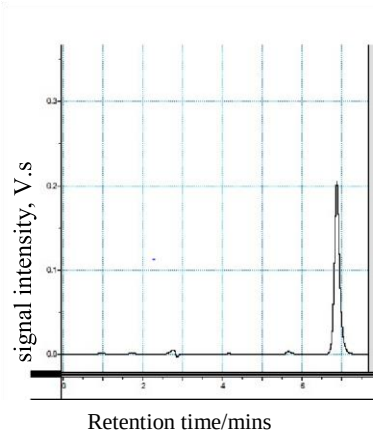

Figure 4.8: Chromatogram of pure Praziquantel (0.4mg/ml)

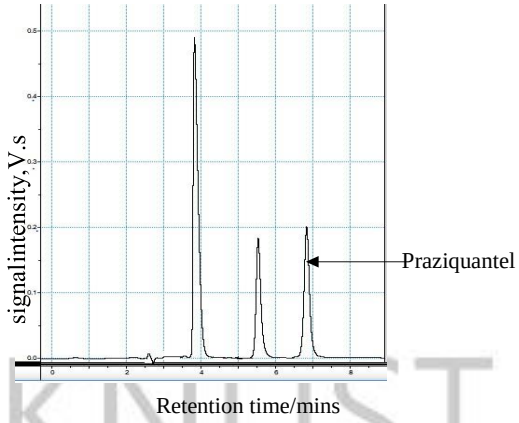

Figure 4.9: Chromatogram of a sample of veterinary product containing Praziquantel, Pyrantel Pamoate and Febantel

4.3 PHARMACOPOEIAL TESTS

4.3.1 Assay of Pure Compounds

Table 4.1: Assay of Pure Compounds

SAMPLE	PERCENTAGE PURITY(%w/w)	SPECIFICATION (% w/w)
Oxytetracycline	99.24	95-102 (BP 2013)
Praziquantel	99.40	98.5–101(USP 35)

4.4 HPLC METHOD DEVELOPMENT

4.4.1 Retention Time

Table4.2: Retention Time of Pure Samples and Formulated Products (Samples)

SAMPLE	RETENTION TIME (MINUTES)
Oxytetracycline	3.326± 0.0590
Praziquantel	6.80 ± 0.0744

Sample Chromatograms Obtained Using the Methods Developed

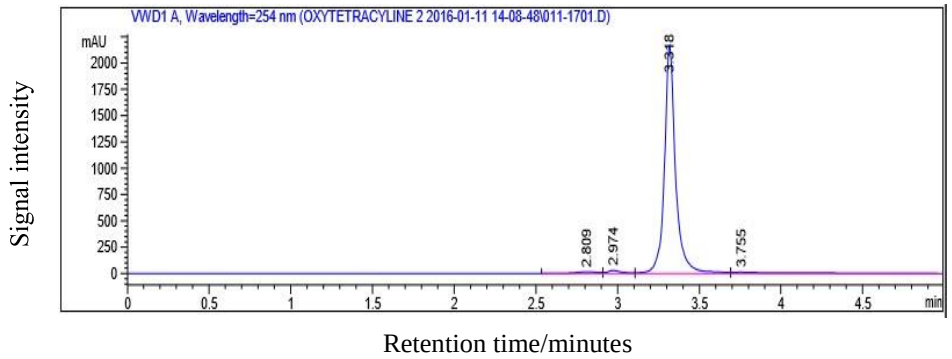

Figure 4.10: Chromatogram of Pure Oxytetracycline (0.4mg/ml)

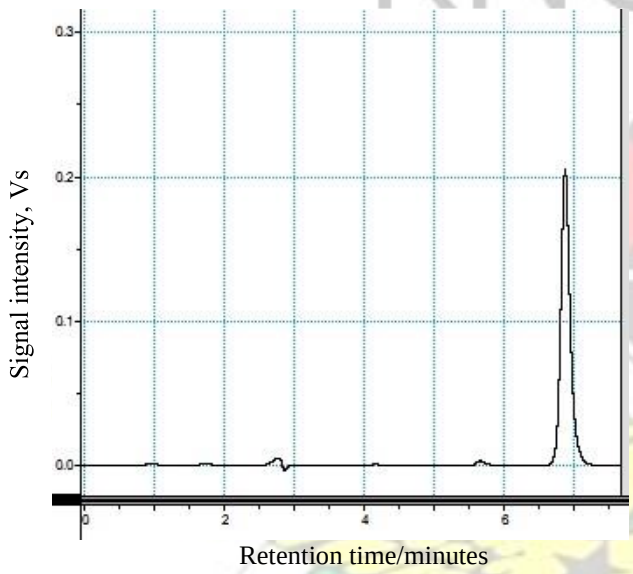

Figure 4.11: HPLC spectrum of pure Praziquantel (0.4mg/ml)

