DISPOSITION KINETICS AND TISSUE RESIDUE STUDY OF SULPHADIAZINE-TRIMETHOPRIM AND AMPROLIUM IN BROILER POULTRY

Thesis

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By

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Pantnagar
July, 2022

(Preeti Bisht)
Authoress

CERTIFICATE-I

This is to certify that the thesis entitled **"DISPOSITION KINETICS AND TISSUE RESIDUE STUDY OF SULPHADIAZINE-TRIMETHOPRIM AND AMPROLIUM IN BROILER POULTRY"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Veterinary Pharmacology and Toxicology** and minor in **Veterinary Physiology**, of the College of Post-Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Ms. Preeti Bisht, Id. No. 31035** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Pantnagar
July, 2022

(A.H. Ahmad)
Chairman
Advisory Committee

CERTIFICATE-II

We, the undersigned, members of the Advisory Committee of **Ms. Preeti Bisht, Id. No. 31035,** a candidate for the degree of **Doctor of Philosophy** with major **Veterinary Pharmacology and Toxicology** and minor in **Veterinary Physiology**, agree that the thesis entitled **"DISPOSITION KINETICS AND TISSUE RESIDUE STUDY OF SULPHADIAZINE-TRIMETHOPRIM AND AMPROLIUM IN BROILER POULTRY"** may be submitted in partial fulfillment of the requirements for the degree.

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ABBREVIATIONS

%	Percentage
@	At the rate of
<	Less than
>	More than
°C	Degree Celsius
µg	Microgram
µL	Microliter
A	Zero-time plasma drug concentration intercept of regression line of distribution phase
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APL	amprolium
AST	Aspartate aminotransferase
$AUC_{0-\infty}$	AUC from time zero to infinity
AUC_{0-t}	AUC calculated from zero time to time of last observed concentration
AUMC	Area under the moment curve
C.V.	Co-efficient of variance
CAPC	Companion Animal Practice Council
CL/F	Clearance when fraction of dose absorbed is not known
CL_B	Total body clearance
C_{max}	Maximum plasma concentration.
C^{ss}_{max}	Maximum steady state concentration
C^{ss}_{min}	Minimum steady state concentration
D	Priming dose
e.g.	Example
EDTA	Ethylene diamine tetraacetic acid
et al	Co-workers
etc	et cetera

FAO	Food and Agricultural Organization
Hb	Hemoglobin
HPLC	High performance liquid chromatography
i.m.	Intramuscular
i.v.	Intravenous
k_{10}	Elimination rate constant
k_a	Absorption rate constant for one compartment
kg	Kilogram(s)
L	Liter(s)
LOD	Limit of detection
LOQ	Limit of quantification
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
mm	Millimeter(s)
MRT	Mean residence time
PCV	Packed cell volume
pg	Picogram
RBC	Red blood corpuscles
S.E.	Standard error
SDZ	sulphadiazine
$t_{1/2k10}$	Elimination half life in one compartment
$t_{1/2ka}$	Absorption half life in one compartment model
TEC	Total erythrocyte count
TLC	Total leukocyte count
T_{max}	Time to reach maximum plasma concentration.
TMP	Trimethoprim
V/F	Volume of distribution where the absorption of drug is not known
viz	Namely
WBC	White blood corpuscles

Introduction

Chapter 1 INTRODUCTION

Broiler industry is one of the profitable agro industries, especially in the rural areas where it can tackle the problems of unemployment and underemployment, particularly of small and marginal farmers. Broiler industry can be adopted under a wide range of climatic conditions and can generally be combined conveniently with other farm enterprises **(Singh *et al.*, 2010).** With 7.3 per cent growth in poultry population the Indian poultry sector, has witnessed one of the fastest annual growth of about 6 per cent in eggs, 10 per cent in meat production and 8.35 per cent in broiler production over the last decade amongst all animal based sectors **(Pawariya and Jheeba, 2015).** Total poultry is 851.81 million during 2019. Over 45.79% increase in backyard poultry and total backyard poultry is 317.07 million in 2019. The commercial poultry has increased by 4.5% and the total commercial poultry is 534.74 million. The total poultry population in urban areas has increased by 26.5% during 2019. Commercial poultry in the urban areas has increased by 17.95% whereas in rural areas the percentage increased is 3.95%. In world including India gastrointestinal parasitism is one of the most important constraints for the growth and development of poultry industry. These parasites cause heavy losses to farmers by affecting growth, production, high mortality amongst young animals. It is caused by protozoa, a parasite of the genus *Eimeria*, and cause intestinal cell disruption, resulting in weight loss or poor weight gain. Intestinal coccidiosis is an economically important infectious disease of poultry and livestock throughout the world caused by *Eimeria* species. Among *Eimeria* species *Eimeria tenella* is the most pathogenic strain of coccidium which is usually located in cecum and causes cecal coccidiosis **(Allen *et al.*, 1998, Augustine, 2001).** Various drugs have been utilized to minimize the possible negative effects of infection **(Chapman, 1989).** In commercial broiler chicken production, chickens are continually exposed to coccidial oocysts found in litter. Moreover, broiler chickens will have multiple stages of coccidial development simultaneously. Once the clinical signs of coccidiosis appear in a broiler chickens, it may be too late to therapeutically control the infection in all birds unless the

therapeutic anti-coccidial is against multiple stages of development **(Allen *et al.*, 2002).**

In modern poultry farms, the chickens are raised indoors and are given antibiotics to overcome the effects of crowded and unsanitary conditions or inadequate diets. Antibiotics can be put in feed, water or can be injected. Amprolium, 1-[(4-amino-2-propyl-5-pyrimidinyl) methyl]-2 methylpiridinium chloride hydrochloride, a coccidiostat still used today was developed in 1950s. Amprolium retards the growth of new protozoa and kills them as well **(Peters *et al.*, 1994**). In addition amprolium compete with thiamine for intestinal absorption (**Hamamoto *et al.*, 1997**). In ruminants excessive administration of amprolium may lead to polioencephalomalacia due to a thiamine deficiency (**Chahar *et al.*, 1993**). Recently, it has been found that some of the microbes causing illnesses have developed a resistance to the antibiotics given to poultry for weeks or months at a time in low doses usually employed to treat the diseases (**Tan *et al.*, 1996**).

Classical combinations of sulphadiazine (SDZ) and trimethoprim (TMP) have been used extensively to treat serious infections of bacterial or protozoal origin in a range of animal species for over many years, with particular use in respiratory and alimentary tract infections **(Bushby, 1980; Nielsen and Gyrd-Hansen, 1994; Ensink *et al.*, 2003)**. These two chemotherapeutics when given in combination has a synergistic antibacterial effect *in vitro* caused by the inhibition of a different step in the bacterial folic acid biosynthetic pathway **(Batzias *et al.*, 2005).** This synergistic combination not only lowers the minimum inhibitory concentration (MIC) of both drugs, but also broadens the bacterial spectrum and decreases resistance occurrence **(Bushby, 1980; Van Duijkeren *et al.*, 1994; Plumb, 2002)**. In veterinary practice, SDZ and TMP are generally used at a ratio of 5:1 **(Spoo and Riviere, 2001; Batzias *et al.*, 2005)** and this has proved effective against a wide range of pathogenic bacteria **(Rogers *et al.*, 1988; Clarke *et al.*, 1989; Gookin *et al.*, 1999; Rothschild *et al.*, 2004; Ensink *et al.*, 2003, 2005).** The combination of these two chemotherapeutic agents has proved to be very useful for the treatment of diseases caused by a wide range of pathogenic bacteria. The pharmacokinetic role for the combination of these

drugs has been described in chickens **(Loscher *et al.*, 1990; Batzias *et al.*, 2000),** camels **(Kumar *et al.*, 1998),** cattle **(Clarke *et al.*, 1989),** donkeys **(Oukessou and Alsouss, 1998),** horses **(Brown *et al.*, 1983; Van Duijkeren *et al.*, 1994),** dogs **(Sigel *et al.*, 1981)**, Japanese quails **(Lashev and Mihailov, 1994),** carp **(Nouws *et al.*, 1993)** and pigs **(Soli *et al.*, 1990; Nielsen and Gyrd-Hansen, 1994; Garwacki *et al.*, 1996; Baert *et al.*, 2001).**

Over the past few decades poultry has gone through tremendous growth, however with the increase in production, use of certain drugs and feed additives has become crucial in order to prevent diseases, their treatment and growth promotion (**Goetting *et al.*, 2011; Rokka *et al.*, 2013; Seri, 2013; Ezenduka *et al.*, 2014**). One of the drawbacks of excessive use of antimicrobial drugs is however, that they get accumulated in the tissues and organs of treated animals as residues and eventually become part of food pyramid **(Oyarzabal, 2008**). For efficient poultry production, use of certain pharmaceutical products such as antibiotics as prophylactic and curatives become necessary to ensure rapid growth and health. Nevertheless, inappropriate and non-judicious use of these drugs results in accumulation of toxic and harmful residues in meat and eggs of treated birds.

Since traditional backyard and intensive poultry farming is a common practice in many developing countries and farmers do have easy access to veterinary drugs and the use of medicines in indiscriminate and inappropriate higher doses of antimicrobial drugs is common, which eventually accumulates harmful residues in edible tissues of poultry (**Goetting *et al.*, 2011**) The other cases where poultry can have harmful residues in their meat and egg products area) unintentional or accidentally cross contaminated feed in feed mills, (b) recirculation through litter and (c) administration of feed ingredients or water contaminated with metals, pesticides or toxic chemicals etc.

For animal health, drugs information on bioavailability as well as excretion, distribution, and depletion from edible tissues is needed when their utility is assessed. In accordance with aforesaid facts, a detailed study regarding pharmacokinetics and

residual study of Sulphadiazine-Trimethoprim and Amprolium in broiler poultry birds has been planned with following objectives:

1. To conduct pharmacokinetic and tissue residue study of Sulphadiazine-Trimethoprim in poultry following single dose intramuscular administration.
2. To conduct pharmacokinetic and tissue residue study of Sulphadiazine-Trimethoprim in poultry following multiple dose intramuscular administration.
3. To conduct pharmacokinetic and tissue residue study of Amprolium in poultry following single dose oral administration.
4. To conduct pharmacokinetic and tissue residue study of Amprolium in poultry following multiple dose oral administration.
5. To conduct biochemical and hematological parameters in poultry following multiple dose intramuscular administration of Sulphadiazine-Trimethoprim.
6. To conduct biochemical and hematological parameters in poultry following multiple dose oral administration of Amprolium.

Review of Literature

Chapter 2 REVIEW OF LITERATURE

Pharmacokinetics is the quantitative study of the time course of drug absorption, distribution, metabolism and excretion. It is also concerned with the relationship of these processes to the intensity and time-course of pharmacologic (therapeutic and toxicologic) effects of drugs and chemicals. In poultry for disease control and treatment antibiotics and antiparasitics are extensively used. As these drugs get accumulated in the tissues and organs of treated animals as residues and eventually become part of food pyramid which is one of the drawbacks of excessive use of these drugs **(Goetting *et al*., 2011; Oyarzabal, 2008),** hence excessive usage has been recognized as illegal and prohibited by the food regulatory and health authorities **(Ezenduka *et al*., 2014).**

2.1 Sulphonamide history

Antibiotics are naturally occurring, semi-synthetic or synthetic compounds with antimicrobial activity and are most widely used drugs in poultry industry. They are administered parenterally or intravenously, topically and orally (**Adela, 2015; Lawal, 2016).** After the discovery of the prototype of sulfonamides (Prontosil) by Gerhard Domagk in 1935, the active portion known as sulfanilamide was found. Sulfonamides group, containing the structure SO_2NH_2 is used for treatment/ prevention of infections in humans and in animals as feed additives to promote growth in animals (**Bishop, 2005).** Over 5400 derivatives of sulfanilamide were created, but approximately 20 derivative forms are generally available. Sulfonamides, the first synthetic chemotherapeutic, play an important role as effective antimicrobials inhibiting both gram-positive and gram-negative bacteria, as well as some protozoa, such as coccidials (**Prescott, 2000; Barragry, 1994).** The fundamental act of sulfonamides was described by Woods and Fildes in 1940, who discovered para - aminobenzoic acid (PABA) as a metabolic substrate for folic acid (**Woods, 1940).** In veterinary practice, sulfonamides are extensively used due to their broad spectrum of activity and low cost **(Saif *et al*., 2003).**

2.1.1 Chemistry

Chemically sulfa drugs are amphoteric. They behave as weak organic acid with pKa 4.79 to 8.56. Though they are weakly soluble in water, their solubility is increased at alkaline pH. Sodium salts are however easily soluble in water. They are structural analogue of PABA.

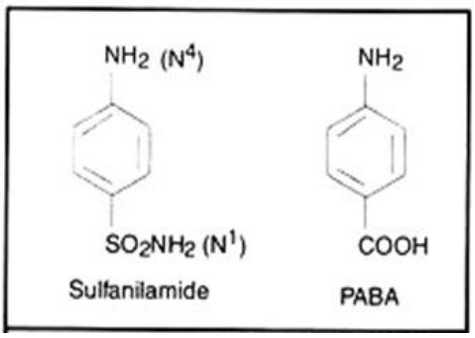


Figure2.1: Structure of sulphanilamide and PABA

2.1.2 Structure activity relationship

At para position the nitrogen of amino group is designated as N^4, while nitrogen of $So_2\ NH_2$ is designated as N^1. By substitution at N^1 position systemic sulfa drugs are evolved whereas, gut active sulfa drugs are produced by substitution at N^4 position. About 5000 compounds are synthesized by substitution at N^1 and N^4 positions. Among them 30 are of clinical significance. Sulfanilamide and its derivatives are popularly known as sulfonamide or sulfa drug.

Free para amino group is essential for antibacterial activity. Substitution of heterocyclic aromatic components at N^4 position produces more potent sulfa drugs. Any substitution in benzene ring causes loss of activity. $So_2\ NH_2$ group is not essential as such however; sulfur atom is directly linked with benzene ring. The more negative So_2 group at N^1 exhibits greater antibacterial activity. Substitutions made in the amide NH_2 (N^1) have variable antibacterial activity. The para NH_2 group (N^4) can be replaced or substituted by such chemical groups that can be converted into free NH_2 group in the body.

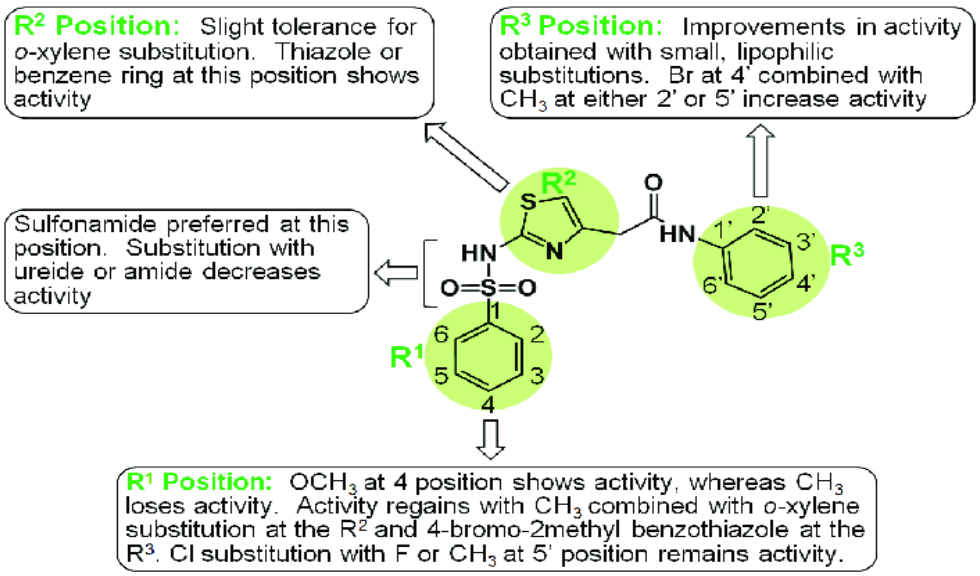


Figure 2.2: Structure activity relationship of sulphonamides

2.1.3 Classification of sulphonamides

Sulphonamides are classified on the basis of their pharmacokinetics and pharmacodynamics **(Mandell *et al.*,1996**) sulphonamides that are rapidly absorbed and excreted (e.g. sulphamethoxazole,sulphamethazine,sulphadiazine, sulphamerazine and sulphachlorphyridazine) **(Allen *et al.*,1993),** sulphonamides that are rapidly absorbed with intermediate excretion viz sulphadimethoxine and sulphasoxazole (**Craig and White, 1976**) sulphonamides that are absorbed rapidly but excreted slowly and thus long-acting (e.g.sulfadoxine) (**Greene, 1998)** potentiated sulphonamides (e.g. a sulphonamide combined with a diaminopyrimidine); (**Plumb, 1995)** agents that are poorly absorbed from the gastrointestinal tract and used for activity in bowel lumen (e.g. sulpha-salazine) (**Wilcke, 1988)** sulphonamides used topically (e.g. sulphacetamide, silver sulphadiazine and mafenide) (**Mandell *et al.*, 1996; Allen *et al.*, 1993; Craig, and White, 1976** ;**Greene, 1998**).