4.5 METHOD VALIDATION

4.5.1 Linearity

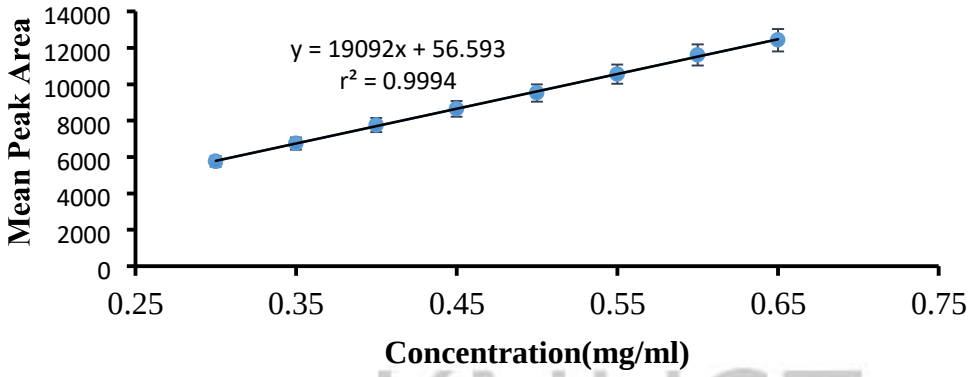

Figure 4.12: Calibration curve for Oxytetracycline

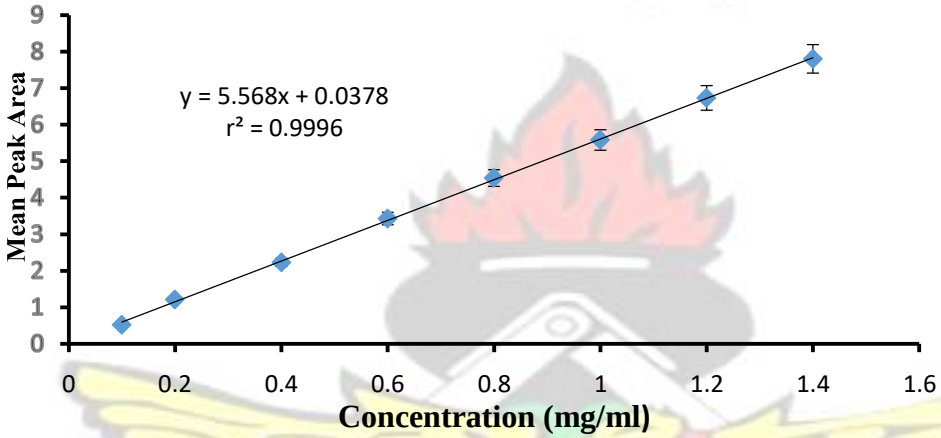

Figure 4.13: Calibration curve for Praziquantel

Table 4.3: Parameters of the calibration curve

Sample	Calibration equation	Correlation coefficient
Oxytetracycline	y = 19092x + 56.63	0.9994
Praziquantel	y = 5.568x + 0.0378	0.9996

Table 4.4: Sensitivity analysis of the various samples

Sample	Linearity range	LOD(mg/ml)	LOQ (mg/ml)
Oxytetracycline	0.30 – 0.60	0.01054	0.03196
Praziquantel	0.10 – 1.40	0.03156	0.09564

4.5.2 Specificity

OXYTETRACYCLINE

Table 4.5: Specificity of Oxytetracycline

Parameter	Response
Diluent	No peak obtained at the retention time for Oxytetracycline (-)
Oxytetracycline	Distinct Peaks obtained with no interference from diluent and mobile phase
Diluent	No peak obtained at the retention time for Oxytetracycline (-)

(-): Not Detected

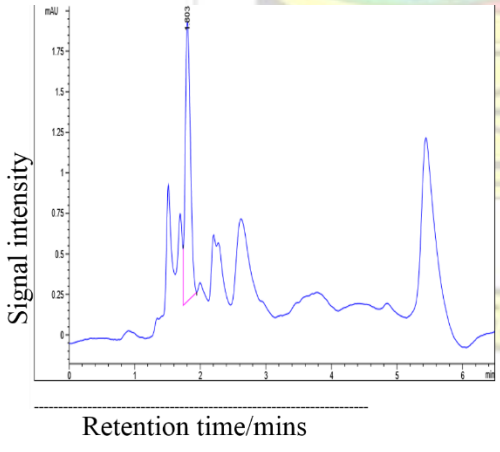

Figure 4.14 Chromatogram of diluent

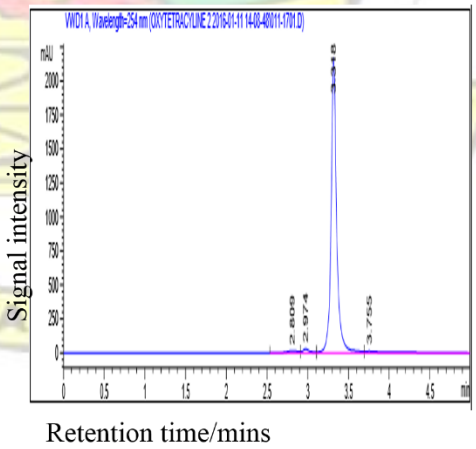

Figure 4.15: Chromatogram of pure Oxytetracycline

PRAZIQUANTEL

Table 4.6: Specificity of Praziquantel

Parameter	Response
Diluent	No peak obtained at the retention time for Praziquantel(-)
Praziquantel	Single Peak obtained
Diluent	No peak (-)