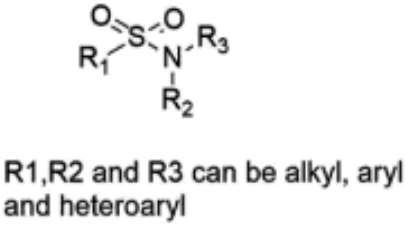


Figure 2.3 : Basic structural unit of sulphonamide

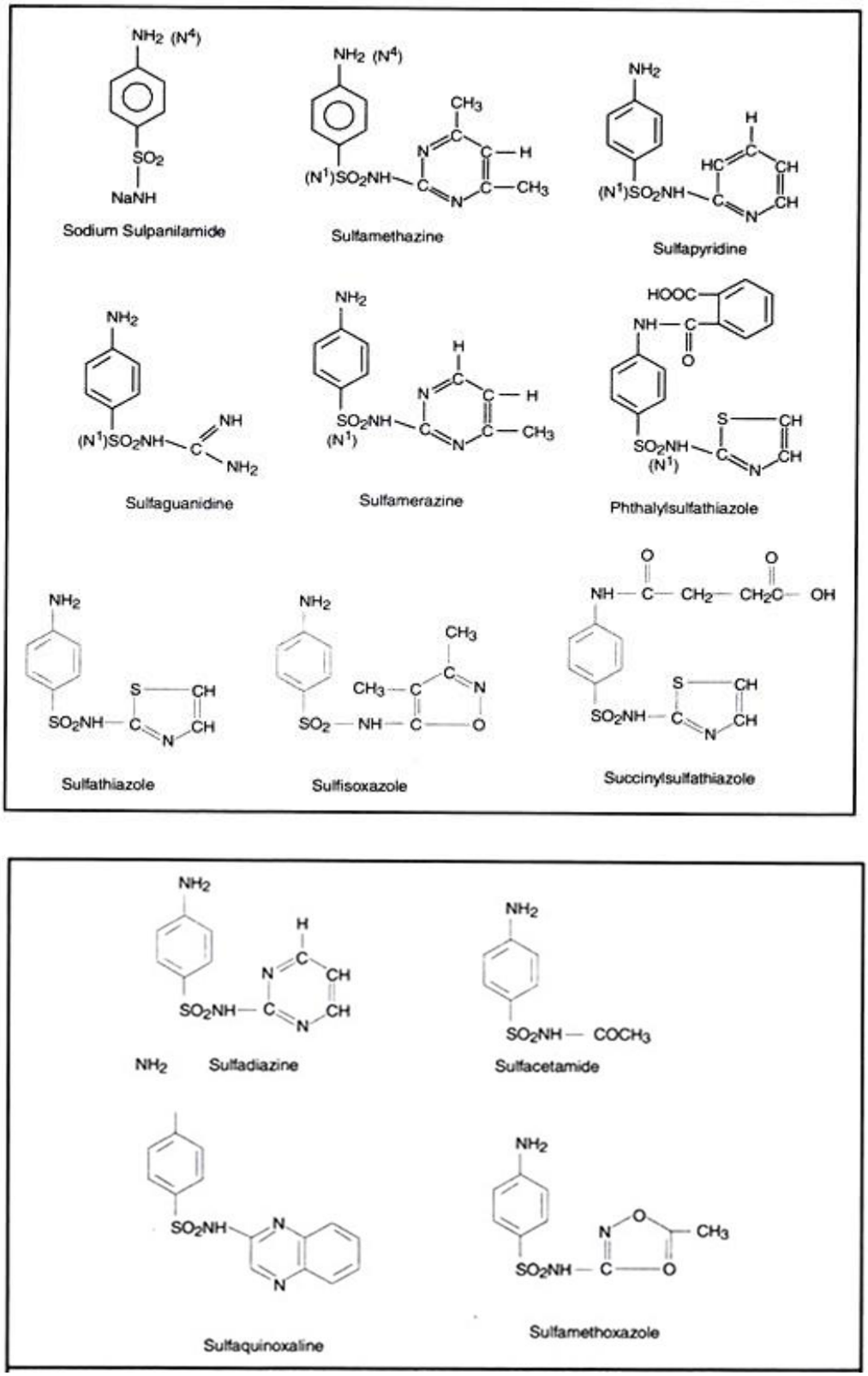


Figure 1.4: Structure of different sulphonamide

2.1.4 Sulphadiazine (SDZ)

SDZ (N-2-pyrimidinyl-4-aminobenzenesulfonamide) has been widely used for prophylaxis and therapeutics of diseases in land and aquatic animals. SDZ is the drug of choice for nocardiosis and is also used for pneumonia, cerebral meningitis, staphylococcal and streptococcal sepsis, and other infectious diseases. Due to its low solubility and certain nephrotoxicity this drug is not recommended for urinary tract infections. Its silver form of salts (sulphadiazine silver) used as an external antibacterial agent, mainly for treating burns. The silver ion which is present in the molecule facilitates increased antimicrobial and wound-healing action. Its main antibiotic mechanism is to compete with dihydrofolate synthetase from aminobenzoic acid leading to the inadequate synthesis of dihydrofolate to inhibit the growth of pathogens (**Chen and Xie, 2018).** Thus, SDZ has a broad-spectrum antibiotic activity.

2.1.4.1 Physical and chemical properties

Generally the sulfonamides are weak organic acids, relatively insoluble in water, more soluble in alkaline than acid pH, with their variability and extent bound to plasma protein (15%-90%) having a wide range of pKa (2.65 to 10.4). Chemically sulfa drugs are amphoteric in nature. They behave as weak organic acid with pKa 4.79 to 8.56 (**Spoo and Riviere, 2001**).

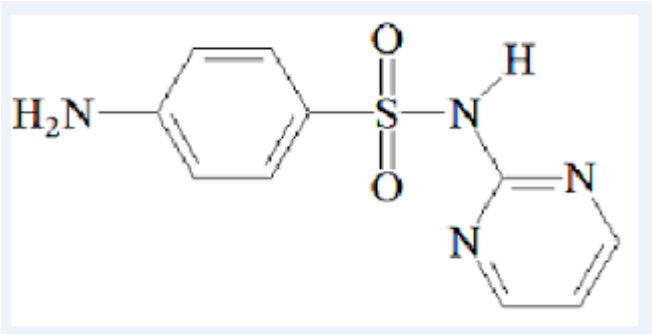


Figure 2.5 Chemical structure of sulphadiazine

2.1.4.2 Mechanism of action

Sulfonamides inhibit dihydropteroate synthase (DHPS) which condenses pteroate and p-aminobenzoic acid (PABA) to form dihydropteroate from which folic acid is generated. The folic acid is an essential enzyme for bacteria which can

synthesize it. The sulfonamide competes with PABA at the active site of the enzyme to act as an alternative substrate and produce pteroate-sulfonamide complex from which the bacteria cannot generate folic acid. As sulphonamides are structurally similar to PABA, sulphonamide action is dependent on the chemical similarity with PABA. Sulphonamides act as a false substrate and compete with PABA for the enzyme dihydrofolate synthase and block the synthesis of dihydrofolic acid (DHFA), and in turn trimethoprim inhibits the synthesis of tetrahydrofolic acid (THFA) and folate cofactor is inhibited. For the synthesis of nucleic acid the folate cofactor acts as a 1- carbon donor. The growth and division of bacteria get stopped as a result of blocking the biosynthesis of folate coenzyme. Preformed folates from the diet used by mammalian cells, whereas bacteria cannot use preformed folates and must synthesise their own folic acid, the sulphonamides, demonstrate selective bacteriostatic toxicity (**Capasso and Supuran, 2014).**

2.1.4.3 Pharmacokinetic of sulfonamides

Absorption and bioavailability

Most sulfonamides are well absorbed by the gastrointestinal tract except some long-acting compounds such as sulfamethoxypyridazine **(Riviere *et al*., 1991).** The other exceptions are some so-called enteric drugs like phthalylsulfathiazole, succinylsulfathiazole, sulfaquinoxaline and sulfaguanidine **(Spoo and Riviere, 2001).** However, the absorption may be delayed in pathological conditions (**Botsoglou and Fletouris, 2001),** in adult ruminants when administered with food to monogastric animals (**Prescott and Baggot, 1993; Plumb, 1991).** In chickens, oral bioavailability of sulphadiazine has been reported at approximately 100 % (**Loscher *et al*., 1990**) and 80% **(Baert *et al*., 2003; Bevill *et al*., 1988).**

Distribution

Sulfonamides penetrate widely into tissue and fluid throughout the body including CNS, synovial fluid, bile, urine and milk **(Spoo and Riviere, 2001).** However, there are many factors which determine individual sulfonamides distribution such as pKa value, ionisation state, the vascularity of the absorption site, lipophilicity, plasma protein-binding properties and species **(Botsoglou and**

Fletouris, 2001). In addition, total plasma protein concentration in domestic mammals is similar to that in humans (range of 6.0-8.5 $g.dL^{-1}$), but it is approximately 3.8-5.2 $g.dL^{-1}$ in galliform species due to the low albumin concentration (**Baggot, 2001).** In ruminants the different degree of plasma-binding protein ranging from less than 20% for sulfanilamide (**Nielson and Rasmussen, 1977**) to 94% for sulfadimethoxine **(Van Gogh, 1980)** has been reported. In chickens, the percentage of plasma protein-binding has been recorded as being 22% for sulfadimidine **(Nouws *et al*., 1988**) and 40-60% for sulfamerazine **(Atef *et al*., 1978; Oshima *et al*., 1964).**

Biotransformation

Sulfonamides are easily and extensively metabolised (**Kahn, 2008**). Liver is the primary site of metabolism, but they are also biotransformed in other tissues like kidney, lung, brain, adrenal, blood, neuron, skin and GIT (**Botsoglou and Fletouris, 2001).** Acetylation and hydroxylation are the principal processes in sulphonamide metabolism which are not uniform in the various species - chicken, turkey, pigeon, goat and swine (**Ginneken *et al*., 1991**; **Nouws *et al*., 1993).** The hydroxylation rate is significantly species-dependent **(Nouws *et al*., 1988; Witkamp *et al*., 1992; Van't Klooster, 1992)**. Acetylation is performed by N-acetylase and acetyl CoA. There are interspecies as well as intra-species, genetically determined differences in the activity of those enzymes, thus dividing individuals to rapid and slow acetylators. The activity of N-acetylase varies among species. Acetylation is important step of the reversible process acetylation-deacetylation, and its equilibrum is species-dependent too.

Excretion

Kidneys play the primarily route of elimination for most sulfonamides either as parent substance or metabolite, by glomerular filtration, active carrier-mediated proximal tubular secretion and passive reabsorption of nonionised drugs from the distal part of the renal tubules (**Prescott, 1993**, **2000**; **Plumb, 1991**, **2002**). Other less significant routes of excretion are via milk, bile, faeces, tears and sweat (**Spoo and Rivere, 2001**). In the case of sulfadimidine it has been reported that 11-37% and 24.5% of the dose was excreted unchanged into the urine in cattle and swine, respectively **(Nouws *et al*., 1988; Bevill *et al*., 1977; Duffee *et al*., 1984).**

2.2 Trimethoprim

Hitchings and his colleagues during the mid-1950's synthesized trimethoprim (2, 4-diamino-5-(3' 4' 5' –trimethoxybenzyl)-pyrimidine which is the most active and selective antibacterial of a series of inhibitors of dihydrofolate reductase (DHFR) (**Hitchings and Bushby, 1961; Roth *et al*., 1962; Falco *et al*., 1951).** Trimethoprim (TMP) is one of the recent antibacterial drugs with action on a unique target. Trimethoprim chemically belongs to the group of diaminopyrimidines (**Yao and Moellering, 2003**). After its synthesis, TMP was used in 1962 and was taken into clinical practice in 1968 with combination of sulfonamide (**Roth *et al.*, 1962; Huovinen, 1987**). Trimethoprim has a wide antibacterial spectrum, used for multiple infections with low toxicity, has fewer side effects and is inexpensive so can be easily produced. This is one of the most commonly used antibacterial agents globally (**Huovinen, 2001**). TMP is not a structural analog to dihydrofolic acid, although other structural analog drugs act on DHFR enzymes. It is well absorbed from the gut and distribute rapidly. Few important TMP analogs that are used clinically, including pyrimethamine, brodimoprim, tetroxoprim, and the racemic drug iclaprim.

2.2.1 Physical and chemical properties

It is a weak base with a pKa of about 7.3, slightly soluble in water, and forms stable salts with a variety of acids most of which have low solubility in water. The acetate and lactate are the most water soluble, solubility of the lactate salt being 25 mg of base/ml water. TMP is stable to autoclaving at 120°C for 20 minutes. It is a white to light yellow, odorless, bitter compound with a molecular weight of 290.32 and the molecular formula $C_{14}H_{18}N_4$.

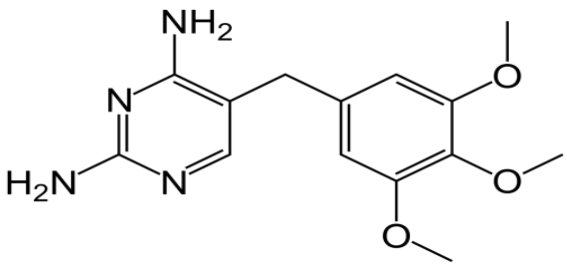


Figure: 2.6 Chemical structure of trimethoprim

2.2.2 Mechanism of action

Trimethoprim is an antifolate antibacterial agent that reversibly inhibits bacterial DHFR, a critical enzyme that catalyzes the formation of tetrahydrofolic acid (THF). Tetrahydrofolic acid is necessary for the biosynthesis of bacterial nucleic acids and proteins and ultimately for bacterial survival, trimethoprim inhibits its synthesis, results in bactericidal activity. Trimethoprim binding with bacterial dihydrofolate reductase is much stronger as compared to its mammalian counterpart, allowing trimethoprim to selectively interfere with bacterial biosynthetic processes. Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) (**Brogden *et al*., 1982).**

2.2.3 Pharmacokinetics

Trimethoprim, as a pyrimidine derivative with an antibacterial and antiprotozoic activity, is applied in combination with various sulphonamides. Formulations intended for oral application are often used for treatment and prophylaxis in birds. After an intravenous application, it shows a first order pharmacokinetics. The one compartment and two-compartment models and a high volume of distribution are typical (**Pashov and Mutafchieva, 1992; Lashev and Mihailov, 1995).** Administered orally, trimethoprim exhibited a comparatively high degree of absorption from the gut of chickens - 80-85% (**Dagorn *et al*., 1991; Pashov and Mutafchieva, 1992)** and Japanese quails - 41.1% (**Lashev, 1994).** Its disposition in tissues is uniform **(Pashov, 1983)** and it is excreted partially unchanged via the kidneys (**Loscher *et al*., 1990).** It undergoes a partial metabolism in the liver as well. The biological half-life of trimethoprim in chickens varies between 3.19 h and 5.17 h (**Pashov, 1983).**

Absorption

Trimethoprim is rapidly absorbed following oral administration. It exists in the blood as unbound, protein-bound, and metabolized forms. Steady-state concentrations are achieved after approximately 3 days of repeated administration **(Bergan et *al*., 1986).** Average peak serum concentrations (C_{max}) 1µg.mL^{-1} was achieved within 1 to 4 hours (T_{max}) following the administration of a single 100mg dose. Trimethoprim

appears to follow first-order pharmacokinetics. Serum concentrations approximately get doubled, when given as a single 200mg dose as compared to that of a 100mg dose. The steady-state area under curve (AUC) of orally administered trimethoprim is approximately 30 $mg.L^{-1}.h$ **(Bergan *et al*., 1986).**

Metabolism

Trimethoprim undergoes oxidative metabolism to a number of metabolites, the most abundant of which are the demethylated 3'- and 4'- metabolites, accounting for approximately 65% and 25% of the total metabolite formation, respectively **(Goldman *et al*., 2015).** Minor products include N-oxide metabolites and benzylic metabolites in even smaller quantities **(Goldman *et al*., 2015).** The parent drug is considered to be the therapeutically active form. 10-20% of trimethoprim is metabolized, primarily in the liver, the remainder is excreted unchanged in the urine. The principal metabolites of trimethoprim are the 1 and 3-oxides and the 3'- and 4'-hydroxy derivatives. The majority of trimethoprim biotransformation appears to involve CYP2C9 and CYP3A4 enzymes, with CYP1A2 contributing to a lesser extent (**Goldman *et al*., 2015**). Trimethoprim half-life ranges from 8-10 hours, but may be prolonged in patients with renal dysfunction.

Excretion

Approximately 10-20% of an ingested trimethoprim dose is metabolized, primarily in the liver, while a large portion of the remainder is excreted unchanged in the urine. Following oral administration, 50% to 60% of trimethoprim is excreted in the urine within 24 hours, approximately 80% of which is unchanged parent drug. Excretion of trimethoprim is primarily by the kidneys through glomerular filtration and tubular secretion. Urine concentrations of trimethoprim are considerably higher than are the concentrations in the blood. After a single oral dose of 100 mg, urine concentrations of trimethoprim ranged from 30 to 160 $\mu g.mL^{-1}$ during the 0 to 4 hour period and declined to approximately 18 to 91 $\mu g.mL^{-1}$ during 8 to 24 hour period.

2.3 Combinations of sulphadiazine and trimethoprim

Classical combinations of sulphadiazine (SDZ) and trimethoprim (TMP) have been used extensively to treat serious infections of bacterial or protozoal origin in a

range of animal species for over 35 years, with particular use in respiratory and alimentary tract infections **(Bushby, 1980; Nielsen and Gyrd-Hansen, 1994; Ensink *et al*., 2003).** Combining these two chemotherapeutics has a synergistic antibacterial effect *in vitro* caused by the inhibition of a different step in the bacterial folic acid biosynthetic pathway **(Batzias *et al*., 2005).** This synergism not only lowers the minimum inhibitory concentration (MIC) of both drugs, but also broadens the bacterial spectrum and decreases resistance occurrence (**Bushby, 1980; Van Duijkeren *et al*., 1994; Plumb, 2002**). In veterinary practice, SDZ and TMP are generally used at a ratio of 5:1 **(Spoo and Riviere, 2001; Batzias *et al*., 2005)** and this has proved effective against a wide range of pathogenic bacteria (**Rogers *et al*., 1988; Clarke *et al*., 1989; Gookin *et al*., 1999; Rothschild *et al*., 2004; Ensink *et al*., 2003, 2005)**. The pharmacokinetic profile of SDZ and TMP used together has been reported for chicken **(Loscher *et al*., 1990; Batzias *et al*., 2000; Baert *et al*., 2003)** and ostrich **(Abu-Basha *et al*., 2008**). Studies in other species such as swine **(Søli *et al*., 1990; Nielsen and Gyrd-Hansen, 1994; Garwacki *et al*., 1996; Baert *et al*., 2001)**, cattle **(Clarke *et al*., 1989)**, camel **(Kumar *et al*., 1998**), horse **(Brown *et al*., 1983; Van Duijkeren *et al*., 1994)**, dog **(Sigel *et al*., 1981**), donkey **(Oukessou *et al*., 1998**), Japanese quail (**Lashev *et al*., 1994)** and carp (**Nouws *et al*., 1993**) have also been performed.

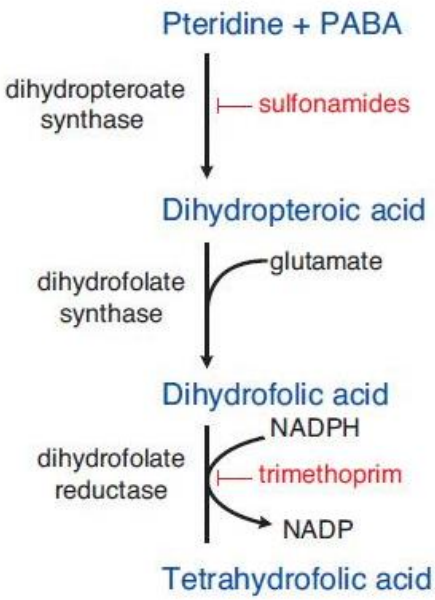


Figure 2.7: Steps in folate metabolism blocked by sulfonamides and trimethoprim

2.3.1 Pharmacokinetics of sulphadiazine and trimethoprim:

Abu-Basha *et al*. (2008) studied the pharmacokinetic and bioavailability of sulphadiazine combined with trimethoprim (sulphadiazine /trimethoprim) in fifteen healthy young ostriches after intravenous (i.v.), intramuscular (i.m.) and oral administration @ 30 $mg.kg^{-1}$b.w (25 and 5 $mg.kg^{-1}$ b.w of sulphadiazine and trimethoprim, respectively). The study followed a single dose, three periods, cross-over randomized design. After an overnight fasting the combination of sulphadiazine /trimethoprim was administered to ostriches on three treatment days, each separated by a 2-week washout period. Blood samples were collected at 0 (pretreatment), 0.08, 0.25, 0.50, 1, 2, 4, 6, 8, 12, 24 and 48 h after drug administration. Following i.v. administration, the elimination half-life($t_{1/2\beta}$), the mean residence time (MRT), volume of distribution at steady-state (Vd_{ss}), volume of distribution based on terminal phase (Vd_z), and the total body clearance (Cl_B/F) were (13.23 ± 2.24 and 1.95 ± 0.19 h), (10.06 ± 0.33 and 2.17 ± 0.20 h), (0.60 ± 0.08, and 2.35 ± 0.14 $L.kg^{-1}$), (0.79 ± 0.12 and 2.49 ± 0.14 $L.kg^{-1}$) and (0.69 ± 0.03 and 16.12 ± 1.38 $mL.min^{-1}.kg^{-1}$), for sulphadiazine and trimethoprim, respectively. No significant difference in C_{max} (35.47 ± 2.52 and 37.50 ± 3.39 $\mu g.mL^{-1}$), t_{max} (2.47 ± 0.31 and 2.47 ± 0.36 h), $t_{½\beta}$ (11.79 ± 0.79 and 10.96 ± 0.56 h), Vd(z)/F (0.77 ± 0.06 and 0.89 ± 0.07 L/kg), Cl_B/F (0.76 ± 0.04 and 0.89 ± 0.07 $mL.min^{-1}.kg^{-1}$) and MRT (12.39 ± 0.40 and 12.08 ± 0.36 h) were found in sulphadiazine after i.m. and oral dosing, respectively. There were also no differences in C_{max} (0.71 ± 0.06 and 0.78 ± 0.10 $\mu g.mL^{-1}$), t_{max} (2.07 ± 0.28 and 3.27 ± 0.28 h), $t_{½\beta}$ (3.30 ± 0.25 and 3.83 ± 0.33 h), Vd(z)/F (6.2 ± 0.56 and 6.27 ± 0.77 $L.kg^{-1}$), Cl_B/F (21.9 ± 1.46 and 18.83 ± 1.72 $mL.min^{-1}.kg^{-1}$) and MRT (3.68 ± 0.19 and 4.34 ± 0.14 h) for trimethoprim after i.m. and oral dosing, respectively. The absolute bioavailability (F) was 95.41% and 86.20% for sulphadiazine and 70.02% and 79.58% for trimethoprim after i.m. and oral administration, respectively.

O'Fallon *et al*. (2020) studied pharmacokinetics of sulphadiazine and trimethoprim suspension in neonatal foals. There is limited investigation of neonatal foal pharmacokinetic parameters for the antimicrobial combination of sulphadiazine (SDZ) and trimethoprim (TMP). In this study the pharmacokinetics of sulphadiazine–trimethoprim was determined in five healthy neonatal foals with oral administration at

24 mg.kg^{-1} every 12 hr for 10 days. Blood samples were collected at serial time points at approximately 72 hr of age (steady-state) and at days 5 and 10 to monitor the influence of age within the neonatal period. Pharmacokinetic parameters were determined using a one-compartment model analysis. C_{max} was 37.8 ± 13.4 μg.mL^{-1} (SDZ) and 1.92 ± 0.25μg.mL^{-1} (TMP). T_{max} was 1.4 ± 0.6 hr (SDZ) and 1.4 ± 0.4 hr (TMP). C_{min} for SDZ and TMP was 16.84 ± 8.46 μg.mL^{-1} and 0.46±0.24 μg.mL^{-1}, respectively. Elimination half-life was 10.8 ± 6.1 hr (SDZ) and 6.5±2 hr (TMP). $AUC_{0-\infty}$ (Area under curve) was 667 ± 424 μg.hr.mL^{-1} (SDZ) and 21.1±5.3 μg.hr.mL^{-1}(TMP). Foals remained healthy, and the plasma concentration of sulphadiazine–trimethoprim reached levels above MIC (90) for *Streptococcus equi* (SDZ/TMP): 9.5/0.5 μg.mL^{-1}).

Baert *et al*. (2003) studied pharmacokinetics and oral bioavailability of sulphadiazine and trimethoprim in broiler chickens and the plasma concentrations of the drugs were determined by validated HPLC methods, and pharmacokinetic parameters were calculated. A combination of sulphadiazine (33.34 mg.kg^{-1} body weight) with trimethoprim (6.67 mg.kg^{-1} b.w) was used in this study and administered both intravenously and orally, according to a crossover design, to 7-week-old healthy broilers. After i.v. or oral administration of TMP and SDZ, both active substances were rapidly eliminated from the plasma. Mean half-life of 1.61h for trimethoprim and 3.2h for sulphadiazine was observed. The apparent volumes of distribution of trimethoprim and sulphadiazine estimated were 2.2 and 0.43L.kg^{-1}, respectively, indicated that the tissue distribution of trimethoprim was more extensive than that of sulpahdiazine. About 80% of bioavailability was found for both components.

Ning Xu *et al*. (2020) studied pharmacokinetics of sulphadiazine in grass carp. This study examined the bioavailability and pharmacokinetic characteristics of sulphadiazine (SDZ) in grass carp (Ctenopharyngodon idellus) after oral and intravenous administrations. The blood samples were collected at predetermined time points of 0.083, 0.17, 0.5, 1, 2, 4, 8, 16, 24, 48, 72, and 96 hr. The samples were extracted and purified by organic reagents and determined by the ultra-performance liquid chromatography. The results demonstrated that the concentration–time profile of SDZ was best described by a one-compartmental open model with first-order

absorption after a single oral dose. The main pharmacokinetic parameters, the absorption rate constant, the absorption half-life, the elimination rate constant, the elimination half-life, and the area under concentration–time profile ($AUC_{0-\infty}$) were 0.3 h^{-1}, 2.29 hr, 0.039 h^{-1}, 17.64 hr, and 855.78 $mg.h.L^{-1}$, respectively. After i.v. administration, the concentration–time curve fitted to a two-compartmental open model without absorption. The primary parameters, the distribution rate constant, the elimination rate constant, the distribution half-life, the elimination half-life, the apparent distribution volume, the total clearance, and $AUC_{0-\infty}$ were 9.62 h^{-1}, 0.039h^{-1}, 0.072 hr, 17.71 hr, 0.33 $L.kg^{-1}$, 0.013 L. $h^{-1}.kg^{-1}$, and 386.23 $mg.h.L^{-1}$, respectively.

Nouws *et al.* (2011) conducted a study to determine pharmacokinetics and renal clearance of sulphadimidine, sulphamerazine and sulphadiazine and their N4acetyl and hydroxy metabolites in pigs. The effect of molecular structure on the drug disposition and protein binding in plasma, the urinary recovery, and the renal clearance of sulphamerazine (SMR), sulphadiazine (SDZ), and sulphadimidine (SDM) and their N4-acetyl and hydroxy derivatives were studied in pigs. Following i.v administration of SDM, SMR and SDZ, their mean elimination half-lives were 12.4 h, 4.3 h and 4.9 h respectively. The acetylated derivatives were the main metabolites; traces of 6-hydroxymethylsulphamerazine and 4-hydroxysulphadiazine were detected in plasma. The urine recovery data showed that in pigs acetylation is the major elimination pathway of SDM, SMR and SDZ; hydroxylation became more important in case of SMR (6-hydroxymethyl and 4-hydroxy derivatives) and SDZ (4-hydroxy derivatives) than in SDM. In pigs methyl substitution of the pyrimidine side chain decreased the renal clearance of the parent drug and made the parent compound less accessible for hydroxylation. Acetylation and hydroxylation speeded up drug elimination, because their renal clearance values were higher than those of the parent drug.

Wang *et al.* (2016) studied comparative pharmacokinetic of three sulphadiazine suspensions by oral administration in chickens. In this study, a pharmacokinetic analysis was performed to compare the bioequivalence of a combined SDZ and TMP product against existing licensed SDZ and TMP formulations in broiler chickens. Three groups of 15 birds were administered a single

dose of either the test formulation or a reference oral suspension. The plasma concentration of SDZ and TMP were determined by reverse-phase high performance liquid chromatography (HPLC), and the maximal plasma concentration (C_{max}), area under the curve (AUC), the peak time (T_{max}), mean residence time (MRT) and elimination half-life, were calculated for SDZ. The combined formulation I and II reference suspension exhibited almost identical concentration-time curves, and ANOVA analyses of the pharmacokinetic parameters identified no significant differences between the reference preparations and the test one. Furthermore the AUC and C_{max} values of the SDZ active ingredient were not significantly different. The I formulation was bioequivalent with both II and III (80-125% and 70–143%, respectively, at the 90% confidence interval). In conclusion, the combined SDZ and TMP product was bioequivalent with both existing commercially available SDZ suspensions and can be used interchangeably in veterinary medical practice.

Wang *et al*. (2015) conducted a study to determine the pharmacokinetic and tissue residue study of sulphadiazine combined with trimethoprim (SDZ/TMP=5/1) in mandarin fish after single (120 $mg.kg^{-1}$) or multiple-dose (an initial dose of 120$mg.kg^{-1}$followed by a 5-day consecutive dose of 60 $mg.kg^{-1}$) oral administrations at 28 °C. The absorption half-life, elimination half-life, volume of distribution, and the total body clearance for SDZ and TMP were 4.3±1.7 to 6.3±1.8 h and 2.4±1.0 to 3.9 ±0.9h, 25.9±4.5 to 53.0±5.6 h and 11.8±3.5 to 17.1±3.4 h, 2.34± 0.78 to 3.67±0.99 $L.kg^{-1}$ and 0.39± 0.01 to 1.33± 0.57 $L.kg^{-1}$, and 0.03±0.01 to 0.06 ± 0.01 $L.kg^{-1}.h^{-1}$ and 0.02 ± 0.01 to 0.05± 0.01 $L.kg^{-1}.h^{-1}$, respectively, after the single dose. The elimination half-life and mean residue time (MRT) for SDZ and TMP were 68.8±7.8 to 139.8± 12.3 h and 34.0± 5.5 to 56.1± 6.8 h, and 99.3± 6.1 to 201.7± 11.5 h and 49.1± 3.5 to 81.0±5.1 h, respectively, after the multiple-dose administration. The daily oral SDZ/TMP administration might cause a high tissue concentration and long elimination half life, thereby affecting antibacterial activity.

Batzias *et al.* (2005) studied bioavailability and pharmacokinetics of sulphadiazine, *N* -acetylsulphadiazine and trimethoprim following intravenous and intramuscular administration of a sulphadiazine/trimethoprim combination in sheep. In this study twelve rams of the Chios breed were used to study the pharmacokinetics

of sulphadiazine, its metabolite N acetylsulphadiazine and trimethoprim after i.v. and intramuscular i.m. administration of a combination of sulphadiazine/trimethoprim (5:1) in sheep at a total dose of 30 mg (25 mg/kg SDZ + 5 mg/kg TMP)/kg b.w. A SDZ/TMP injectable solution (Tribrissen, 400 mg SDZ + 80 mg TMP/ml, Cooper/Schering-Plough, UK) was used for this study. Bioavailability for sulphadiazine observed was 69.00%±10.51%. The half-life of the terminal phase (4.10±0.58h after i.v, and 4.03±0.31h after i.m. administration) was significantly higher than that observed for trimethoprim (0.59±0.19 h) after i.v. administration. The maintenance of constant plasma concentration ratio after i.v. administration was therefore impossible. AUC ratio between N-acetylsulphadiazine and sulphadiazine determined the acetylation capacity in sheep, was very low (less than 4%). In this study trimethoprim was not detected in sheep plasma after i.m. injection.

Garwacki *et al.* (1996) studied pharmacokinetics and tissue residues of an oral trimethoprim/sulphadiazine formulation in healthy pigs. Twenty-six healthy female pigs weighing 19.5-33 kg were used in three separate experiments. Feed was given twice a day individually to the animals. TMP/SDZ formulation was added to feed in the amount of 6 mg/kg b.w (TMP) and 30 mg/kg b.w (SDZ). Concentrations of TMP and SDZ in blood plasma, muscles, liver and kidneys were measured. Pharmacokinetic parameters showed that the absorption of TMP from the alimentary tract in pigs was faster than the absorption of SDZ, and the elimination of TMP was slower than that of SDZ. The absorption half-lives were 0.96 and 2.24 h for TMP and SDZ, respectively, whereas elimination half-lives were 5.49 (TMP) and 4.19 h (SDZ). The observed SDZ: TMP ratios in blood plasma after multiple dose administration ranged from 11.4: 1 to 23.2: 1. One day after administration of the last dose of SDZ/TMP the plasma concentration ratio was 15.5: 1, but in muscles, liver and kidneys it was much lower: 0.79: 1, 0.14: 1 and 1.53: 1 respectively. Tissue concentrations one day after the last multiple dose administration were very low for TMP and SDZ (maximum TMP: 0.29 $\mu g.g^{-1}$ in liver: maximum SDZ: 0.23 $\mu g.g^{-1}$ in kidneys). 8^{th} day after the last administration of TMP/SDZ neither drug was detected in any tissue.

2.3.2 Tissue residue

Antibiotics are mostly administered either through feed or in drinking water. Poultry receiving therapeutic/prophylactic doses of antibiotics without observance of recommended withdrawal times have shown, deposition of significantly higher concentrations of various antibiotic residues in edible tissues **(Kabir *et al*., 2004**). **Mwangi *et al*. (2011)** reported the accumulation of oxytetracycline (88.217 $ng.g^{-1}$), enorfloxacin (18.32 $ng.g^{-1}$), quinolones (30.81$\mu g.kg^{-1}$), chloramphenicol (0.021 and 0.008 $\mu g.kg^{-1}$) residues in chicken meat. **Amjad *et al*. (2005)** reported considerably higher concentrations of enrofloxacin (due to lipophilic nature) in broilers meat. The deposition of ciprofloxacin (34%), enorfloxacin (22%) and tetracycline (20%) residues in meat along with amoxicillin (26%) in thigh muscles, ciprofloxacin (30%), tetracycline (24%), amoxicillin (22%) and enorfloxacin (18%) residues in breast muscles of layers and broilers reported by **Sattar *et al*. (2014)**.

Poultry meat and eggs are very commonly consumed by humans. However, there may be situations where contaminations of drug residues in poultry products occur. Drugs and feed additives which, are used in veterinary practice, especially anticoccidials and antibacterials e.g. sulfonamides are drugs most commonly used on poultry farms. They can be easily absorbed and distributed through the body of chickens, accumulated in various tissues and transferred into their products **(Kan and Petz, 2000; Weiss *et al*, 2007**). Sulphadiazine (SDZ) and trimethoprim (TMP) combinations are commonly used for the treatment of various infections of respiratory, gastrointestinal and urogenital system in food producing animals. Due to the large scale of application of this combination has led to the occasional occurrence of residues in edible tissues. If the recommended withdrawal times are not respected these residue values could be particularly high presenting a hazard to human health**.** The MRLs fixed for pig and broiler tissues are 100 $\mu g.kg^{-1}$ and 50 $\mu g.kg^{-1}$ for SDZ and TMP, respectively. Residue of various sulphonamide eg. sulfadimethoxine has been observed in broiler tissues following dietary administration at 25, 50 and 100 $mg.kg^{-1}$ (**Nagata and Fukuda, 1994**). The residue was very rapidly eliminated from tissues with less than 0.1 $\mu g.g^{-1}$ remaining within two days after the drug withdrawal. This result is in the range of the study of **Takahashi *et al.* (1991**), who reported that

sulfadimethoxine was rapidly disappeared from plasma and tissues (except skin) on the 3rd-5th day post dosed given in drinking water. Compared to sulfadimethoxine residue in laying hens, it has been found that it quickly distributed through the body and reached a constant level in various tissues at 8 hours after feed (**Furusawa and Mukai, 1995).** However, it may appear below 0.1 $\mu g.g^{-1}$ in yolk and albumen up to 7 and 3 days after the drug withdrawal, respectively (**Nagata *et al.,* 1992).** The withdrawal period is the necessary interval between the last administration of the drug under normal conditions of use and the time when treated animals can be slaughtered for the production of safe foodstuffs. The withdrawal period should provide a high degree of assurance both to the producers and the consumers that the concentration of residues in foods derived from treated animals do not exceed the Maximum Residue Levels (MRLs).