(-): Not Detected

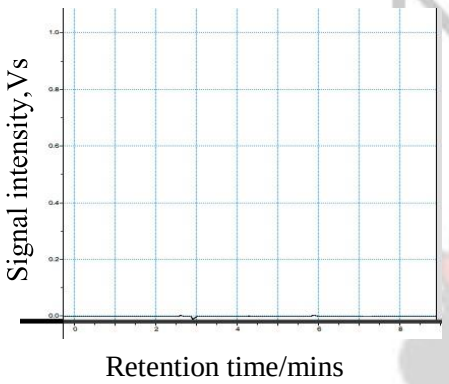

Figure 4.16: Chromatogram of diluent

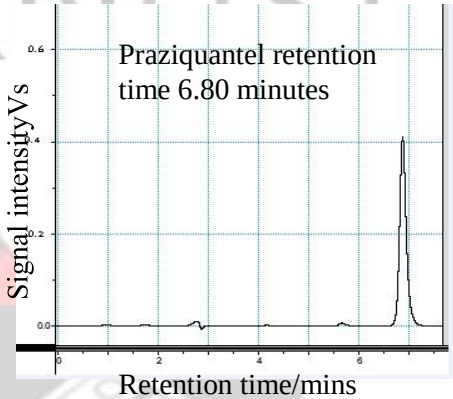

Figure 4.17 :Chromatogram of pure Praziquantel

Figure 4.17 Chromatogram of pure Praziquantel

Selectivity of Method for Praziquantel

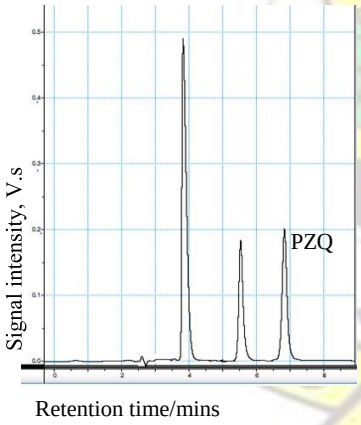

Figure 4.18: Selectivity of method for sample PA

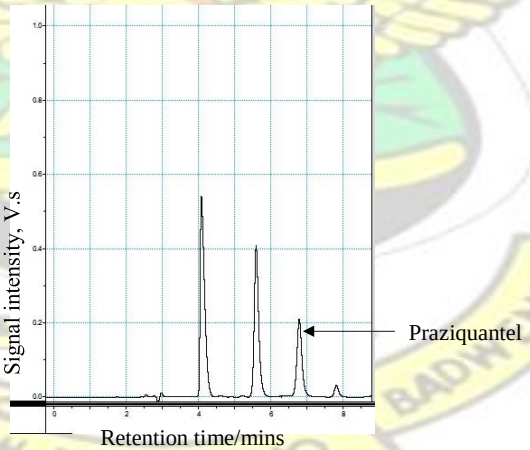

Figure 4.19: Selectivity of method for sample SB

4.5.3 Precision

Table 4.7: Intra-day precision of method for Oxytetracycline and Praziquantel

Sample	Time (hrs.)	Mean peak area ±SD(V.s)	Relative standard deviation (%)
Oxytetracycline	0	9617.36 ± 47.29	0.49
	2	9599.95 ± 23.77	0.25
	6	9607.80 ± 31.39	0.33
Praziquantel	0	2.22 ± 0.01	0.37
	2	2.22 ± 0.01	0.44
	6	2.22 ± 0.01	0.25
Acceptance criteria		**RSD ≤ 2%**	

Table 4.8: Inter-day precision of method for Oxytetracycline and Praziquantel

	Time (hrs.)	Mean peak area ±SD(V.s)	Relative standard deviation (%)
Oxytetracycline	1	9571.23 ± 69.94	0.73
	2	9576.53 ± 83.28	0.87
	3	9622. 54 ± 50.00	0.51
Praziquantel	1	2.23 ± 0.01	0.34
2	2.22 ± 0.01	0.37	
3	2.22 ±0.01	0.23	
	Acceptance criteria		**RSD ≤ 2%**

4.5.4 Accuracy

Table 4.9: Accuracy of Oxytetracycline and Praziquantel

Sample	Concentration (%w/w)	Amount taken (mg/ml)	Amount added (mg/ml)	Percentage recovery ± SD
Oxytetracycline	80	0.50	0.40	99.98 ± 0.59
	100	0.50	0.50	99.96 ± 0.80
	120	0.50	0.60	100.30 ± 0.43
Praziquantel	80	0.40	0.32	99.54 ± 0.53
	100	0.40	0.40	100.18 ± 0.45
	120	0.40	0.48	99.78 ± 0.33
Acceptance Criteria 98% - 102%				

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4.5.5 Robustness

Table 4.10: Robustness of Oxytetracycline and Praziquantel

Sample	Original condition (mean % content)	Varied condition (mean % content)	P –value (t – test)	F- test
Oxytetracycline	100.64 ± 0.03	100.59 ± 0.08	0.1954	0.0970
Praziquantel	99.63 ± 0.77	99.33 ± 0.47	0.4369	0.3002
RSD	0.03	0.07	Acceptance of null hypothesis p> 0.05	