2.3.3 Adverse effect of residue

Veterinary drug residues can cause allergic or toxic reactions in humans; penicillin is one crucial example of this. The occurrences of penicillin-induced hypersensitivity after milk or beef consumption have been cited **(Ormerod *et al.,* 1987; Reyes-herrera *et al.,* 2005**). However, with regard to other antibiotics, macrolide and cepharosporin residues are unlikely to be an allergenic hazard (**Dewdney *et al.,* 1991).** Although sulfonamides potentially induce skin hypersensitivity reactions (**Choquet-kastylevsky *et al.,* 2002),** however, no allergic cases involving of exposure to residues of sulfonamides in food have been reported **(Paige *et al.,* 1999**). Fortunately, exposure of antibiotics via oral channels usually sensitises to a lesser extent than the parenteral route does (**Dewdney *et al.,* 1991).**

2.3.4 Tissue residue study

Roncada *et al.* (2011) studied residue depletion of sulphadiazine and trimethoprim in pigs and broilers after oral administration. The residual study was conducted in to know the behaviour of sulphadiazine (SDZ) and trimethoprim (TMP) combination in fourteen pigs and twenty-eight broilers. In pigs and broiler feed the drug combination was added in the amount of 700 $mg.kg^{-1}$ (SDZ) and 140 $mg.kg^{-1}$ (TMP) and 300 $mg.kg^{-1}$ (SDZ) and 60 $mg.kg^{-1}$ (TMP), respectively. The medicated

feeds were supplied for 5 consecutive days. The tissue SDZ/TMP concentrations were measured by a HPLC method. To ensure safe residue levels in all target tissues, withdrawal time of 8.6 days and 6.0 days should be applied to pigs and broilers, respectively, treated with SDZ and TMP in feed. The calibration curves for the two test antibacterial drugs were linear over the concentration ranges examined (SDZ 0.05-2.0 $\mu g.mL^{-1}$; TMP 0.02-1.0 $\mu g.mL^{-1}$) with correlation coefficients always greater than 0.999. Recoveries in the target tissues ranged from 76.79±0.74 (broiler) and 78.82±1.04% (pigs) for SDZ and between 77.02±0.62% (broilers) and 80.49±1.15% (pigs) for TMP. In pigs, the inter-day precision ranged from 1.12% (liver) to 2.83% (skin/fat) for SDZ and from 1.97% (liver) to 2.88% (kidney) for TMP. In broilers, the inter-day precision ranged from 1.91% (skin/fat) to 3.44% (muscle) for SDZ and from 1.35% (muscle) to 3.37% (liver) for TMP. The LOD was defined for all tissues at 0.025 $\mu g.\ mL^{-1}$ for SDZ and at 0.020 $\mu g.\ mL^{-1}$ for TMP. Ten days after the end of treatment both SDZ and TMP concentrations were lower than the LOQ in all tissues for all animals.

Vandenberge *et al*. (2012) studied residues of sulphadiazine and doxycycline in broiler liver and muscle tissue due to cross-contamination of feed. In this study, broilers received an experimental feed containing sulphadiazine or doxycycline at cross-contamination levels of 2.5, 5 and 10% of therapeutic dose in feed. Breast, thigh muscle and liver samples were collected during treatment and depletion period and analyzed via liquid chromatography–tandem mass spectrometry (LC–MS/MS). Concentrations reached a plateau phase 3–5 days after the start of experimental feeding. A rapid depletion of residues was noted after withdrawal of the experimental feed. No significant differences in measured concentrations were observed between the various muscle types. Residue concentrations for some experimental groups; the 10% group of sulphadiazine and 5% group of doxycycline, however, exceeded their corresponding maximum residue limits (MRLs). For both compounds studied, no significant differences were found between the concentrations in breast and thigh muscle. In breast muscle the residue concentrations were observed slightly higher than the concentrations in thigh muscle.

Tzivara *et al*. (2013) conducted a study to determine residues depletion study and withdrawal period determination of sulphadiazine and trimethoprim premix in pigs. Purpose of this study was to detect and quantify the concentration of the residues of Sulphadiazine (SDZ) and Trimethoprim (TMP) in edible tissues of pigs and to determine the withdrawal period after oral administration of optiprime® premix 40%, containing 66.7 g TMP and 333.3 g SDZ per kg to healthy pigs. Optiprime® was orally administered @ 1.5 kg per ton of feeding stuff, for 5 consecutive days. A total of 22 pigs at age 65±2 days and from 27.1-33.0 kg were used. Into 4 groups (5 pigs per group) the experimental animals were divided, while 2 pigs were kept as control animals. All medicated pigs were sacrificed at 1, 4, 7 and 11 days after the last administration and muscle, fat, liver and kidney tissues were collected and analyzed using a validated liquid chromatography-mass spectrophotometry method. On the 1st day post medication SDZ was found in muscle and fat at higher concentrations than TMP, whereas higher concentrations of TMP were found in the liver, while both substances were found in high concentrations in kidney samples. On 4th day SDZ and TMP could not be quantified or detected in any tissue. On 11th day, all observations were below the Limit of Quantification (LOQ) of the method. LOQ observed for SDZ, 0.025µg.g^{-1} and for TMP, 0.013µg.g^{-1} in all tissues. Both substances deplete rapidly in all tissues. A withdrawal period of 5 days is justified for the commercial product optiprime® 40% premix in pigs.

Atta and EL-zeini, 2001 studied depletion of TMP and SDZ in egg albumen and yolk of laying hens after oral administration of TMP/SDZ at a dose rate of 0.2 and 0.4g.L^{-1} of drinking water for 5 successive days. HPLC was used for analysis of drug. After a dose of 0.2 g.L^{-1} of drinking water maximum concentration of TMP and SDZ in egg yolk found were 0.43ug.g^{-1} and 0.15 ug.g^{-1} and in egg albumen 0.24 ug.g^{-1} and 0.22 ug.g^{-1} respectively. Maximum concentration of TMP and SDZ in egg yolk found 0.81ug.g^{-1} and 0.18µg.g^{-1} and in egg albumen were 0.43 and 0.32 µg.g^{-1}, respectively, after a dose of 0.4g.L^{-1}. TMP was detected upto day 5 and 7 in egg yolk and day 4 and 6 in albumen respectively after following the small and large doses of drug. SDZ was detected upto day 4 and 6 in egg yolk and day 5 and 7 in egg albumen after following the small and large doses of drug respectively. The withdrawal time to

tolerance level (0.05 and 0.1 µg.g^{-1} for TMP and SDZ respectively) is not less than 3 and 4 days after the use of 0.2 and 0.4 g.L^{-1} of drinking water.

2.3.5 Effect on biochemical and haematological parameters

Saganuwan (2006) studied haematological and biochemical effects of sulphadimidine in Nigerian mongrel dogs. Five nigerian mongrel dogs of either sex weighing between 7 and 12 kg were used. Before administration of sulphadimidine i.m @ 100mg.kg^{-1} b.w for a period of 7 days, the pretreatment blood and serum samples were collected and body weight of animals taken. The results showed that there was no significant difference between pre administration and post administration weights of dogs. Packed cell volume decreased significantly with duration sampled dogs. Liver function test revealed significant decrease of total bilirubin and alkaline phosphatase. Other indices of liver function and electrolytes indices were normal. Sulphadimidine caused anaemia of moderate value (26.4 ±3.36%) in the treated samples as compared to pretreated samples (46.4±6.27). Total bilirubin (12.32 ±1 4µmol.L^{-1}) in pretreatment samples was decreased in comparison with treated (18.5 ±2.0 µmol.L^{-1}) samples. Alkaline phosphatase was decreased in pre administration samples (114.2 ±5.7µg.L^{-1}) as compared to post administration samples (130 ±9.61µg.L^{-1}). Therefore longtime administration of sulphadimidine in anaemic mongrel dogs may aggravate anaemic condition. Sulphadimidine may increase renal excretion of bilirubin and decrease bone mineralization in mongrel dogs during bone formation.

Youssef *et al.* (1981) studied some pharmacokinetic and biochemical aspects of sulphadiazine and sulphadimidine in ewes. Studies were carried out on eight clinically healthy non-pregnant ewes. Each animal was injected i.v. with either SDZ or sulphadimidine at a dose rate of 100 mg.kg^{-1} b.w. Two-compartment pharmacokinetic model described the disposition of these drugs. The elimination half-lives were 7.15 ± 0.58 h and 9.51 ± 0.59 h and the distribution half-lives were 0.56 ± 0.07 h and 0.42 ± 0.05 h for sulphadiazine and sulphadimidine, respectively. The apparent specific volumes of distribution were less than 1L.kg^{-1} (0.410 and 0.501L.kg^{-1} for sulphadiazine and sulphadimidine, respectively) which indicates a

relatively lower distribution of these drugs to tissues than in plasma in sheep. The degree of plasma protein binding was similar for both drugs (19.15 ± 0.55% and 23.12 ± 0.32%) for sulphadiazine and sulphadimidine, respectively. Serum concentrations of ketone bodies, total lipids and calcium were significantly reduced, and blood glucose concentration significantly increased following administration of both of these sulphonamides, whereas serum total protein concentration was unaltered. The serum cholesterol concentration was significantly reduced following sulphadiazine administration, but not after sulphadimidine.

2.4 Role of coccidiostat:

Coccidiosis is a parasitic disease with the greatest economic impact on poultry industries worldwide **(Allen and Fetterer, 2002)** due to production losses and cost of treatment and prevention **(Shirley *et al.*, 2004).** In replacement birds such as broiler breeders and egg producing stock the control of coccidiosis is a continuing problem, which includes broiler mortalities (6–10%) and also the global economic losses occur as a result of reduction in growth rate and feed conversion efficiency **(Weber, 1997; Banfield *et al*., 1999)**. In order to prevent coccidiosis various coccidiostats are used in broiler chicken farms **(Vrba *et al*., 2011; Barbour *et al*., 2015)**. Globally, coccidiostats are used in controlling avian diseases; the poultry industry has been under constant pressure to decrease its dependence on antimicrobials including coccidiostats **(Abdelrahman *et al*., 2014**). Ionophore and non-ionophore are two classes of coccidiostats **(Varga *et al*., 2017).** Ionophore coccidiostats, such as salinomycin, monensin, lasalocid, maduramicin, and narasin, are monocarboxylic polyether antibiotics (**Ebrahimnezhad *et al*., 2010**), they form dimeric groups by binding monovalent and divalent cations that facilitate metal ions crossing hydrophobic membranes. Therefore, ionophore antibiotic dietary supplements may change the bioavailability of some nutrients, affecting intestinal absorption in the body **(Elsasser, 1984; Ebrahimnezhad *et al*., 2010)**. On the other side synthetic drugs, such as the non-ionophore coccidiostats diclazuril, amprolium, halofuginone, nicarbazin, robenidine, and decoquinate have specific response mechanisms against parasitic metabolism **(Mortier *et al*., 2005).** Parasites belonging to the genus *Eimeria* commonly affect swine, poultry, cattle, sheep and rabbits **(Akpo *et al*., 2012),** when

intensively farmed in warm humid conditions. Overcrowding, poor hygiene practices and a failure to isolate infected animals allow disease proliferation. Parasites are transmitted via oocysts, which are shed in the feces of infected hosts and ingested by uninfected animals (**Sharman *et al*., 2010).** The disease can lead to intestinal lesions, diarrhea, poor weight gain, poor feed conversion and in some cases death. Intensive poultry production imposes a particularly high risk of disease occurrence with the result that coccidiosis ranks first among the diseases with the greatest impact in terms of economic losses (**Barreto *et al*., 2017).**

2.4.1 Amprolium

Amprolium (APL), a thiamine analogue, which is used for treatment and prevention of coccidiosis in poultry, cattle and rabbits. It is white to off-white coloured powder. Amprolium exists as positively charged ion in aqueous solution. The solubility values of amprolium hydrochloride in twelve pure solvents were determined at temperature range from T = 273.15 to 313.15 K. As expected, it increased with rising temperature in all of the selected solvents. The values obeyed the following order: methanol > acetone > cyclohexanone > ethanol > 1, 4-dioxane > n-propanol > ethyl acetate > n-butanol > isopropanol > i-butanol > acetonitrile > n-octanol. Amprolium is rapidly eliminated from the organism via kidneys. It is commercially available in formulations for oral administration. It has no adverse effects and is considered one of the safest anticoccidial drugs. Amprolium is marketed only for veterinary use, sold alone or in combination with a substituted aminobenzoic methyl ester or in combination with sulfonamides or pyrimethamine. Its use in dogs (300–400 mg total for 5 days, or 110–200 mg single dose (s.i.d.) for 7–12 days), cats (60–100 $mg.kg^{-1}$ bw, s.i.d. for 7 days), and domestic ferrets (19 $mg.kg^{-1}$ bw, s.i.d. for 2 weeks or longer) is only off-label (**CAPC, 2013).** Amprolium combination with ethopabate, sulphaquinoxaline and pyrimethamine extended and strengthened the spectrum of activity. Amprolium is compatible with vitamins, antibiotics, minerals and other ingredients commonly used in poultry ration but it should not be mixed in concentrates containing high levels of choline, because of tendency for it to break down into picric acid.

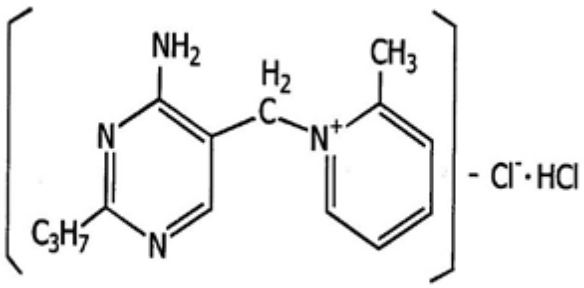


Figure 2.8: Chemical structure of Amprolium.

2.4.2 Mechanism of action

Amprolium is thiamine analogue and blocks the thiamine transporter of Eimeria species. By blocking thiamine uptake it prevents carbohydrate synthesis. It blocks the thiamine receptors due to its close structural similarity, so acts as thiamine antagonist. This blockage of receptors prevents coccidia from utilizing thiamine and as a result thiamine is unavailable to coccidian (competitive inhibition of thiamine uptake). There are two sites at which amprolium could act to lower yolk thiamine levels, first in the intestine where amprolium compete with thiamine for intestinal absorption **(Hamamoto, *et al*., 1997),** and may prevent thiamine absorption, and/or at the yolk membrane, decreasing thiamine deposition into yolk. **(Polin *et al*, 1963).** Amprolium stops the growth of new protozoa and kills them as well **(Peters *et al*., 1994).** As rapidly dividing coccidian has relatively high requirements for thiamine so amprolium exerts their greatest coccidiostatic activity against the asexual stages **(Kahn, 2008).** It has a coccidiostatic effect at lower doses and coccidiocidal at higher doses. APL is used to prevent and treat intestinal coccidiosis by blocking the thiamine transporter of Eimeria spp., which causes disruption of cell metabolism. It inhibits the development of merozoites and formation of second-generation meronts. It also has some activity against the sexual stages and oocyst sporulation (inhibits development of sporozoites) **(Clarke *et al*., 2014).**

2.4.3 Pharmacokinetics of amprolium

Absorption and distribution

Amprolium shows its effect mainly in the gastro-intestinal tract, but some is absorbed and retained in eggs and other organs **(Lang, 1989).** Many water-soluble

drugs are not well absorbed from the digestive tract as explained by the pH partition theory. However, some drugs like thiamine are absorbed from the intestine **(Rindi *et al*., 1966; Thomson and Leevy, 1972)**. Therefore, the possibility exists that APL may be absorbed into the body of the chicken by an active transport mechanism **(Polin *et al*., 1963).** The tissue permeability of a drug is determined by number of factors including the physiochemical properties of drug, binding to plasma and tissue protein, blood flow etc. The distribution and elimination of APL was rapid, as indicated by the mean alpha half-life value of 0.037 h and by the mean beta of 0.212 h after bolus i.v. administration of 13 mg APL/kg b.w. **(Hamamoto *et al*., 2000).**

Bioavailability

The bioavailability of APL after oral administration of 26 mg/ kg to chickens in a nonfasting condition was about twice that after 13 mg APL/kg **(Hamamoto *et al*., 2000)**. The bioavailability value of APL was 2.3±2.6% under the nonfasting condition and 6.4% in the fasting condition. The bioavailability of APL was therefore decreased by food intake. Amprolium is structurally similar to vitamin B1 **(Botsoglou and Fletouris, 2001**). When administered orally to chickens its bioavailability is low **(Hamamoto *et al*., 2000),** but absorbed amprolium is widely distributed to tissues **(Alam *et al*., 1987)** and rapidly eliminated in the urine and faeces **(Polin *et al*., 1963).**

Biotransformation and excretion

Biotransformation is the process, whereby a substance is changed from one chemical to another by a chemical reaction within the body. Metabolism or metabolic transformations are terms frequently used for the biotransformation process. Excretion is the irreversible loss of a substance from the system. In most cases, all drug-related material, including parent drug and metabolites are eventually cleared from the body. It is important to characterize, which routes of excretion are most important. Excretion commonly occurs by function of the kidney or liver (bile/feces), but the drug can also be excreted through sweat, tears, or breath. At very low plasma concentration, it is cleared by the kidney at a rate approximating renal plasma flow in the dog. Its renal clearance is not depressed by organic acids (p-aminohippurate or probenecid), but is reduced by the quaternary base, mepiperphenidol. Acetate and pantothenate may influence the clearance of amprolium. Its clearance decreased as

urinary pH is increased. APL clearance was not altered over a substantial range in urine at either high or low urinary pH (**Beyer and Gelarden, 1975).**

2.4.4 Pharmacokinetic studies of amprolium

The pharmacokinetic behavior of amprolium is studied in chicken **(Hamamoto *et al*. 1997; El-sayed, 2014; Hormazabal and Yndestad, 2000)** rabbits **(Ivey, 2000**). **Hamamoto *et al*. (1997)** studied detection of amprolium in chicken plasma by post-column HPLC with fluorescence detector. APL was administered orally @ 13 or 26 $mg.kg^{-1}$ body weight to chicken. HPLC method used was simple, sensitive, reproducible and able to treat numerous samples in a short period of time. HPLC showed excellent precision, accuracy and speed with a detection limit of $2ng.mL^{-1}$ and the limit of quantitation was $5ng.mL^{-1}$. The extraction recoveries from plasma containing 20, 50 and 100 $ng.mL^{-1}$ APL were 98, 99 and 105%, respectively. The results of the accuracy and precision (intra-day and inter-day) and are all below 15%, which is an acceptable range for validated HPLC methods.

Hamamoto *et al*. (2000) studied bioavailability of amprolium in fasting and nonfasting chickens after intravenous and oral administration. Bioavailability of APL was measured in chickens after intravenous and oral administration. A dose of 13 $mg.kg^{-1}$ b.w intravenously given to twelve healthy chickens weighing 1.28±1.41 kg and 13 or 26 $mg.kg^{-1}$ b.w orally in both a fasted and a non fasted condition. The half-life beta, volume of distribution and total body clearance after intravenous administration were 0.21h, 0.12 $L.kg^{-1}$ and 1.32 $L.h^{-1}.kg$, respectively. The elimination half-life after oral administration was 0.292±0.654h which is 1.5±3.2 times longer than after intravenous administration, suggesting the presence of a `flip-flop' phenomenon in chickens. The maximum plasma concentration (C_{max}) of 13 $mg.kg^{-1}$ APL administered orally to chickens during fasting was significantly (about four times) higher than that during nonfasting. Bioavailability during nonfasting was from 2.3 to 2.6%, and 6.4% during fasting.

Drug residue:

The European Commission sets Maximum Residue Limits (MRLs) after adoption by the Standing Committee, following advice from the Committee for Veterinary Medicinal Products (CVMP). Owing to the widespread use of these drugs

in farms, there is a risk that the amprolium residues will be present in animal products intended for human consumption. The presence of their residues in animal food products may have side effects to consumers. Coccidiostats are anticoccidial feed additive approved for use in feeds for broiler chickens, sheep, cattle, calves, goats, and are approved to be used in the prevention of coccidiosis in ruminating and nonruminating calves, including veal calves and cattle caused by *Eimeria* protozoa. (**Sanchez *et al.*, 2008**). Continuous administration of coccidiostats often leads to the accumulation of veterinary drug residues in food products for human consumption (**Reig and Toldra, 2008; Girardi and Odore, 2008)**. Presence of residue of veterinary drugs has received much attention because of the growing concern for safety by consumers. **Nose *et al.* (1982); Kan *et al*. (1989)** found that amprolium administered to laying hens is deposited primarily in the egg yolk and residues can be detected in eggs for 2 weeks or more after cessation of treatment, depending on the dose and assay sensitivity. The Korea Food and Drug Administration established maximum residue limits (MRLs) in chicken and cattle's muscle for the amprolium (0.5 $mg.kg^{-1}$). APL residues may occur in poultry products if adequate withdrawal times for the animals have not been observed. The U.S.Food and Drug Administration (FDA) has established tolerances for APL in the muscle (0.5 ppm) and liver (0.3 ppm) in the Code of Federal Regulations in order to prevent these residues in chicken products.

2.4.5 Residue studies of amprolium

Furusawa, (2002) developed a method for the routine monitoring of amprolium residue in edible chicken tissues (muscle and liver). Tissues with residual amprolium from meat breeder chickens that were fed a diet containing 200 ppm APL for 7 days were also used in order to validate the method for routine monitoring. The LOQs for the muscle and liver samples were 0.22 and 0.25$\mu g.g^{-1}$, respectively. The LOQs were below the tolerances (0.5 $\mu g.g^{-1}$ for muscle and 0.3 $\mu g.g^{-1}$ for liver).

Byung *et al*. (2012) conducted a study for the simultaneous determination of veterinary medicines (amprolium and decoquinate) in cattle and chicken's muscle by HPLC/UV-vis. Samples, 10g of cattle or chicken's muscle, were taken. Good linearity in the concentration range of 0.13-12.0 $mg.kg^{-1}$ with a correlation coefficient r^2=0.997

was observed. Relative recovery (accuracy) and LOQ for amprolium were in the range of 78.5-107.1% and 0.13-0.42 mg. kg^{-1}, respectively. The LOD and LOQ of amprolium in cattle and chicken's muscle were in the concentration range of 0.04-0.05 $mg.kg^{-1}$ and 0.13-0.18 $mg.kg^{-1}$, respectively. The LOQs were above the MRL in chicken and cattle's muscle. The survey was performed in the concentration level of LOQ 6 times of MRLs. The precision and accuracy of amprolium in the concentration range of 0.13-3.0 $mg.kg^{-1}$ from the spiked muscles was 2.2- 7.9% and 96.6-102.8%, respectively.

Hormazabal and Yndestad, (2000) conducted a study to determine residues of Amprolium and Ethopabate (ETB) in chicken meat by HPLC. Samples of chicken meat (3g) were used. The calibration curves for AMP and ETB were obtained by spiking muscle tissue samples with standard solutions, to yield 5, 10, 15, 20, 30, 50, 100, 200, 300 and 500 $ng.g^{-1}$ and 2, 5, 10, 20, 30 and 50 $ng.g^{-1}$ of AMP and ETB, respectively. Recovery rates were determined by comparing results of analysis of the spiked muscle samples with those of standard solution. Using peak-height measurements the linearity of the standard curves for AMP and ETB in muscle was tested. The standard curves were found linear at a range of 5 - 500 and 1 - 50 $ng.g^{-1}$ for AMP and ETB in meat, respectively. The linearity of the standard curves was 0.9998 for AMP. 99% recovery for AMP and varied from 98 to 99% for ETB in meat. In meat the precision of these recovery studies varied from 0.8 to 1 .0% and from 0.2 to 0.8% for AMP and ETB, respectively. The LOQ in meat was found 5 $ng.g^{-1}$ and 1 $ng.g^{-1}$ for AMP and ETB respectively.

Yamamoto and Kondo, (2001) conducted a study to determine residues of Halofuginone (HFN) and Amprolium in chicken muscle and egg by liquid chromatography. In this study, whole egg and breast tissue was used. Recoveries of HFN and APL from chicken muscle spiked at 0.5$\mu g.g^{-1}$ were 74.8 ± 17.7 and 94.2 ± 5.0%, respectively (Mean ± SD). In chicken muscle, the lower limit of determination for both APL and HFN was 0.03$\mu g.g^{-1}$. Recoveries of HFN and APL from chicken egg spiked at 0.5 mg/g by a cleanup procedure using SPE were 54.6 ± 3.4 and 85.0 ± 2.4%, respectively. In chicken egg, the lower limit of determination for both APL and HFN was 0.04 $\mu g.g^{-1}$.

Song *et al.* (2007) conducted a study to determine the concentration of amprolium, carbadox, monensin, and tylosin in surface water by liquid chromatography/ tandem mass spectrometry. For analysis surface water samples of Lansing, Michigan agricultural farm were collected, and to remove solid particles water samples were filtered through a 0.4-mm glass fiber filter. To extract analytes from water a hydrophilic-lipophilic balanced (HLB) cartridge (Waters Oasis, 30 mm, 6 cc/200 mg) was used, same cartridge was also used for cleanup and concentration step in sample preparation. For evaluating the effectiveness of extraction and interday variation, the antibiotics stock solutions were spiked into the river water at three concentration levels, level I: 10, 10, 1.0, 2.0 $ng.mL^{-1}$; level II: 30, 30, 3.0, 6.0 $ng.mL^{-1}$; and level III: 50, 50, 5.0, 10 $ng.mL^{-1}$ for amprolium, carbadox, monensin and tylosin, respectively. The spiked antibiotics were extracted from river water using the SPE and quantified by LC/MS/MS. Each spiked sample was repeated six times, twice per day for three consecutive days. Among the four antibiotics, amprolium is a cationic compound, and manifested a quite short retention time on the column. Amprolium was detected in nine out of the eleven water samples with the concentration range from 10–288 $ng.L^{-1}$. To establish one LC/MS/MS run for all four antibiotics, it is necessary to enhance the affinity of amprolium with the column hence extending the retention time. Amprolium spiked into deionized water generally resulted in lower recoveries, 50% compared to that spiked into river water.

Barreto *et al*. (2017) conducted a study for determination and confirmation of 14 coccidiostats in poultry muscle and eggs using liquid chromatography quadrupole linear ion trap tandem mass spectrometry (HPLC–QqLIT-MS/MS). Compounds were analyzed in a single run include lasalocid A, maduramicin, monensin, narasin, salinomycin, semduramicin, robenidine, diclazuril, toltrazuril, trimethoprim,, clopidol, amprolium, diaveridine and nicarbazin (as the marker residue dinitrocarbanilide). The method was fully validated according with Commission Decision 2002/657/EC and was applied for more than 100 samples from the Brazilian National Residue Control Plan (NRCP). Various parameters such as precision, reproducibility, trueness, the decision limit and detection capability were determined. The range of trueness values were within 73–115%. Precision values (repeatability and intermediate precision)

ranged from 0.4% to 21% and intra laboratory reproducibility ranged from 6.3% to 27%, depending on matrix. LOD and LOQ of APL were 3.3 *s*/*S* and 10 *s*/*S*, respectively, where, *s* is the standard deviation of the intercept of the regression line and *S* the slope of calibration curve. Calibration curves ranged from 0.25 to 2.0 times the MRL or validation level.

Basha *et al.* (2016) used novel potentiometric application for the determination of amprolium HCl in its single and combined dosage form and in chicken liver. Three novel amprolium HCl-selective electrodes were investigated with 2-nitrophenyl octylether as a plasticiser in a polymeric matrix of polyvinyl chloride (PVC). Sensor I was fabricated using potassium tetrakis (4-chlorophenyl) borate (TpClPB) as a cationic exchanger without incorporation of an ionophore. Sensor II used 2-hydroxy propyl b-cyclodextrin as an ionophore while sensor III used p-tert-butylcalix arene as an ionophore. Nernestian response were shown by these three proposed sensors with slopes of 29.2 ± 0.8, 29.3 ± 0.6 and 30.2 ± 0.4 mV/decade over the concentration range from 10–6 to 10–2 mol/l , respectively. These sensors displayed useful analytical characteristics for the determination of APL in different pharmaceutical formulations, bulk powder and chicken liver and in the presence of ethopabate. For its linearity, accuracy, precision and robustness the proposed method was validated according to ICH guidelines.

Takashaki *et al.* (1994) conducted simultaneous study for determination of Amprolium, Ethopabate (EB), Sulfaquinoxaline (SQ) and N4-Acetylsulfaquinoxaline in chicken tissues by using HPLC. A reversed-phase high-performance liquid chromatographic method is described for the quantitative simultaneous residue determination of amprolium with fluorometric detection using post-column reaction, and ethopabate, sulfaquinoxaline and its major metabolite, N4-acetylsulfaquinoxaline with UV detection, in chicken muscle, liver, kidney, skin and plasma. Average recoveries from chicken tissues fortified with 0.1 $\mu g.g^{-1}$ of the four compounds tested were ranged from 81.0 to 103.8 % for individual compounds from individual tissues. Coefficients of variation were ranged from 1.1 to 8.6 %. Detection limits were 0.002–0.004 $\mu g.g^{-1}$ for each compound. The applicability of this method was demonstrated by determining concentrations of the four compounds in tissues from chickens

administered with the three parent compounds. Two White Leghorn chickens of 7 weeks old were used. They were kept in cages individually and provided non-medicated feeds and water ad libitum. They were administered 0.4 $g.kg^{-1}$ Pancoxin (Dainippon Pharmaceutical Co.) AMP (200 $mg.g^{-1}$) , EB (10 $mg.g^{-1}$) and SQ (120 $mg.g^{-1}$) orally with catheter, 6 and 24 hours after the administration they were sacrificed after bleeding, and the muscle, liver, kidney and skin of trunk were removed. Plasma and tissue samples were stored frozen at -80°C until analysis. Concentration in different tissues ($\mu g.g^{-1}$) for amprolium determined were 0.45 $\mu g.g^{-1}$, 3.76 $\mu g.g^{-1}$, 3.29 $\mu g.g^{-1}$, 0.59 $\mu g.g^{-1}$, 0.31 $\mu g.g^{-1}$ for muscles, liver, kidney, skin and plasma after 6 hr of drug administration and 0.37 $\mu g.g^{-1}$, 1.41 $\mu g.g^{-1}$, 0.59 $\mu g.g^{-1}$, 0.28 $\mu g.g^{-1}$ and 0.21 $\mu g.g^{-1}$ after 24hrs of drug administration, respectively.

2.4.6 Effect of amprolium on biochemical and haematological parameters in poultry

Ghasemi-Sadabadi *et al*. (2020) conducted a study for comparing the effect of ionophore and non-ionophore coccidiostats on performance, carcass characteristics, blood biochemical parameters and gut microbial flora in broiler chickens. Ethoamprox (recommend level 500 grams per ton), was used which contained 250 gm amprolium per kg. A total number of 300 one day old broilers were randomly allocated into five treatments including: (1) basal diet (control group); (2) basal diet with 60 ppm salinomycin; (3) basal diet with 3.75 ppm maduramicin; (4) basal diet with 1 ppm Diclazuril; and (5) basal diet with 125 ppm amprolium. Improved weight gain was seen with diclazuril compared to all diets containing ionophore coccidiostats (feed intake significantly increased with broilers fed on diclazuril containing feed compared with broilers fed on maduramicin at 1 to 42 days. For female chicks, the highest carcass yield value was recorded in the control group, followed by the salinomycin, and maduramicin treatments respectively; however, thigh yield was higher in the diclazuril treatment group. Highest carcass and breast yields for male broilers were obtained in the diclazuril treatment group ($P<0.05$). Lactobacilli and Coliform bacterial populations were significantly higher in the diclazuril and control groups when compared to the salinomycin group at 28 and 42 days ($P<0.05$). Blood sodium and potassium concentrations were affected by treatment in male chickens

(P<0.05). The use of diets containing non-ionophore coccidiostats, particularly diclazuril, had beneficial effects on the overall growth performance of broiler chickens. Six birds (three males and three females) from each pen starved 12 h before blood sampling from the brachial vein in nonheparinized tubes. The blood was centrifuged at 2000 × g for 15 min to obtain serum. All the samples were properly labeled and stored for further analysis at -20 ˚C. Serum biochemical parameters including glucose, total protein (TP), albumin, uric acid, calcium and phosphorus were calculated using a Technicon RA-1000 Auto-analyzer (Technicon Instruments Corporation, Tarrytown, New York, USA) and the kit package (Pars Azmoon Co Tehran, Iran). Plasma globulin concentration was determined by the difference between total protein and albumin (Coles, 1986). Blood sodium and potassium concentration was analyzed by AOAC (2005) methods with a Flame photometer (**Ebrahimnezhad *et al*., 2010**).

Materials and Methods

Chapter 3 MATERIALS AND METHODS

3.1 Pharmacokinetic study in broiler poultry birds:

The experiment was carried out to investigate pharmacokinetic and tissue residue study of sulphadiazine, trimethoprim and amprolium in broiler poultry.

3.1.1 Experimental animals:

This study was conducted in thirty six white leg horn broiler birds of age 4-6weeks, weighing approximately 1.50±0.20 kg procured from local market. Birds were maintained on pre-experimental duration of 15 days prior to the commencement of experimentation in animal shed of Department of Veterinary Pharmacology and Toxicology for acclimatization to the ambient surroundings. During this pre-experimental period, all the birds were dewormed with Albendazole (Albendazole 10% suspension) @ 10mg/kg body weight orally 7 days prior to the experiment. The birds were reared under uniform management and husbandry setting, maintained on standard ration free of any antibiotic and water was offered *ad libitum*. The birds were clinically examined daily prior to the commencement of the experiment. Experiments were carried out as per the recommendation of Institutional Animal Ethics Committee (IAEC), Pantnagar accredited by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi vide approval number IAEC/VPT/CVASc./448 dated 12.12.2020 (CPCSEA Reg.No.330/GO/Ere/ SL/01/CPCSEA03.01.2001)

3.1.2. Drug

Biotrim®-I.M. [combination of sulphadiazine (400mg) and trimethoprim (80mg), Zydus Animal Health (Cadila Healthcare Ltd.)]

Amprolium® oral powder (amprolium 20%, Vetoquinol India Animal Health Pvt. Ltd.) were used in this study.

3.1.3 Standards

Sulphadiazine (Sigma® pure technical grade 99.9 percent),

Trimethoprim (Sigma® pure technical grade 99.9 percent) and

Amprolium (Sigma® pure technical grade 99.9 percent) were used.

3.1.4 Chemicals:

1) Methanol (HPLC grade)
2) Water (HPLC grade)
3) Acetonitrile (HPLC grade)
4) Heparin (Loba Chemie®)
5) 0.5% Hexane sulphonic acid
6) Potassium dihydrogen phosphate
7) Sodium acetate
8) Perchloric acid
9) Heparinized vials
10) HPLC grade water was prepared in the laboratory using Millipore water purification assembly (Milli-Q)

3.1.5 Apparatus

- HPLC system (M/S Shimadzu Corporation, Kyoto, Japan) comprising of,

- Solvent delivery unit – Parallel double micro plunger type, model number LC-20AD.
- Column oven – Model number CTO-10 ASVP, temperature setting range: 4^0C to 80^0C.
- Sample injector – Rheodyne manual injector with a 20 µl loop and 50 µl Hamilton® microliter syringe
- Detector – Diode array detector, model number SPDM10AVP.
- Communication module – Model number CBM – 20 A
- Chromatography column – LiChroCART® 125-4 RP-18 end capped (5µm) chromatography column was purchased from M/S Merck KGaA, Germany.
- Guard column – LiChroCART® 4-4, 5 µm, RP-18 guard column purchased from M/S Merck KGaA, Germany.

- UV – VIS Spectrophotometer (Model no. v3.0013e SmartSpecTM Plus, M/S BioRad laboratories).
- Compound microscope (MLX U, Magnus).
- Cuvettes (trUViewTM50-2000 µl cuvette, M/S Bio-Rad laboratories.
- pH meter (PB 20, Sartorius).
- Refrigerated centrifuge (Eppendorf 5804 R).
- Biological incubator (JSGW).
- Ultra refrigerator (Remi RQFV-26D).
- Homogenizer (Type-127 a high speed homogenizer, RPM=8000; by Remi Motors Mumbai-53, India)
- Ultrasonic tissue disintegrator Sanyo, U.K. (Sonipre 150)
- Centrifuge machine
- Supelco® solid- phase extraction C_{18} cartridges

HPLC instrumentation used for pharmacokinetic studies

3.2. Method validation

3.2.1 Chromatographic method

The method was validated according to ICH, 2006 guidelines by documenting their linearity, accuracy, precision, specificities, limits of detection and quantification. Using these methods, the cited drugs could be determined without any interference from their degradation products.

3.2.2 Specificity

The specificity of the method was assessed by subjecting the blank plasma samples (n = 6) for each drug (amprolium, sulphadiazine and trimethoprim) collected from the animals to HPLC analysis in order to verify the presence of any potential interfering compounds.

3.2.3 Linearity and range

The linearity of the proposed methods was evaluated by analyzing a series of different concentration of each drug. Good linearity is indicated by the correlation coefficient values. For determination of linearity, the standard calibration curves were plotted with drug concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 $\mu g.mL^{-1}$ against peak area of amprolium and with drug concentration 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 $\mu g.mL^{-1}$ against the peak area for both sulphadiazine and trimethoprim. Calibration curves are depicted in **Figure 3.6, 3.7 and 3.8** for sulphadiazine, trimethoprim and amprolium, respectively.

3.2.4 Precision

Precision and accuracy describes the closeness of the individual results, when the method is repeated several times. The intra-day precisions of the proposed methods were determined by the analysis of six different concentrations each of sulphadiazine, trimethoprim and amprolium within the linearity range of three replicates each on a single day, while the inter- day precisions were determined by the analysis of six different concentrations of the proposed drugs, within the linearity ranges of three replicates each on three consecutive days. The standard concentrations of 0.05, 0.1, 0.25, 0.5, 1 and 2.5 $\mu g.ml^{-1}$ were subjected to the same extraction

procedure described earlier and the precision co-efficient variance (C.V.%) was measured for sulphadiazine, trimethoprim and amprolium.