4.5.6 Stability of Solution

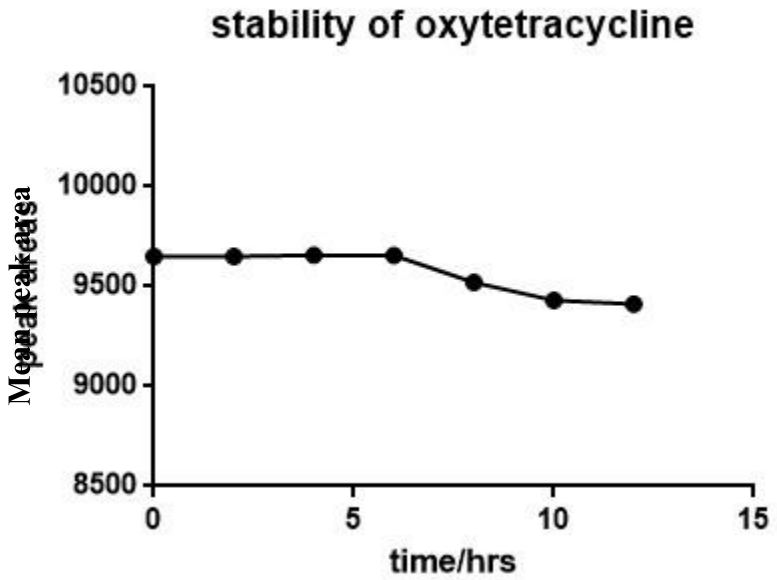

Figure 4.20: Stability of Oxytetracycline

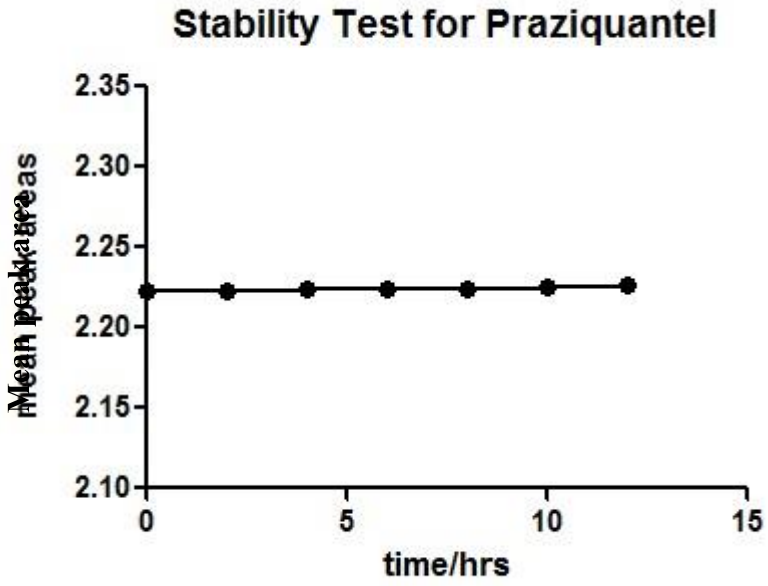

Figure 4.21: Stability of Praziquantel

4.6 ASSAY OF FORMULATED PRODUCTS

Table 4.11: Assay of Formulated Products of Oxytetracycline

SAMPLE	SOURCE	DOSAGE FORM	PERCENTAGE CONTENT (% w/w for powders and %w/v for injectables) ± SD	ACCEPTANCE CRITERIA (BP 2013)
Sample A	Manufacturer	Powder	71.49 ± 0.22	95-110%
Sample B	Farm	Powder	52. 45 ± 0.12	95-110%
Sample C		Powder	34.11 ± 0.56	95-110%
Sample D		Injectable	99.99 ± 0.25	95-110%
Sample E		Powder	61.94 ± 0.62	95-110%
Sample F	Open Markets	Powder	111.38 ± 0.42	95-110%
Sample G		Powder	79.46 ± 0.50	95-110%
Sample H		Powder	61.94 ± 0.32	95-110%
Sample I		Powder	115.76 ± 0.47	95-110%
Sample J		Injectable	99.44 ± 0.14	95-110%
Sample K		Injectable	106.31 ± 0.02	95-110%
Sample L		Powder	115.48 ± 0.16	95-110%
Sample M		Powder	61.55 ± 0.22	95-110%
Sample N	Veterinary	Injectable	99.45 ± 0.02	95-110%
Sample O	Hospitals	Injectable	99.91 ± 0.08	95-110%
Sample P		Injectable	101.02 ± 0.04	95-110%
Sample Q		Injectable	100.61 ± 0.12	95-110%
Sample R		Powder	100.31 ± 0.44	95-110%
Sample S		Powder	65.49 ± 0.72	95-110%
Sample T		Powder	103.76 ±0.64	95-110%

Table 4.12: Results for the Sampling and Analysis of Praziquantel

SAMPLE	DOSAGE FORM	PERCENTAGE CONTENT (% w/v) ± SD	ACCEPTANCE CRITERIA (USP35)

Sample PA	Tablet	99.47 ± 0.35	90 - 110%
Sample PB	Tablet	99.39 ± 0.37	90 - 110%
Sample PC	Tablet	101.86 ± 0.40	90 - 110%
Sample SA	Tablet	62.50 ± 0.60	90 - 110%
Sample SB	Tablet	82.52 ±0.44	90 - 110%

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Comparison of Percentage Content of Samples Containing Oxytetracycline from Different Sampling Sites

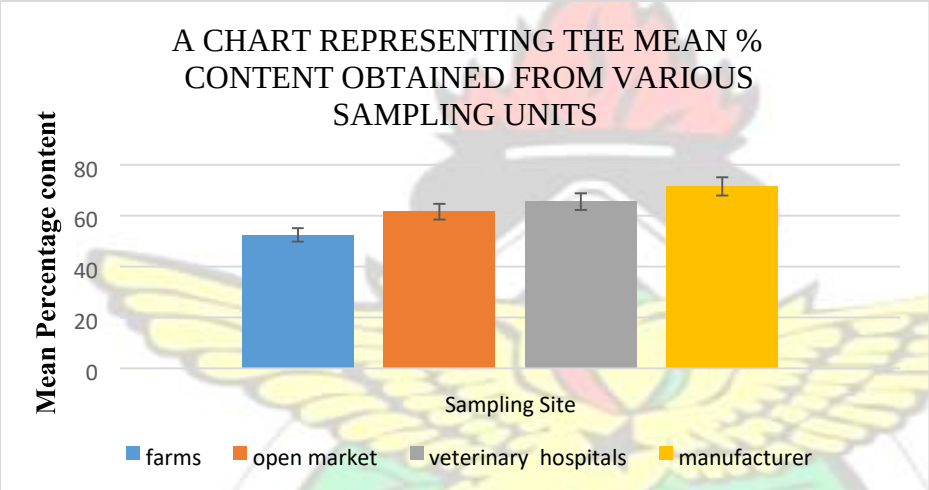

Figure 4.22: Mean Percentage Content Obtained From Various Sampling Units

Sample Calculation for Percentage Content of Oxytetracycline in Samples In HPLC;

The peak area (A) in a chromatogram is directly proportional to the concentration of the analyte. This

suggests Aα C

For similar compounds,

$$\frac{area\ of\ standard}{concentration\ of\ standard} = \frac{area\ of\ test}{concentration\ of\ test}$$

$$\text{concentration of the test} = \frac{concentrationofstandard}{areaofstandard} \times area\ of\ test$$

Concentration of pure powder (C) $= 0.5mg/ml$

Area of pure powder = 9540.00

Area of test = 6820.42

$$Concentration\ of\ test = \frac{0.5}{9540}\ x\ 6820.42$$

= **0.3575mg /ml**

Percentage content

$$= \frac{actual\ weight}{expected\ weight}\ x\ 100\%$$

$$= \frac{0.3575}{0.5000} x\ 100$$

= **71.49**%.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1Sampling

Sampling was done with respect to the sites veterinary pharmaceuticals could be obtained. (See Figure 4.1). Farmers use the veterinary pharmaceuticals for their animals when required. Individuals also purchase veterinary pharmaceuticals such as Praziquantel for their pets such as dogs. Very few

pharmacies that stock mainly medicines for humans stock veterinary pharmaceuticals.

Out of the number of samples analysed, 40% of samples were obtained from the open markets, 35% of samples were obtained from veterinary hospitals, 20% was obtained from farms visited and the remaining was obtained directly from the manufacturer. Various dosage forms of Oxytetracycline were sampled while tablets were sampled for veterinary pharmaceuticals containing Praziquantel. The predominant dosage form of Praziquantel sampled was tablets. (See Appendix B).

Veterinary shop owners contacted reported that there was no license involved in the operation of a veterinary shop. The only requirement was registration with the Registrar General's Department. The activities in the various premises of sale of the veterinary medicines were not regulated by any recognized institution. This was found to be in contrast to the regulation of human medicine and activities in pharmacies mainly for human medicines.

The Public Health Act, 2012, Act 851 Part seven (7) clearly defines the functions of the Food and Drugs Authority (FDA) in Ghana. The functions of the FDA includes the assurance of the quality of drugs, which includes veterinary pharmaceuticals. However, most of the products sampled had no FDA registration numbers.

Procurement of veterinary medicines by farmers is done from veterinary shops and from the veterinarians positioned in various districts. Some of the drugs were sold to farmers in the veterinary hospitals or even on the farm after the animals had been diagnosed.

In the open market, storage of veterinary medicines was a challenge. Many veterinary medicines were not stored under the required storage conditions. A common observation made was the poor ventilation in some shops and the exposure of some of the pharmaceuticals to sunlight.