3.2.5 Selectivity

Selectivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the calibration curve concentration.

3.2.6 Robustness

The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH and mobile phase composition, flow rate. It was found that the method was robust, when the mobile phase ratio and flow rates were varied. The method was not affected by small changes in the conditions used, indicating the reliability of the method during the routine work, however the areas and peak symmetry were conserved.

3.2.7 Detection and quantification limit

The approach based on the standard deviation of the response and the slope of the regression equation was used for determination of detection and quantification limits [Limit of Quantification (LOQ) and Limit of Detection (LOD)]. The limit of quantification of an analytical method is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The limit of detection of an analytical procedure is defined as the lowest amount of analyte in a sample, which can be detected as an exact value. LOD and LOQ were determined by subjecting the standard solution containing the lowest amount of analyte to HPLC analysis.

3.2.8 Recovery

The accuracy of an analytical method is usually determined by the recovery (%) of a drug quantitatively added to blank biological specimens before sample preparation and assay following the corresponding procedure. A high and constant recovery is particularly important and relevant for a standard HPLC analysis.

Recovery of the drug was done by deproteinizing the plasma having the above mentioned drug concentrations. The estimation of drugs was done as per the

procedure outlined above. The peak areas of various supernatant drug concentrations recorded and recovery was estimated by the formula:

$$\text{Recovery \%} = \frac{N\left(\sum xy\right) - \left(\sum x\right)\left(\sum y\right)}{N\left(\sum x^2\right) - \left(\sum x\right)^2} \times 100$$

Where, x = amount of standard drug added, y = amount of drug found by proposed method, N = number of observations.

3.2.9 Calculation of correction factor

Correction factor (C.F.) for a particular residue factors was calculated by the following formula for each of residual concentration **(Leoni, 1992).**

C.F. = 100/ Percent recovery

3.3 Experimental design:

3.3.1 Pharmacokinetic study of sulphadiazine-trimethoprim following single dose administration.

For single dose pharmacokinetic study of sulphadiazine and trimethoprim, a combination of sulphadiazine-trimethoprim containing sulphadiazine 400mg and trimethoprim 80mg in the ratio of 5:1 was administered once @ 24mg.kg^{-1} body weight (20mg.kg^{-1} sulphadiazine and 4mg.kg^{-1} trimethoprim) intramuscularly (i.m.) in 9 broiler birds. The blood samples were collected from jugular vein or wing vein of each bird in heparinized microcentrifuge tubes at time intervals of 0, 5,10,15 and 30min, and at 1,2,4,8,12,24 and 48hr. The blood was centrifuged at 2500rpm for 10 min at 25°C to collect plasma. The plasma was separated in eppendorf tubes and stored at -20 °C till further analysis. Same plasma samples were utilized for estimation of sulphadiazine and trimethoprim separately. Thereafter, the birds were sacrificed at different time intervals and tissues were collected for further analysis of drug residue.

3.3.2 Pharmacokinetic study of sulphadiazine-trimethoprim following multiple (5) dose i.m. administration.

A total of 9 new birds were used to conduct multiple dose kinetic study of sulphadiazine and trimethoprim. A combination of sulphadiazine-trimethoprim (containing sulphadiazine 400mg and trimethoprim 80mg in the ratio of 5:1) was

administered i.m. @ 24mg.kg^{-1} (20mg.kg^{-1} sulphadiazine and 4mg.kg^{-1} trimethoprim) for 5 days at every 24h interval. During 2nd, 3rd and 4th day blood was collected twice to study peak (maximum plasma concentration) and trough (minimum plasma concentration) concentrations of drug in plasma. On 1st and 5th day of drug administration, blood samples were taken at 0 (predose), 5,10,15 and 30min, and at 1,2,4,8,12,24 and 48hr.The blood was centrifuged at 2500rpm for 10 min at 25°C to collect plasma. The plasma was separated in eppendorf tubes and stored at -20 °C till further analysis. Same plasma samples were utilized for estimation of sulphadiazine and trimethoprim separately. Birds were sacrificed at different time intervals for tissue residue analysis.

3.3.3 Pharmacokinetic study of amprolium following single oral dose administration.

Amprolium given as a single dose at a dose rate of 30 mg.kg^{-1} body weight orally using sterile dropper in nine broiler birds. The blood samples were collected either from jugular vein or wing vein of each bird in heparinized microcentrifuge tubes for amprolium at time intervals of 0, 15 and 30 min, and at 1, 1.5, 3, 5, 7, 13 and 24 hr. The blood was centrifuged at 2500rpm for 10 min at 25°C to collect plasma. The plasma procured was separated in eppendorf tubes and stored at -20 °C till further investigation. Birds were sacrificed at different time intervals for tissue residue analysis.

3.3.4 Pharmacokinetic study of amprolium following multiple dose oral administration.

A total of 9 new birds were used to conduct multiple dose study. Amprolium was administered orally using sterile dropper at a dose of 30 mg.kg^{-1} body weight once daily for 5 consecutive days. The blood samples were collected from jugular vein or wing vein of each bird in heparinized microcentrifuge tubes at time intervals 0min, 15min, 30 min. and at 1, 1.5, 3,5, 7, 13 and 24 hr after first and last multiple dose oral administration. During 2nd, 3rd and 4th day blood was collected twice to study peak (maximum plasma concentration) and trough (minimum plasma concentration) concentrations of drug in plasma. The blood was centrifuged at 2500rpm for 10 min at 25°C to collect plasma. The plasma was separated in

eppendorf tubes and stored at -20 °C till further analysis. Birds were sacrificed at different time intervals for tissue residue analysis.

3.3.5 Tissue Residue study of sulphadiadize-trimethoprim

Drug residues in various tissues (liver, kidney, muscles, and intestine) were evaluated in broiler birds.

For estimation of tissue residue concentration following single dose drug administration, same 9 birds in which sulphadiazine-trimethoprim combination was administered i.m @ 24mg.kg^{-1} b.w for single dose pharmacokinetic study were utilized. Birds were sacrificed at different time intervals. 3 birds each sacrificed at 24, 48 and 72h post drug administration and tissue samples were collected.

For multiple dose tissue residue study of sulphadiazine and trimethoprim, tissue (liver, kidney, muscles, and intestine) samples were collected from the same 9 birds in which multiple (5) dose pharmacokinetics of sulphadiazine-trimethoprim was conducted. 3 birds each were sacrificed at 48, 72 and 96h post drug administration, and tissue (liver, muscles, kidney and intestine) samples were collected for further analysis.

3.3.6 Tissue Residue study of amprolium

Drug residues in various tissues (liver, kidney, muscles, and intestine) were evaluated in broiler birds. For estimation of tissue residue concentration following single dose 30mg.kg^{-1} b.w oral administration of amprolium, same 9 birds which were used for single dose pharmacokinetic study were utilized. The tissue (liver, muscle, kidney and intestine) samples from three birds each were collected, at 24, 48 and 72hr for drug residue analysis.

For estimation of tissue residue concentration following multiple (5) dose oral administration of amprolium, same 9 birds were utilized, as that used in multiple (5) dose pharmacokinetic study. A dose of 30mg.kg^{-1} b.w amprolium was administered orally for five days at every 24hr interval. Tissue (liver, muscle, kidney and intestine) samples were collected at 48, 72 and 96hr of last dose of multiple dose administration for analysis of drug residue.

3.4 Extraction of drugs from plasma samples:

Extraction of sulphadiazine and trimethoprim from plasma was performed according to the procedure reported by **Wang *et al.*, 2016**. The plasma proteins were precipitated by adding, 2ml acetonitrile to 0.5ml plasma, then vortexed for 5min. followed by centrifugation for 15mins at 5000rpm. Extract was evaporated to dryness using rotary evaporator at 45^0C, residues re-dissolved in 1ml mobile phase, and then centrifugation was performed for 15mins at 16000rpm. The supernatant was syringe filtered by 0.22 µm millipore cellulose acetate membrane filter paper. 20ul of aliquot thus obtained was injected for further analysis. Procedure was depicted in **Figure 3.1**.

Extraction of amprolium from plasma was performed according to the procedure reported by **(Hamamoto *et al.*, 2000)** with slight modifications**.** Following thawing of plasma samples the plasma proteins were precipitated by adding, 0.5 mL of 0.33 M perchloric acid solution to 0.2 mL of plasma. The mixture was vortexed for 30seconds and centrifuged for 10 min at 2150 rpm. The supernatant was separated and allowed to stand for 3h. The supernatant was syringe filtered by 0.22 µm millipore cellulose acetate membrane filter paper. A 20 µl aliquot was injected into the high-performance liquid chromatography (HPLC). Procedure depicted in **Figure 3.2**.

Blood samples (0.5 mL)

⇩

Centrifuged at 5000 rpm for 15 minutes to separate plasma

⇩

Extract was evaporated to dryness using rotary evaporator at 45^0C

⇩

Residues re-dissolved in 1ml mobile phase

⇩

Centrifuged at 16000rpm for 15 min

⇩

Supernatant filtered through 0.22 µm filter paper

⇩

20 µL injected into HPLC system

Figure 3.1. Extraction procedure for analysis of plasma samples for sulphadiazine and trimethoprim.

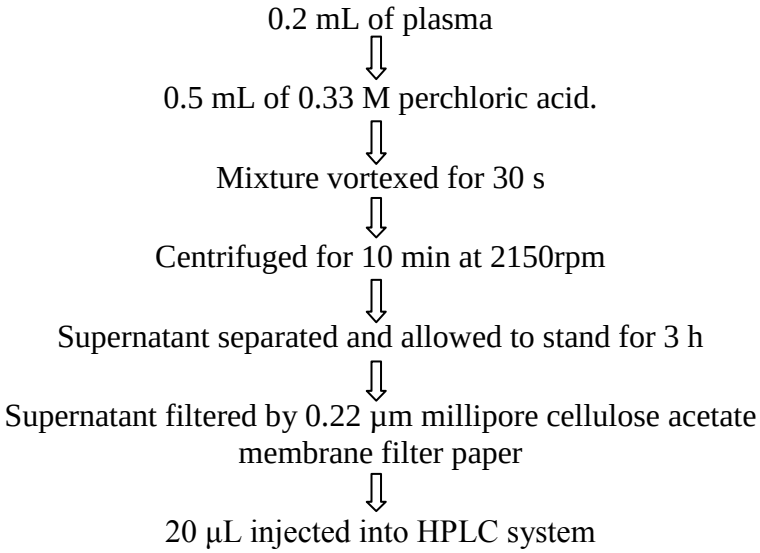


Figure 3.2. Extraction procedure for analysis of plasma samples for amprolium.

3.5. Extraction and clean up of drugs from tissue samples:

Extraction of sulphadiazine was done as per the methods of **Roncada *et al*. (2012)** with slight modifications**.** Target tissues were cut into small pieces and approximately 2g of tissue was weighed for further analysis and in the ratio of 1:3 water (1mL) and methanol (3mL) was added to the sample for homogenization. Centrifugation of homogenate was performed at 1000rpm for 15min. Supernatant thus obtained after centrifugation was evaporated to dryness under a stream of nitrogen to get the residue. The residue was dissolved in 20mL of 0.5 M NaCl (pH 2.5) and applied to a SPE-C18 cartridge, prewashed with methanol (2mL), water (2mL) and 0.5 M NaCl (pH 2.5) (2mL). The cartridge was washed with 1mL of 0.5 M NaCl (pH 2.5) and sulphadiazine was eluted with 2mL of methanol:water (1:1 v:v). The eluate was evaporated to dryness under a stream of nitrogen, then redissolved with 500µL of methanol: water (1:1v: v) and transferred into vials for HPLC analysis.

Extraction of trimethoprim was done with slight modification of method by **Roncada *et al*. (2012).** Target tissues were cut into small pieces and approximately 2g of tissue was weighed for further analysis and in a ratio of 1:3 water (1mL) and methanol (3mL) was added to the sample for homogenization. The homogenate was diluted with 0.2 M sodium acetate (20 mL) and then centrifuged at 1000rpm (15 min).

The supernatant was cleaned on SPE-C18 cartridge prewashed with methanol (2mL) and water (2mL). After the sample loading, the cartridge was washed with a 0.025 M potassium dihydrogen-buffer solution (pH 4.5) (2mL) and TMP was eluted with 2mL of a mixture 0.025 M potassium dihydrogen phosphate-buffer solution (pH 3.5): methanol (10:90 v:v). The eluate dried under vacuum was dissolved in 400 µL of 0.025 M potassium dihydrogen phosphate -buffer solution (pH 4.5) and transferred into vials for HPLC analysis.

Amprolium extraction from poultry tissues (liver, kidney, muscle and intestine) was executed as per the modified procedure of **Hormazabal and Yndestad (2006).** 0.5 ml water and 6 ml acetone-tetrahydrofuran (6 + 4) was added to 3g of sample. The mixture was homogenized for approximately 4 min and the extract sonicated till sandy suspension. After centrifugation for approximately 5 min. (5000 rpm), a 5 ml volume of the supernatant (corresponding to 1.5 g, was pipetted into a conical centrifuge tube, and 6mL diethyl ether-hexane (6 + 4) added. The mixture was shaken vigorously for approx. 5 sec. After centrifugation for 3 min (3500 rpm), the upper layer (organic phase) was transferred into another glass-stoppered tube; the bottom water layer was retained for subsequent analysis of amprolium. One ml acetone and 5 ml dichloromethane was added to the water based sample. The mixture was shaken vigorously for 10 sec, and centrifuged for 3 min. The upper layer (water) was transferred to a graduate glass-stoppered tube. The supernatant was evaporated with a gentle stream of nitrogen in a water bath at 45–50°C.

The dried samples obtained after evaporation under the stream of nitrogen were reconstituted in 5ml of acetonitrile and loaded onto the conditioned SPE (Solid phase extraction) cartridges pre-treated with 10 ml each with HPLC grade water and acetonitrile and allowed to pass through vacuum (20mmHg). The eluate thus obtained was filtered through 0.22 µm cellulose acetate membrane filter. 20µl aliquot injected into the HPLC for further analysis.

3.6. Analysis of drugs

In the present study, data acquisition and chromatogram analysis was carried out by ‘’LC solution software” (M/S Shimadzu Corporation, Kyoto, Japan).

3.6.1 HPLC conditions

For suphadiazine

Analysis of sulphadiazine was done according to the method reported by **Wang *et al*. (2016)** with slight modifications**.** The mobile phase consisted of acetonitrile and 10mM potassium dihydrogen phosphate in a ratio of 14:86, pH was adjusted to 3.5-3.7 by adding glacial acetic acid. The mobile phase was filtered through a 0.45µm membrane and degassed. The mobile phase was eluted at a flow rate of 0.7ml.min^{-1} and progress was monitored using a UV detector at a wavelength of 240 nm at room temperature.

For trimethoprim

Trimethoprim analysis was done as per the method described by **Roncada *et al*. (2012)** with slight some modifications**.** The HPLC mobile phase consisted of water (0.01M sodium acetate) and acetonitrile in the ratio of 70:30. The pH was adjusted to 3.5-3.7 by adding glacial acetic acid. The flow rate was maintained at 0.6ml.min^{-1}. The detection wavelength was 254 nm and chromatography performed at ambient temperature of oven.

For amprolium

The analysis of plasma and tissue samples for amprolium was done as per the method described by **Ali *et al*. (2017)** with slight modifications. The isocratic mobile phase consisted of methanol and purified water in the proportion of 60:40 (v/v) containing 0.5% Hexanesulfonic acid sodium at pH of 3.7, which was adjusted by using glacial acetic acid. The flow rate was maintained at 0.5ml.min^{-1}. The detection wavelength was 270nm and chromatography was performed at ambient temperature 25^{0}C of oven.

3.7 Sulphadiazine,Trimethoprim and Amprolium standards and their calibration curves

The standards for sulphadiazine and trimethoprim was prepared by dissolving 1mg of each pure standard in 1ml of methanol (HPLC grade). The standards for amprolium, was prepared by dissolving 1mg of pure amprolium in 1ml of methanol: water (7:3) and final concentration of stock solution was made 1000µg.ml^{-1}. Further

dilutions for all the three drugs were prepared from their respective stock solution in acetonitrile in the concentrations of 10.0, 5.0, 2.5, 1, 0.5, 0.25, 0.1, 0.05 and 0.025 $\mu g.ml^{-1}$. 20µl of these concentrations was injected into HPLC under the HPLC conditions mentioned above. **Figure 3.3, 3.4 and 3.5** represent the chromatogram obtained for sulphadiazine, trimethoprim and amprolium standard, respectively. A standard calibration curve acquired by representing concentration against average of peak area under the curve was derived. . Standard calibration curve obtained for sulphadiazine, trimethoprim and amprolium are represented in **Figure 3.6, 3.7 and 3.8,** respectively.

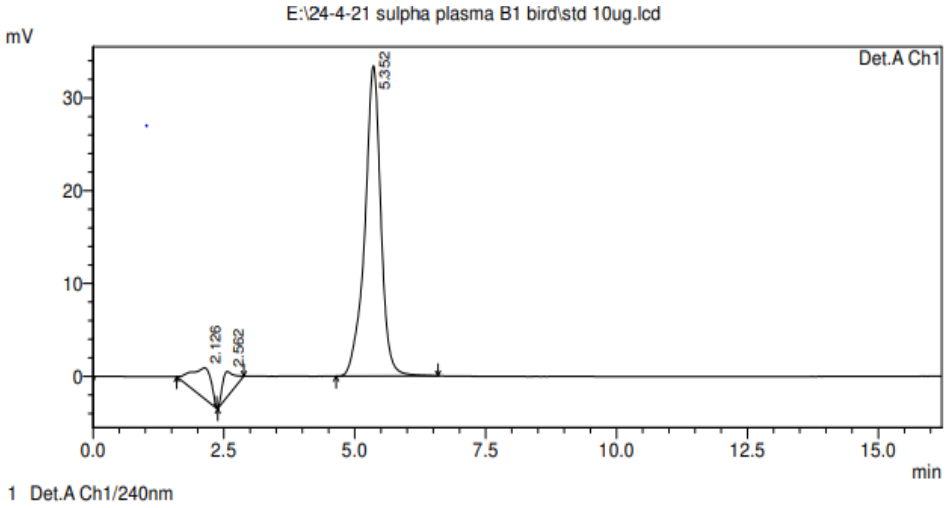


Figure 3.3. Chromatogram showing standard peak of Sulphadiazine with a retention time of 5.352 minutes in the mobile phase (acetonitrile and 10mM potassium dihydrogen phosphate in a ratio of 14:86).