5.1.2 Identification Tests

- **Identification of Pure Samples**

Identification tests play a significant role in ensuring the identity and purity of compounds in medicines. Establishment of the true identity of a compound is necessary in order to analyse a product. Identification tests are also used to ascertain whether the compound of interest is present in a sample as specified by the label claim. In a chemical laboratory, establishment of the identity of any substance is key. Some compounds could bear superficial resemblance to the sample being worked on. Identification tests thus provide a means by which the compound of interest can be distinguished from other unwanted substances.

Subjection of the samples to identification tests enables samples being worked on to be used sometimes as reference compounds in both qualitative and quantitative analysis. This is because the authenticity of results can be obtained from results of confirmed samples.

Identification tests comprise various forms ranging from measurement of physical properties of compounds, chemical reactions or the use of equipment like the IR spectrophotometer and the HPLC. Detection of adulteration and chemically related substances can be achieved with HPLC. With such techniques, chemically related compounds that could significantly affect results obtained for both qualitative and quantitative work could be handled without

any interferences. Identification tests were performed prior to analysis to ensure that the right compound was analysed.

- **Identification of Pure Samples Using UV**

The identity of pure sample of Oxytetracycline was confirmed using UV/Visible Spectroscopy and the maximum wavelength of absorption was 268nm in aqueous acid (0.1M HCl) as stated in literature (Mofatt , Jackson , Moss , & Widdop , 2005)(See Figure4.2).

The identity of Praziquantel was also confirmed using 96% ethanol, which gave a maximum wavelength of absorption at 264nm as specified by literature (Mofatt , Jackson , Moss , & Widdop , 2005). (See Figure 4.3).

- **Identification of Pure Samples using Infrared Spectroscopy**

The IR spectrophotometer was used to confirm the presence of Oxytetracycline (Mofatt , Jackson , Moss , & Widdop , 2005) and Praziquantel (British Pharmacopoeia Commission, 2013). The spectra for both Oxytetracycline and Praziquantel showed bands as specified in literature. The bands in the fingerprint region for both compounds corresponded to that specified in literature. This confirmed the identity of the pure powders. (See Figure 4.4 and 4.5).

- **Identification of Pure Sample of Oxytetracycline Using Chemical Reaction**

The identity of Oxytetracycline pure powder used was confirmed by the positive reaction for the chemical reaction described in the method for the identification of Oxytetracycline pure powder.

- **Identification of Samples of Oxytetracycline and Praziquantel Using HPLC**

The retention time of a compound under specified conditions could be used to identify a substance. The retention times of the HPLC chromatograms of the working concentrations of Oxytetracycline and Praziquantel in both the pure reference sample and the formulation were identical and consistent (3.326 ± 0.0590 and 6.80 ± 0.0744 minutes respectively) when analysed with the chromatographic conditions specified in this study. This confirmed the presence of Oxytetracycline and Praziquantel in the formulation. (See Figures 4.6, 4.7 and Figures 4.8 and 4.9).

- **Identification of Samples of Oxytetracycline Using Chemical Reaction**

The presence of Oxytetracycline in the formulated products (samples) used was confirmed by the positive reaction for the chemical reaction described in the method for the identification of samples of Oxytetracycline.

5.1.3 Pharmacopoeial Tests

- **Uniformity of Weight of Praziquantel Tablets**

Uniformity of weight ensures consistency of dosage units produced from a batch. The weight of a tablet is determined by the quality of die filling in the tableting process, bulk density of granules and uniformity of particulate flow into the die. Consistency of dosing could be ensured using uniformity of weight. In practice, small variations between individual preparations are accepted and the limits are defined in the official compendia.

With reference to the (British Pharmacopoeia Commission, 2013), twenty tablets should be randomly selected for the uniformity of weight determination and not more than two of the tablets should deviate from the average weight of the tablets by ± 10% for a sample containing 80mg or less and none of the tablets should deviate by more than ± 20% for tablets containing 80mg or less of the active ingredient. (See Appendix A4)

The results obtained indicated that all the batches of Praziquantel tablets analysed passed the uniformity of weight test.

- **Assay**

The purity of the pure reference standards of Oxytetracycline and Praziquantel were ascertained using UV/ Visible spectroscopic methods as stated in (Mofatt , Jackson , Moss , & Widdop , 2005), and in the in- house validated method of a reputable pharmaceutical company in Ghana.

The respective percentage purities of Oxytetracycline and Praziquantel were 99.24% w/w and 99.40% w/w. The BP (2013) specifies a range of 95 – 102 % for Oxytetracycline HCl and 97.5% -102% for Praziquantel. The USP 35 specifies 98.5% - 101% for Praziquantel pure powder (United States Pharmacopoeial Convention, 2012).

Oxytetracycline and Praziquantel pure powders were within the acceptable limits and therefore passed the test. Oxytetracycline and Praziquantel pure powders used were of high purity.

5.1.4 HPLC Method Development

Literature search was done on the various methods developed for the analysis of Oxytetracycline and Praziquantel. A search was also done on the physicochemical properties of the medicines being studied. Various trials were done based on findings from literature. The choice of mobile phase was an essential part of this study. The method developed and validated for Oxytetracycline analysis was a mobile phase composition of acetonitrile: water: trifluoroacetic acid (TFA) (85:14.5:0.5(1%v/v); v/v/v) respectively. The stationary phase employed was an Altima Cyano 100A 5µ, (250mm x 4.6mm) column at a wavelength of detection, 254nm. The retention time was 3.326 ± 0.05 minutes. A flow rate of 2ml/min was used for the analysis of Oxytetracycline.

Chromatographic conditions developed for Praziquantel was mobile phase composition of acetonitrile: water: TFA (45:54.95:0.05(1%v/v); v/v/v) respectively. The stationary phase employed was ReproSil 100 CN 5µ, (250mm×4.6mm) and was used at a detection wavelength of 230 nm. The retention time was 6.80 ± 0.07 minutes.

A mixture of solvents including acetonitrile, water and orthophosphoric acid in different combinations using a C-18 column form part of the trials for Oxytetracycline. Repeatability of results was however a challenge under the conditions. Several other combinations were tried until the final methods presented in this work were obtained.