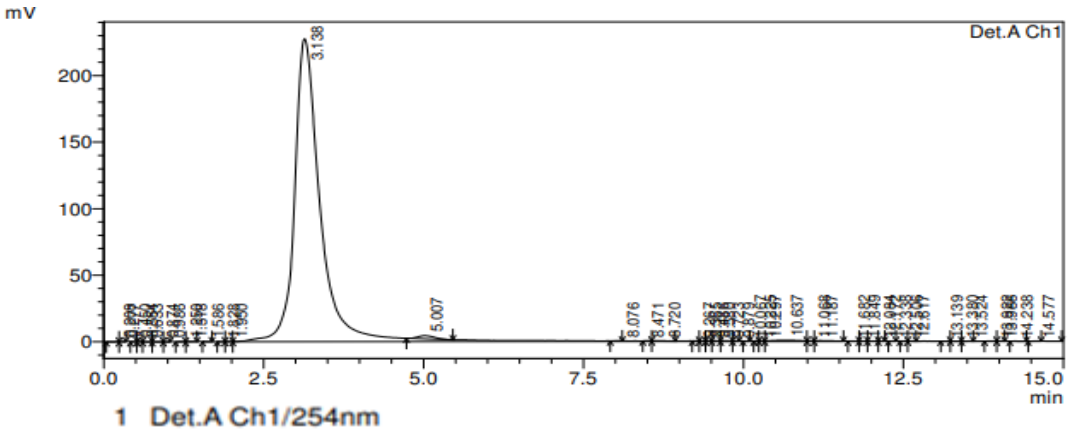


Figure 3.4. Chromatogram showing standard peak of Trimethoprim with a retention time of 3.138 minutes in the mobile phase (0.01M sodium acetate and acetonitrile in the ratio of 70:30)

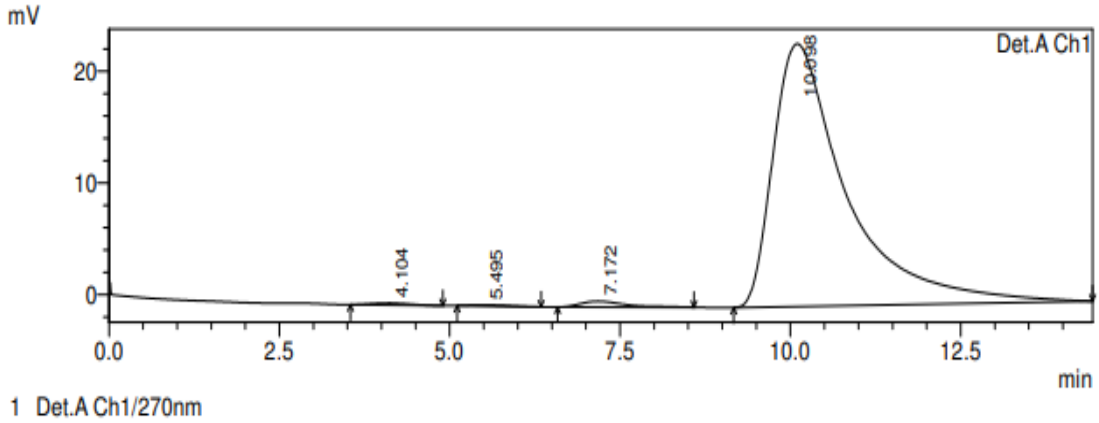


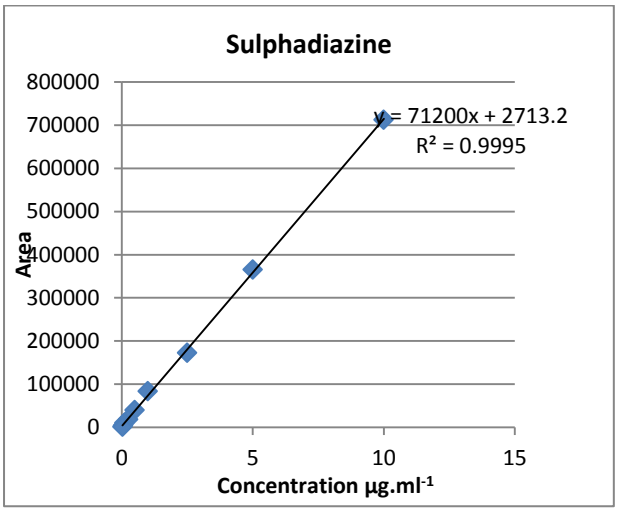
Figure 3.5. Chromatogram showing standard peak of Amprolium with a retention time of 10.098 minutes in the mobile phase (methanol and purified water in the proportion of 60:40 (v/v) containing 0.5% Hexanesulfonic acid sodium).

3.7.2 Drugs standards in plasma

To the drug free plasma samples, drug concentration from the above described dilutions was added to make plasma drug concentration as, 10, 5, 2.5, 1.0, 0.5, 0.25, 0.1, 0.05, 0.025 $\mu g.ml^{-1}$ each for sulphadiazine, trimethoprim and amprolium by applying serial ten times dilution (100 µl standard + 900 µl drug free plasma) of 100, 50, 25, 10, 5, 2.5, 1.0, and 0.1 $\mu g.ml^{-1}$ of standards, in equal volumes of drug free plasma, each time. The sulphadiazine, trimethoprim and amprolium, detection from plasma was done according to the procedures mentioned above. The peak value obtained after chromatography was represented against concentration so as to obtain a standard calibration curve.

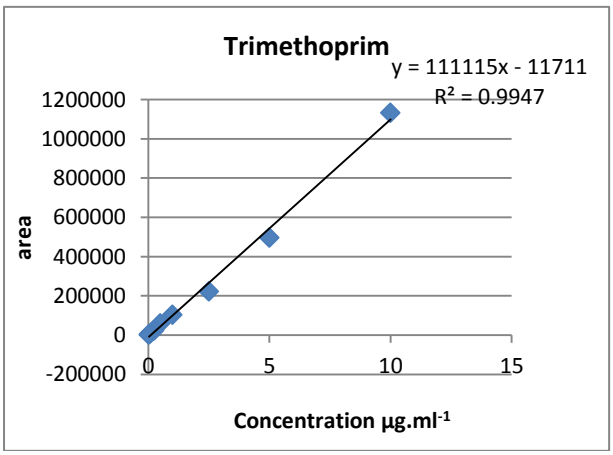
3.7.3 Drug standards in tissue samples

To the drug free homogenized tissue, drug concentration (sulphadiazine-trimethoprim and amprolium) was added to it to make the tissue concentration as, 10, 5, 2.5, 1.0, 0.5, 0.25, 0.1, 0.05, 0.025 $\mu g.g^{-1}$. The detection of sulphadiazine, trimethoprim and amprolium, from plasma was done according to the procedures mentioned above. The peak value obtained after chromatography was represented against concentration so as to obtain a standard calibration curve.



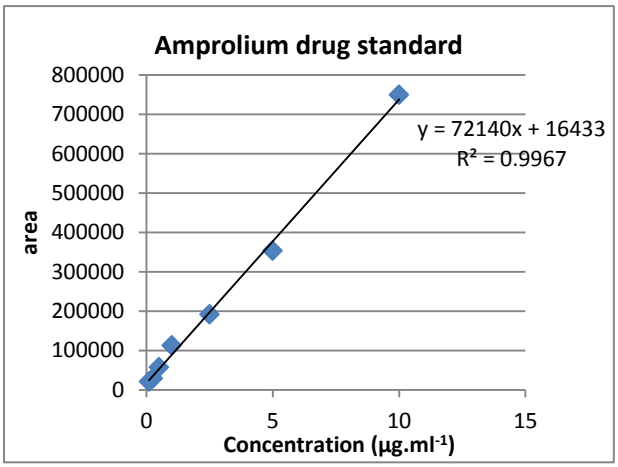
Concentration ($\mu g.mL^{-1}$)	Area
10	712377
5	365593
2.5	172342
1	83769
0.5	39822
0.25	18530
0.1	9879
0.05	3843
0.025	1328

Figure 3.6: **Standard calibration curve of Sulphadiazine**



Concentration ($\mu g.mL^{-1}$)	Area
10	1132907
5	496112
2.5	221868
1	102762
0.5	58860
0.25	23457
0.1	9875
0.05	5035
0.025	2134

Figure 3.7: Standard calibration curve of Trimethoprim



Conc($\mu g.ml^{-1}$)	area
10	748766
5	353237
2.5	191238
1	112345
0.5	56748
0.25	28240
0.1	20364

Figure. 3.8: Standard calibration curve of Amprolium.

3.8. Calculation of pharmacokinetic parameters

In the current study, the data acquisition and chromatogram analysis was carried out by "LC Solution software" (M/S Shimadzu Corporation, Kyoto, Japan). Following data acquisition various pharmacokinetic parameters of sulphadiazine, trimethoprim and amprolium were determined by employing a menu driven add-in program for Microsoft Excel called "PK Solver v2.0" as per the method developed and standardized by **Zhang *et al.*, 2010** for kinetic studies of drugs, but the basic principles for calculation of pharmacokinetic parameters are given below. The fit of the semi log plasma drug concentration time profile of sulphadiazine, trimethoprim and amprolium was adequately described by using a one-compartmental model. The block diagram describing the drug kinetics in a one-compartment model is given in **Figure 3.9**.

Figure 3.9: Model description for pharmacokinetic analysis

3.8.1 Pharmacokinetic analysis of data following intramuscular and oral administration (one compartment model) are given below:

1. $C_{(t)}$ = Drug concentration at time 't'

$$C_{(t)} = A.\ e^{-k_e t}$$

A = Zero-time blood drug concentration intercept of distribution phase.

k_e = Elimination rate constant; t = dosing time interval; e = base of natural log

2. k_a = Absorption rate constant.

3. $t_{1/2ka}$ = Absorption half-life.

4. k_{10} = Elimination rate constant.

5. $t_{1/2k10}$ = Elimination half-life.

$$t_{1/2\ k10} = -\ 0.693/k_{10}$$

6. **T_{max}** = Time to reach maximum plasma concentration.

7. **C_{max}** = Maximum plasma concentration.

8. **AUC** = Area under curve**.** Total area under the plasma drug concentration- time profile curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

9. **AUC_{0-t}** = AUC calculated from zero time to time of last observed concentration.

10. **$AUC_{0-\infty}$** = AUC from time zero to infinity;

11. **AUMC** = Area under the moment curve; It is the total area under the first moment of the plasma drug concentration-time curve.

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

12. **MRT** = Mean residence time. It is the ratio of AUMC to AUC, it represents the average time a drug molecule spends in the body.

$$MRT = \frac{AUMC}{AUC}$$

13. **A** = Zero-time blood drug concentration intercept of distribution phase

14. **V_d/F** = Volume of distribution when the fraction of drug absorbed is not known

$$V_d/F = \frac{\textbf{amount of drug in the body fluid at a given time } t}{\textbf{plasma concentration at time } t}$$

15. **Cl_B/F** = Plasma clearance/ total body clearance when the fraction of drug absorbed is not known.

$$Cl_B = k_{10} \bullet V_d$$

k_{10} is the elimination rate constant and V_d being volume of distribution.

3.9. Calculation of dosage regimen

The pharmacokinetic data obtained as above was used to calculate the dosage regimen for a single dose study as follows **(Verma, 2008).**

1.	Priming dose	$D = C_{therapeutic} . V_d . e^{\beta.t}$
2.	Maintenance dose	$D = C_{therapeutic} . V_d . (e^{\beta.t} – 1)$
3.	Minimum steady state conc.	$C^{ss}_{min} = D / V_d . (e^{\beta.t} – 1)$
4.	Maximum steady state conc.	$C^{ss}_{max} = D / V_d . (1 - e^{-\beta.t})$

3.10. Effect on biochemical and hematological parameters

This experiment was designed to study the effect on biochemical and hematological parameters in poultry following multiple (5) dose i.m. administration of combination of sulphadiazine-trimethoprim containing (sulphadiazine 400mg and trimethoprim 80mg) in the ratio of 5:1 (20mg sulphadiazine and 4mg trimethoprim) at a dose rate of 24mg/kg for 5 days at every 24h interval and oral administration of amprolium at a dose rate of 30mg/kg bw for 5 days at every 24h interval.

3.10.1 Chemicals and reagents

The chemicals and reagents employed for hematology were procured from Merck Ltd., Mumbai, Loba Chemie Pvt. Ltd., Mumbai and Sisco Research Laboratories Pvt. Ltd., Mumbai, whereas, the parameters for biochemical analysis determined using diagnostic kits (Span Diagnostics Ltd., Surat).

3.10.2 Experimental design

Blood samples were collected at 48, 72h, and 96h post administration of amprolium and sulphadiazine-trimethoprim combination drug from the same poultry birds in which multiple dose administration of drugs was done to evaluate hematological and biochemical parameters.

Table 3.1: Experimental design for hematological and biochemical parameters after multiple (5 days) dose (30mg.kg^{-1}) oral administration of Amprolium in poultry (n=3)

Group	Drug	Dose rate(mg.kg^{-1})	No. of poultry
I (Control)	-	NIL	3
II (48h)	Amprolium	30	3
III (72h)	Amprolium	30	3
IV (96h)	Amprolium	30	3

Table 3.2: Experimental design for hematological and biochemical parameters after multiple (5) dose (24mg.kg^{-1}) i.m. administration of combination of Sulphadiazine-Trimethoprim (Biotrim) (20mg sulphadiazine and 4mg trimethoprim/kg b.w) (n=3).

Group	Drug	Dose rate(mg.kg^{-1})	No. of poultry
I (Control)	-	NIL	3
II (48h)	Biotrim®	24	3
III (72h)	Biotrim®	24	3
IV (96h)	Biotrim®	24	3

3.11. Hematological analysis

A complete blood count was performed as per the method described by **Jain (1993),** as per the following procedures.

3.11.1 Total erythrocyte count (TEC)

The whole blood sample was diluted (1:101) in red blood cell pipette with Hayem's diluting fluid. Total erythrocyte count was done using improved Neubauer's haemocytometer and expressed as millions per microliter (10^6 /µl).

3.11.2 Hemoglobin (Hb)

Estimation of hemoglobin was carried out by Drabkin's method wherein the concentration of cyanmet-hemoglobin formed was measured using spectrophotometer at 540 nm wavelength. The hemoglobin (Hb) concentration is expressed in $g.dL^{-1}$.

Hb ($g.dL^{-1}$ of blood) = OD at 540 nm x 32.6

3.11.3 Packed cell volume (PCV)

Estimation of PCV was carried out using microhematocrit method as described by **Sharma and Singh (2000)**. The whole blood sample was drawn into microhematocrit capillaries up to two third of its length and one end of the capillary was sealed with moulding clay. These micro capillaries were centrifuged at 10,000 rpm for 3 minutes. Packed cell volume was directly read by using citro cap microhematocrit reader and expressed in percentage.

3.11.4 Total leukocyte count (TLC)

In this method whole blood is appropriately diluted (1:10) and cells were counted under Neubauer's chamber of the haemocytometer and expressed as thousands per microliter (10^3 /µl).

3.12 Biochemical parameters

6-7 ml of blood samples from all the birds were collected in non-heparinized test tubes and serum was harvested (as described below) after 3-4 hours. The following biochemical parameters estimated in the serum:

1. Total protein
2. Albumin
3. Globulin
4. Creatinine
5. Cholesterol
6. Alanine Aminotransferase (Serum Glutamic Pyruvate Transaminase)
7. Aspartate Aminotransferase (Serum Glutamic Oxaloacetate Trans- aminase)
8. Urea
9. Glucose

3.12.1 Separation of serum

After collection, blood was allowed to clot in non heparinized test tubes kept in slanting position (to provide larger surface area) for 3-4 h at room temperature (18-20ºC). After clotting of blood, as the serum oozed out of the retracted clot, it was collected with the help of micropipette in marked, microcentrifuge tube. The serum thus obtained was centrifuged at 4ºC for 20 minutes at 4000 rpm in a refrigerated centrifuge. The top layer of serum was collected in another microcentrifuge tube with the help of micropipette and stored at -20 ºC in deep freezer. The biochemical parameters were estimated within 7 h (enzymes within 24 h).

3.13. Analysis of data

The plasma concentration and disposition kinetics parameters of amprolium, sulphadiazine and trimethoprim are indicated as Mean ± Standard error. The disposition kinetics of the plasma concentrations after oral and i.m. administration in this investigation were calculated with the help of pharmacokinetic software. Means of several parameters were assessed and variability among them were calculated by virtue of relevant statistical tests. Differentiation amidst the treatment batches were done. The pharmacokinetic parameters analysed by Wilcoxon's rank sum test (**Steel and Torrie, 1960).** The statistical analysis of hemato-biochemical profile was carried out by using one way ANOVA technique followed by Tukey's Multiple Comparison Test by using Graph Pad Prism Statistical Software and level of significance was determined at 95% confidence interval **(Snedecor and Cochran, 1967).**

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

4.1. Method validation

Chromatographic method

Drugs (sulphadiazine, trimethoprim and amprolium) were determined without any interference from their degradation products.

Specificity

The blank plasma samples (n=6) for each drug (sulphadiazine, trimethoprim and amprolium) were analyzed for the presence of any potential interfering compounds and no significant interference was observed in the blank plasma sample at the retention time of the standard and the analyte.

Linearity and range

For determination of linearity, the standard calibration curves were plotted with drug concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 $\mu g.ml^{-1}$ for amprolium and 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 $\mu g.ml^{-1}$ for sulphadiazine and trimethoprim against their peak area and were found to be linear and reproducible over this concentration range with a mean R^2 value of 0.996 for 0.999 and 0.994 for amprolium, sulphadiazine and trimethoprim, respectively.

Precision

The intra-day precisions of the proposed methods were determined by the analysis of six different concentrations each of, sulphadiazine, trimethoprim and amprolium within the linearity range of three replicates each on a single day, while the inter- day precisions were determined by the analysis of six different concentrations of the proposed drugs, within the linearity ranges of three replicates each on three consecutive days. The standard concentrations of 0.05, 0.1, 0.25, 0.5, 1 and 2.5 $\mu g.ml^{-1}$ were subjected to the same extraction procedure described earlier and the precision co-efficient variance (C.V. %) was measured for sulphadiazine, trimethoprim and amprolium. The intra-day assay results are illustrated in **Table 4.1, 4.3, 4.5** and interday assay in **Table 4.2, 4.4, 4.6** for sulphadiazine, trimethoprim and amprolium, respectively.