It was realized that frequent degassing was not needed when acetonitrile was used instead of methanol. Acetonitrile cools the temperature by absorbing heat. Air bubbles are later generated, as the solution gradually returns to room

temperature (Shimadzu, 2016). Also, the back pressure with acetonitrile was moderate as compared to methanol mixed with water.

TFA, a mobile phase modifier and an ion - pairing reagent, was used to lower the pH of the mobile phase without requiring a salt to maintain the buffer at that pH. Trials without TFA produced peaks that were tailing. TFA prevents secondary interactions and therefore reduces tailing. Ionized silanol groups interact through ion exchange with ionized bases and are a major factor in peak tailing. Suppression of ionization was therefore a way of minimizing this problem.

Cyano column can be used as an intermediate phase (reverse phase or normal phase). In this study, the cyano column was employed as a reverse phase. Cyano column is compatible with water and all common organic solvents (Agilent Technologies, 2011). Cyano columns can be used between pH 1 and 8(Henry, Santasania, Campbell, & Roe, 2010). This makes it very useful when dealing with low pH mobile phases. The moderate retention characteristics of a cyano column allows the use of less organic solvent in the mobile phase, giving faster chromatography.

In the selection of wavelength, factors like interference from the sample matrix such as excipients, other APIs or other impurities present as well as the wavelength cut off point of the solvent system were considered.

Mobile phase did not show a strong background absorption at the selected wavelengths. A wavelength of 254nm was used for Oxytetracycline while that for Praziquantel was 230nm. There is a benzene ring in the structure of Oxytetracycline which allows for absorption at 254nm. The UV/Vis spectrum

also confirmed an extent of absorption at 254 nm for Oxytetracycline and 230 nm. The wavelength employed for the analysis of Oxytetracycline in this work has been used in the BP and some previous methods of analysis developed for Oxytetracycline (Tatar, Ates, & Kucukguzel, 2015). In the development of method for Praziquantel, several trials were made with the detection wavelength in the BP (210nm); however, the other components of the sample matrix interfered. The wavelength was thus modified until 230nm was obtained as the ideal wavelength for the analysis. This wavelength yielded distinct peaks of the components of the sample with no interference from the diluent.

A number of trials were made using different flow rates, starting from 1ml/min. For optimum results, the flow rates employed were 2 ml/min for Oxytetracycline and 1ml/min for Praziquantel.

At this rate, column back pressure was moderate, peak resolution was good and retention times were suitable allowing enough time for interaction of the analyte with stationary phase to achieve a good separation.

5.1.5 Validation of Analytical Method

- **Linearity**

Linearity refers to the capability of an analytical method to obtain a direct proportional relationship between the concentration of analyte in a sample and the test results (peak areas in terms of HPLC). It is expected that a linear relationship is obtained across the scope of an analytical procedure. A correlation co-efficient greater than 0.99 suggest a non-significant deviation from linearity and also a strong correlation between response and analyte concentration. From the results, the correlation coefficient (r^2) of

Oxytetracycline and Praziquantel were 0.9994 and 0.9996 respectively. This shows that there is a strong correlation between the response and analyte concentration over the specified concentration range (See Figures 4.12 and 4.13).

- **Sensitivity**

The smallest concentration of analyte that can be reproducibly and dependably identified (LOD) and quantified (LOQ) were computed for Oxytetracycline and Praziquantel. The LOD and LOQ values obtained for Oxytetracycline were 0.01054mg/ml and 0.03196mg/ml respectively. The LOD and LOQ values for Praziquantel were 0.03156mg/ml and 0.09564mg/ml. respectively.

From the results, the methods can be said to be sensitive.

- **Specificity**

Specificity is the capacity of the method to identify the analyte of interest regardless the presence of other significant components anticipated to be present in the sample.

Selectivity refers to the ability of the method to obtain the response of the analyte of interest, separated from all other responses. Selectivity of a method alludes to the degree to which it can distinguish specific analyte regardless of the interferences present. Selectivity and specificity are measures of the reliability of measurements in the presence of interferences.

Since methods specific for only one analyte are rare, the term selectivity is generally more suitable. For Praziquantel, the method could determine distinctly Praziquantel from several other components in the sample. The formulated

tablets are presented as multi-component formulations containing Praziquantel, Febantel and Pyrantel Pamoate. The method was able to distinguish Praziquantel from the other components which are likely to be Febantel and Pyrantel Pamoate. (See figure 4.18 and 4.19)

A confirmation was not done with respect to the exact identity of the other components of the Praziquantel formulation.

The methods can thus be said to be specific and selective. (See Figures 4.14, 4.15 and 4.16, 4.17).

- **Precision**

The precision of a systematic methodology portrays the closeness of agreement between autonomous test outcomes.

The intra- day results of Oxytetracycline gave RSD of 0.49,0.25% and 0.33% and Praziquantel were 0.37%,0.44% and 0.25% for 0, 2 and 6 hours respectively (See Tables 4.7)

The inter-day results of Oxytetracycline gave RSD values of 0.73%, 0.87% and 0.51% while that of Praziquantel gave RSD values of 0.34%, 0.37% and 0.23% for days 1, 2 and 3 respectively.

The RSD values obtained were less than 2%, indicating the closeness of agreement between the results. The methods developed for Oxytetracycline and Praziquantel are precise.

- **Accuracy**

Accuracy of the methods were determined by recovery tests using standard addition. The accuracy criteria as defined by FDA is that mean recovery should

be 100 % ± 2 at each concentration over the range of 80 – 120 % of target concentration.

Results showed high degree of agreement between the true value and estimated values. The method can be said to be accurate within the desired recovery range (See Table 4.9).

- **Robustness**

The chromatographic methods were subjected to intentional but little variations in the chromatographic conditions in order to estimate the robustness of the methods developed.

The original column (Altima Cyano 100A 5µ, (250mm x 4.6mm) was changed to a ReproSil 100 CN 5µ, (250mm×4.6mm) column and a sample of Oxytetracycline was analysed using both conditions (columns).

For Praziquantel, the original flow rate of the method was 1 ml/min and this was changed to 1.2 ml/min. A sample was analysed using both flow rates and the results were subjected to the t-test and f- test. (See Table 4.10).

The results showed no significant difference when the conditions were changed using GraphPad Prism 6. This shows that the methods developed for Oxytetracycline and Praziquantel can be defined as robust.

- **Stability of Solution**

The time period for analysis is very important. Stability of solution determines the period for which the analysis is valid and accurate.

The RSD was determined for each compound at 0, 2, 4, 6, 8, 10 and 12 hours to measure the degree of variation in the concentration of each component with time.

For Oxytetracycline, it was observed that there was a stable mean peak area until after six hours when a significant variation in the mean peak area was observed (Figure 4.20). This means that in the analysis of Oxytetracycline, fresh solutions should be prepared and analysis done within six (6) hours of preparation of solutions.

However the mean peak areas of Praziquantel was stable and showed no significant variation in peak area and concentration for the time considered (Figure 4.21). Praziquantel can therefore be analysed within twelve (12) hours of sample preparation. Stability of the compound after twelve hours cannot be assured as that was not considered in this study (Kalra, 2011). (See Figures 4.24 and 4.25).

5.1.6 Assay of Sampled Formulated Products of Oxytetracycline and Praziquantel

Twenty-five (25) commercial brands of veterinary products containing Oxytetracycline HCl and Praziquantel were sampled and analysed (See Table 4.11, figure 4.22, Appendix C). All products sampled and analysed were within their shelf life.

According to the British Pharmacopoeia 2013, the acceptance criteria for Oxytetracycline HCl formulations is 95% - 110% (See Table 4.11).

Storage conditions of medicines is very important. Improper storage of products could lead to breakdown of active ingredients in a formulation. For

Oxytetracycline, reported degradation products include 4-epioxytetracycline, α- and β-apooxytetracycline. Research has shown that Oxytetracycline can be broken down by moisture, light, different pH (Xuan, Arisi, Wang, Yates, & Biswas, 2010).

The existence of acid – base active phenolic hydroxyl and amine groups in Oxytetracycline makes it amphoteric in nature. Neutral pH solution has been found to favour the hydrolysis the most, followed by alkaline solution (Xuan, Arisi, Wang, Yates, & Biswas, 2010).

Among these conditions, hydrolytic degradation and photolytic degradation have been reported to be the conditions with the highest rate constants with respect to degradation. Oxytetracycline has strong absorbance at 200–400 nm, making Oxytetracycline susceptible to sunlight irradiation (Lunestad, Samuelson, Fjelde, & Erwik, 1995). Photolysis may be a primary degradation pathway for Oxytetracycline. Rapid photolysis of Oxytetracycline has been observed under sunlight irradiation. As the temperature increases, Oxytetracycline degradation also increases. Photolysis rate is said to increase with increasing sunlight intensity (Xuan, Arisi, Wang, Yates, & Biswas, 2010).

Solid Praziquantel has been reported to exhibit high stability at high temperatures. However, Praziquantel breaks down upon exposure to sunlight and about 50% of its amount can be lost over a period of four (4) months. (Schepmann & Blaschke, 2001). A report has also shown that there is breakdown of Praziquantel in aqueous media with the presence of heat and light. (Horvat, et al., 2011). Monohydroxylated metabolites trans-4´hydroxypraziquantel, cis-4-hydroxypraziquantel and 8-hydroxypraziquantel

have been found as breakdown products in the body; with the trans-4 -OHPZQ being the main metabolite in humans (Schepmann & Blaschke, 2001).

The dosage forms of Oxytetracycline products used in this study were injectables and powders. All the injectables were within specification; including the injectables that had already been opened and were being used in the veterinary hospitals. Packaging is a factor to be considered in ensuring quality standards of medicines. The injectables of veterinary Oxytetracycline come in amber-coloured bottles with a tight seal reducing the chance of breakdown either by photolysis or hydrolysis. Even though some of the injectables had been used once or a couple of times, and were kept on the shelves in the veterinary hospitals, they still were within the accepted percentage content range. However, Oxytetracycline powders were either packaged in rubber or plastic containers. Once opened, proper storage was quite difficult as one would have to be intentional in storing them under the required conditions.

Lack of effective regulation could lead to the production of substandard products. The use of less API and/or more excipients than the actual amount required for production could result in the production of poor quality medicines.

Furthermore, analysis was done using a particular product with similar batch numbers to compare the difference in percentage content with respect to the sampling sites. The results indicated that there was significant difference between the percentage content from the manufacturer to the end user using the One way ANOVA from GraphPad Prism 6. (See Figure4.22). The results indicated that even from the manufacturer, the percentage content was not within specification. The percentage content obtained from veterinary hospitals,

open markets and the farm was quite low as compared to that obtained from the manufacturer. The variation with respect to percentage content could be as result of storage conditions of the sample at their respective sources or possible conditions of storage during the transportation of the product from the manufacturer.

5.2 CONCLUSION

The survey has been successfully conducted. Oxytetracycline and Praziquantel were selected for this study because of the high frequency of use. It was realized that Oxytetracycline was frequently used in animals on the farms visited and Praziquantel was in high demand in the open market.

A simple, precise and reliable reverse phase high performance chromatographic method has been successfully developed using a mobile phase of acetonitrile: water: trifluoroacetic acid (TFA) (85:14.5:0.5(1%v/v); v/v/v) and Altima Cyano 100A 5µ, (250mm x 4.6mm) column at a wavelength of 254nm for the analysis of Oxytetracycline.

Similarly, a simple, precise and reliable RP-HPLC method has been successfully developed using a mobile phase of acetonitrile: water: TFA (45:54.95:0.05(1%v/v); v/v/v) and ReproSil 100 CN 5µ, (250mm×4.6mm) column at a wavelength of 230nm for the analysis of Praziquantel.

The methods are capable of achieving good separation of Oxytetracycline and Praziquantel respectively.

The methods can also be used for general analysis of single and multicomponent formulations containing Oxytetracycline and Praziquantel respectively using the stated conditions for each of them.

The methods were successfully validated under linearity, accuracy, precision, specificity, robustness and stability per the ICH protocols.

All pure reference samples used passed the tests for their identification and were of high purity.

Significant variations in the percentage content of the veterinary pharmaceuticals were observed with respect to the sampling sites.

There is a great need for the regulation of veterinary pharmaceuticals, as it is done for medicines for human use. Lack of regulation of veterinary pharmaceuticals could lead to serious negative outcomes in humans. Possible residues or disease treatment failure due to the use of sub-standard veterinary products could lead to the development of antimicrobial resistance.

5.3 RECOMMENDATIONS

It is recommended that the effective regulation of veterinary pharmaceuticals should be a major concern. All institutions involved in the regulation of veterinary pharmaceuticals should be empowered to do so.

Also, the factors that affect Oxytetracycline and Praziquantel breakdown should be studied with respect to the conditions in Ghana.

It is also recommended that further studies on the residues of Oxytetracycline in poultry be carried out.

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APPENDICES

APPENDIX A

Table A1: F and T-Test Using Graph Pad Prism 6 for Oxytetracycline (Robustness)

Column A	Original Condition
Unpaired t test	
P value	0.1954
P value summary	Ns

Significantly different ?(P< 0.05)	No
One- or two-tailed P value?	Two tailed
t,df	t=1.388 df=10

How big is the difference?

Mean ± SEM of column A	100.6±0.01358 N=6
Mean ± SEM of column B	100.6±0.03077 N=6
Difference between means	-0.04667±0.03363
95% confidence interval	-0.1216 to 0.02827
R square	0.1615

F test to compare variances

F, DFn , Dfd	5.132,5,5
P value	0.0970
P value summary	Ns
Signicantly different? (P< 0.05)	No

A2: Robustness (F and T-Test Using Graph Pad Prism 6 for Praziquantel)

Column B	Varied Condition
Unpaired t test	
P value	
P value summary	0.4369
Significantly different ?(P< 0.05)	Ns
One- or two-tailed P value?	No
t,df	Two-tailed t=0.8098 df=10

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How big is the difference?	
Mean ± SEM of column A	99.63 ± 0.3153 N=6
Mean ± SEM of column B	99.33 ± 0.1920 N=6
Difference between means	-0.2989 ± 0.3692
95% confidence interval	-1.122 to 0.5236
R square	0.06154
F test to compare variances	
F, DFn , Dfd	2.697, 5, 5
P value	0.3002
P value summary	Ns
Signicantly different? (P< 0.05)	

A3: Robustness of Oxytetracycline using a varied column

Test	Original condition (Altima Cyano)		Varied condition (ReproSil Cyano)	
	Peak area	Percentage content	Peak area	Percentage content
1	9600.102	100.63	9604.872	100.68
2	9596.286	100.59	9593.424	100.56
3	9598.194	100.61	9600.102	100.63
4	9602.964	100.66	9583.884	100.46
5	9602.01	100.65	9599.148	100.62
6	9604.872	100.68	9596.286	100.59

Mean ± SD	9600.74 ± 3.17	100.64 ±0.03	9596.29 ±7.19	100.59 ± 0.08
RSD (%)	0.03	0.03	0.07	0.07
Acceptance Criteria	RSD ≤ 2%			

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A4: Uniformity of weight of Praziquantel Tablets

Tablet number	Individual t weight/g (x)	Deviation (x-a)	(x-a)/a	%Deviation (x-a)/a×100
1	0.684	0.0115	0.0171	1.71003717
2	0.676	0.0035	0.005204	0.5204461
3	0.681	0.0085	0.012639	1.26394052
4	0.653	-0.0195	-0.029	-2.8996283
5	0.676	0.0035	0.005204	0.5204461
6	0.666	-0.0065	-0.00967	-0.9665428
7	0.677	0.0045	0.006691	0.66914498
8	0.669	-0.0035	-0.0052	-0.5204461
9	0.678	0.0055	0.008178	0.81784387
10	0.687	0.0145	0.021561	2.15613383
11	0.674	0.0015	0.00223	0.22304833
12	0.669	-0.0035	-0.0052	-0.5204461
13	0.677	0.0045	0.006691	0.66914498
14	0.656	-0.0165	-0.02454	-2.4535316
15	0.665	-0.0075	-0.01115	-1.1152416
16	0.684	0.0115	0.0171	1.71003717
17	0.668	-0.0045	-0.00669	-0.669145
18	0.675	0.0025	0.003717	0.37174721

19	0.662	-0.0105	-0.01561	-1.5613383
20	0.667	-0.0055	-0.00818	-0.8178439

Weight of twenty (20) tablets = 13.45g

$$average\ weight\ of\ tablet = \frac{13.45}{20} = 0.6725g$$

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0.6725g of powdered tablet ≡ 50mg of PZQ

$$\frac{0.4 \times 0.6725}{50} \equiv 0.4\text{mg of PZQ}$$

$$= 0.00538\text{g}$$

A5: One way Analysis of variance to compare content of OTC from the various sampling sites.

Table Analysed	Unpaired t test data		
One-way analysis of variance			
P value	< 0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	4		
F	55.35		
R squared	0.9540		
ANOVA Table	SS	df	MS
Treatment (between columns)	574.2	3	191.4
Residual (within columns)	27.67	8	3.458
Total	601.9	11	

Table A6: Questionnaires Administered To the Various Farms Visited

Question	
Who manages the farm?	
What is the educational level of the farm manager?	
Farm environment and location	
What is the source of farm water?	
What diseases on the farm are treated with antibiotics and other medications?	
Do you have any inventory of the drugs used?	
Dosage form of the antibiotics and other medication	
Routes of antibiotic and other medications administration	
Storage sites of the antibiotics	
Disposal site of farm waste water	
Do you wash yourself after administration antibiotic use or after using other medications?	
Do you use protection during administration?	
Types of protection used	
Is there direct contact with medications during medication handling?	
Where do you buy the antibiotics from?	
Is the source registered? Under which license?	
How is the antibiotic stored at the source? Storage conditions at the point of purchase	
How is the antibiotic stored on the farm?	

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What is the withdrawal period?	

APPENDIX B

Some Selected Samples Collected For Analysis

APPENDIX C

Calculations for the Preparation of 0.1M HCl

$36.4500g\ in\ 1000ml \equiv 1M\ HCl$

$3.6450g\ in\ 1000ml \equiv 0.1M\ HCl$

$0.3645g\ in\ 100ml \equiv 0.1M\ HCl$

$Assay = 36\%$

$36\% = 0.3645g$

$$100\% = \frac{100 \times 0.3645}{36} = 1.0125g$$

Specific gravity = 1.18g/ml

$1.18g = 1ml$

$$1.0125g = \frac{1.01250g \times 1ml}{1.18g} = 0.858ml$$

= 0.86 ml

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