Table 4.1: Intra -day precision and accuracy of sulphadiazine

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.025	0.023 ±0.0003	0.024	99.99
0.05	0.050 ±0.001	0.049	99.99
0.1	0.10 ±0.008	0.143	99.99
0.25	0.21 ±0.006	0.054	99.98
0.5	0.46 ±0.011	0.043	99.99
1	0.95 ±0.003	0.006	99.97

Table 4.2: Inter -day precision and accuracy of sulphadiazine

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.025	0.023 ±0.0005	0.025	99.98
0.05	0.048 ±0.0005	0.020	99.98
0.1	0.094 ±0.0023	0.042	99.92
0.25	0.23 ±0.0057	0.043	99.80
0.5	0.48 ±0.0057	0.020	99.80
1	0.96 ±0.0088	0.015	99.70

Table 4.3: **Intra -day precision and accuracy of trimethoprim**

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.025	0.021 ±0.0008	7.050116	99.99
0.05	0.047 ±0.001	11.61513	99.99
0.1	0.090 ±0.005	21.12676	99.99
0.25	0.226 ±0.003	6.415003	99.98
0.5	0.51 ±0.023	17.64706	99.99
1	0.936 ±0.012	4.081698	99.97

Table 4.4: **Inter -day precision and accuracy of trimethoprim**

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.025	0.022 ±0.0005	4.54	99.98
0.05	0.046 ±0.001	6.54	99.94
0.1	0.088 ±0.0008	1.72	99.97
0.25	0.22 ±0.005	4.54	99.80
0.5	0.476 ±0.037	13.48	98.76
1	0.913 ±0.006	1.26	99.77

Table 4.5: Intra -day precision and accuracy of amprolium

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.05	0.047 ± 0.0006	0.810	99.99
0.1	0.096 ± 0.002	1.379	99.99
0.25	0.240 ± 0.024	5.948	99.99
0.5	0.453 ± 0.055	7.032	99.98
1	0.948 ± 0.017	1.037	99.99
2.5	2.459 ± 0.111	2.611	99.97

Table 4.6: Inter -day precision and accuracy of amprolium.

Standard concentration ($\mu g.mL^{-1}$)	Concentration measured ($\mu g.mL^{-1}$)	Precision coefficient of variance (%)	Accuracy (%)
0.05	0.047 ±0.0006	1.276596	99.9
0.1	0.098 ±0.001	1.020408	99.9
0.25	0.231 ±0.016	6.926407	99.0
0.5	0.449 ±0.049	10.91314	99.8
1	0.948 ±0.017	1.793249	99.6
2.5	2.429 ±0.082	3.375875	99.7

Selectivity

The retention time were found to range between minutes, 5.352 to 5.543, 3.132 to 3.754 and 10.098 to 10.689 minutes for sulphadiazine, trimethoprim and amprolium, respectively.

Robustness

The robustness of the HPLC methods for the drugs (sulphadiazine, trimethoprim and amprolium) were investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH, mobile phase composition and flow rate. The minor modifications in the HPLC conditions did not change area and peak symmetry, indicating the reliability of the method during the analysis of samples.

Detection and quantification limit

The limit of quantification (LOQ) of an analytical method is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy that fall within the range recommended by the EMEA (1996)**.** The limit of detection (LOD) of an analytical procedure is defined as the lowest amount of analyte in a sample, which can be detected as an exact value. In the present study, the limit of detection and limit of quantification were calculated as 0.025μg.ml^{-1} and 0.075 μg.ml^{-1}, respectively, for all the three drugs.

Recovery

The recovery percentages in plasma sample using this method were 94.2%, 96.4% and 97.7% for sulfadiazine, trimethoprim and amprolium, respectively.

4.2 Pharmacokinetics of sulphadiazine

4.2.1 Pharmacokinetics of sulphadiazine following single dose (24mg.kg^{-1}) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The plasma concentration-time profile of sulphadiazine following single dose (24 mg.kg^{-1}) i.m. administration of sulphadiazine-trimethoprim combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry is depicted in **Table 4.7** and semi logarithmic

plasma concentration (Mean±SE) time profile of sulphadiazine in **Figure 4.1**. The chromatogram of sulphadiazine obtained in plasma following single dose i.m. administration is depicted in **Figure 4.2**.

The mean sulphadiazine concentration ranged from 0.08±0.02 to 24.06±1.30 $\mu g.ml^{-1}$. The pharmacokinetic parameters describing the disposition kinetics of sulphadiazine following single dose i.m. administration are presented in **Table 4.8**. A one-compartment model adequately (R^2=0.98) described plasma concentration-time profile of sulphadiazine in poultry following single dose i.m. administration. Other workers have also described plasma concentration time profile of sulphadiazine by one compartment model in broiler chicken (**Baert *et al*., 2003)** and in mandarian fish (**Wang *et al*.,2016)**

In the present study, the elimination rate constant (k_{10}) of sulphadiazine estimated was 0.14±0.009h^{-1}, was similar to that reported in grass carps (0.14h^{-1}) after i.v administration of sulphadiazine **(Ning Xu *et al*, 2020),** in broiler chicken (0.19 ± 0.03 h^{-1}) following oral administration of SDZ /TMP combination **(Baert *et al.*, 2003)** and in sheep (0.173± 0.18 h^{-1}) after i.m administration of SDZ /TMP combination @ 30$mg.kg^{-1}$ b.w (25mg/kg SDZ + 5mg/kg TMP) **(Batiaz *et al*., 2005)**.

The rate constant of absorption phase (k_a) observed in the present study was 8.02±1.338h^{-1} with an absorption half-life ($t_{1/2ka}$) of 0.11±0.027h. The absorption half-life of the present study was similar to the reported in horses (0.27 ±0.21h) after i.v. administration of SDZ/TMP combination @30$mg.kg^{-1}$ b.w **(Van Duijkeren *et al.*, 1994).** However absorption rate constant value observed in the present study was higher than that obtained (2.72h^{-1}) in broiler birds following oral administration of sulphadiazine given @ 33.34$mg.kg^{-1}$ b.w (**Baert *et al.*, 2003**).

The mean peak concentration (C_{max}) of sulphadiazine in the present study was 22.95±0.72 $\mu g.ml^{-1}$, lower than that obtained (35.47 ± 2.52 $\mu g.ml^{-1}$) in ostrich following i.m administration of SDZ/TMP @ 30$mg.kg^{-1}$ b.w (**Abu-Basha *et al.*, 2008**) and in broiler (39.32$\mu g.ml^{-1}$) after oral administration of sulphadiazine @ 33.34 $mg.kg^{-1}$ b.w (**Baert *et al*., 2003)**.

Table 4.7: Plasma concentration (μg.mL^{-1}) of sulphadiazine after single dose (24 mg.kg^{-1}) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ±SE
0.08	15.13	13.04	16.23	11.22	17.11	14.51	14.12	12.51	15.44	14.36 ±0.622
0.16	18.21	15.96	16.67	13.09	17.62	15.12	16.45	14.78	16.04	15.99±0.512
0.25	19.13	18.14	17.34	14.12	18.1	16.54	18.12	17.64	17.65	17.42±0.473
0.5	21.7	22.71	18.31	16.43	19.84	18.39	20.13	19.88	20.21	19.73±0.622
1	22.35	24.9	22.45	18.54	20.1	16.32	21.23	23.32	24.76	21.55±0.948
2	25.84	28.63	16.11	27.77	24.02	26.12	22.64	25.21	20.22	24.06±1.308
4	15.94	16.12	13.89	15.56	14.54	13.99	13.56	14.56	11.32	14.38±0.490
8	8.63	10.06	6.44	4.12	5.99	6.51	6.28	7.01	7.09	6.90±0.556
12	3.5	4.47	2.2	1.76	2.12	3.76	2.22	3.32	4.02	3.04±0.326
24	0.25	0.198	0.24	0.54	0.58	0.44	0.31	0.42	0.38	0.37±0.044
48	0.037	0.021	0.025	0.13	0.11	0.09	0.041	0.087	0.22	0.08±0.021

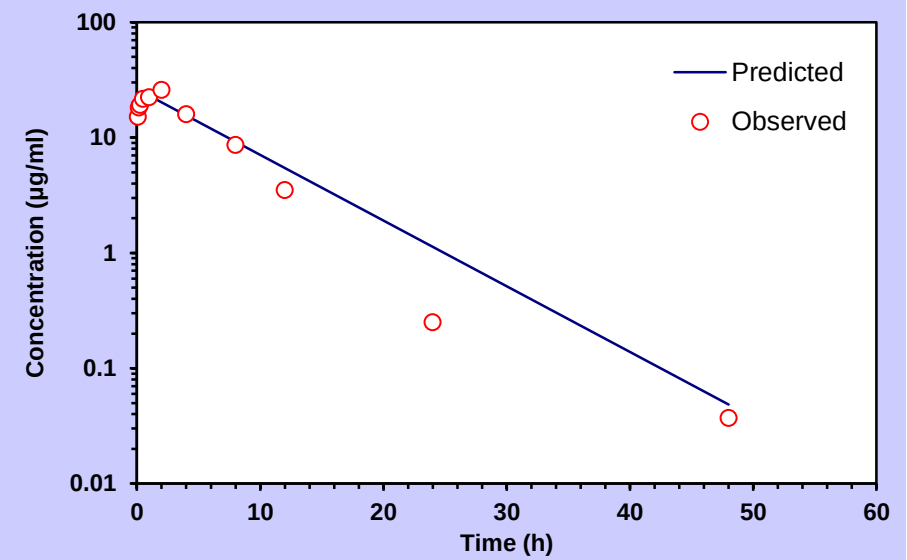


Figure 4.1: Semi logarithmic plot of plasma concentration time profile of sulphadiazine after single dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

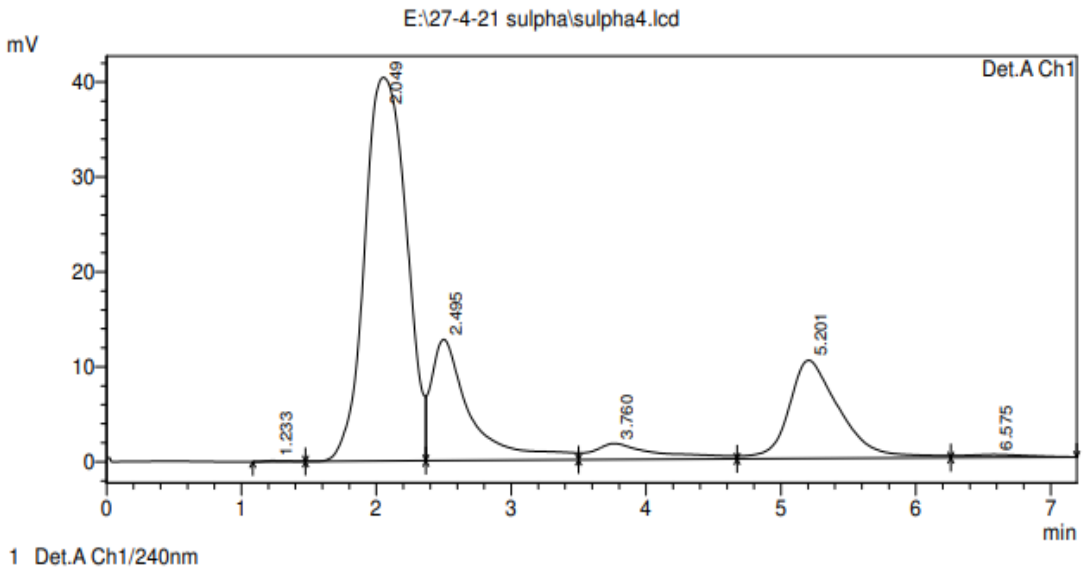


Figure 4.2: Chromatogram of sulphadiazine obtained in plasma following single dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Table 4.8: **Pharmacokinetic parameters of sulfadiazine after single dose (24 mg .kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Unit	I	II	III	IV	V	VI	VII	VIII	IX	MEAN±SE
$t_{1/2ka}$	h	0.086	0.156	0.046	0.315	0.053	0.074	0.088	0.145	0.087	0.11±0.027
$t_{1/2k10}$	h	5.285	4.821	5.049	3.236	5.130	5.791	4.512	4.363	4.469	4.73±0.241
V/F	L.Kg^{-1}	0.773	0.665	0.958	0.636	0.879	0.927	0.990	0.877	0.986	0.85±0.044
CL/F	L.Kg^{-1}.h^{-1}	0.101	0.095	0.131	0.136	0.118	0.110	0.152	0.139	0.152	0.12±0.007
T_{max}	h	0.522	0.799	0.319	1.173	0.357	0.472	0.512	0.739	0.504	0.59±0.088
C_{max}	µg.mL^{-1}	24.138	26.792	19.971	24.427	21.673	20.381	22.38	24.323	22.5000	22.95±0.725
AUC $_{0-t}$	µg.h.mL^{-1}	196.736	208.848	151.789	146.631	168.096	179.622	157.5	172.112	156.781	170.91±6.996
AUC $_{0-\infty}$	µg.h.mL^{-1}	197.106	209.066	152.000	146.636	168.355	180.206	157.6	172.199	156.875	171.12±7.026
AUMC	µg.h^2.mL^{-1}	1527.605	1501.422	1117.497	751.338	1259.196	1525.026	1046.668	1120.185	1031.15	1208.89±89.218
MRT	h	7.750	7.181	7.351	5.123	7.479	8.462	6.638	6.505	6.573	7.00±0.315
A	µg.mL^{-1}	26.279	31.062	21.062	34.792	22.985	21.846	24.705	28.300	24.8148	26.20±1.498
k_a	h^{-1}	8.007	4.433	14.795	2.199	12.905	9.342	7.817	4.756	7.961	8.02±1.338
k_{10}	h^{-1}	0.131	0.143	0.137	0.214	0.135	0.119	0.153	0.158	0.155	0.14±0.009

The calculated value of time of peak concentration (T_{max}) 0.59±0.088h in the present study was lower than the T_{max} (2.47 ± 0.31h) observed in ostrich by **Abu-Basha *et al.* (2008**) and (1.4 ± 0.6) observed after oral administration of sulphadiazine/trimethoprim suspension (24 mg/kg every 12 hr) in neonatal foals (**O'Fallon *et al*., 2020)**.

In the present finding, the mean value of zero time intercept of distribution phase (A) calculated was 26.20±1.498 µg.mL^{-1}. The value of MRT observed in the present study was 7.00±0.315h, similar to that obtained (6.29h) in broiler chicken following oral administration of sulphadiazine @ 33.34mg.kg^{-1} (**Baert *et al.*, 2003**).

In the present study, the elimination half-life (4.73±0.241h) calculated can be compared to that obtained in equines (4.65± 0.88h) **(Van Duijkeren *et al*., 1994),** in sheep (4.03h) **(Batiaz *et al*., 2005)** and in broiler chicken (3.71h) **(Baert *et al.*, 2003).** However comparatively higher elimination half-life than our study have been reported in ostrich (11.79 ± 0.79h) when the combination of SDZ/TMP (25 mg⁄kg SDZ and 5 mg ⁄kg TMP b.w) was administered @ 30 mg.kg^{-1} b.w **(Abu-Basha *et al*., 2008).** Other workers have also reported a higher elimination half lifein grass carps (17.64h) after i.v. administration of sulphadiazine @ 5mg.kg^{-1} b.w **(Ning Xu *et al*., 2020)** and in mandarin fish (25.9± 4.5h) when sulphadizine given orally @ 25mg.kg^{-1} b.w **(Wang *et al*., 2016)**. The differences in half-life of sulphadiazine could be due to variations in the rates of hepatic biotransformation in different species.

The area under curve (AUC) is the parameter that integrates both time and intensity of drug concentration. The AUC characterizes the relative availability of drug in the body. It is used for calculating drug clearance and other kinetic variables. An AUC value of 170.91±6.99h.µg.mL^{-1} was observed following single dose i.m. administration of sulphadiazine in the present study which is quite comparable to that obtained in quines (238.02 ± 31.10 h.µg.mL^{-1}) after i.v. administration of sulphadiazine @ 25mg.kg^{-1} b.w (**Van Duijkeren *et al*., 1994).** However, higher (352.9 ±54.8 h.µg.ml^{-1}) AUC values have been documented in broiler chicken after i.v administration of sulphadiazine @ 33.34mg.kg^{-1} b.w (**Baert *et al*., 2003)** and in ostrich (526.44 ± 22.96 h.µg.ml^{-1}) after i.m administration of sulphadiazine @ 25mg.kg^{-1} b.w **(Abu-Basha *et al*., 2008).**

The volume of distribution (V/F) of sulphadiazine observed in the present study was 0.85±0.04 $L.kg^{-1}$ which can be corroborated to that obtained in pigs (0.83 + 0.16 $L.kg^{-1}$) when sulphadiazine was given i.v. @ 40$mg.kg^{-1}$ bw **(Nouws *et al*., 2011).** However comparatively lower values than present study findings have been reported (0.71 $L.kg^{-1}$) in sheep (**Batiazs *et al*., 2005),** in neonatal foals (0.52 ± 0.15 $L.kg^{-1}$) **(O'Fallon *et al*., 2020**) and in broiler chicken (0.66±0.10 $L.kg^{-1}$) (**Baert *et al*., 2003)**. Higher volume of distribution has been reported in mandarin fish (2.34±0.78 $L.kg^{-1}$) when sulphadiazine given @ 100$mg.kg^{-1}$ b.w (**Wang *et al*., 2016)**. A low volume of distribution of sulphadiazine may be attributed to relatively high ionization state of sulphadiazine at physiological pH and/or its plasma protein binding reported in other species.

Clearance of drug is the volume of blood or plasma cleared of the drug by metabolism and excretion per unit of time. Body clearance is considered a better index of efficiency of drug elimination than half life as it gives the clearance of drug from blood per unit time, however half-life and clearance are not correlated **(Prescott and Baggot, 1988).** The total body clearance of sulphadiazine in poultry observed in the present study was 0.12±0.007$L.h^{-1}.kg^{-1}$ following single dose i.m. administration which could be compared to that obtained in broiler chicken (0.09 $L.h^{-1}.kg^{-1}$) after i.v. administration of sulphadiazine @ 33.34$mg.kg^{-1}$ b.w **(Baert *et al*., 2003)**, in pigs (0.14 ± 0.04 $L.h^{-1}.kg^{-1}$) after i.v. administration of SDZ/TMP combination(25 mg/kg SDZ and 5 TMP mg/kg b.w) @ 30$mg.kg^{-1}$ b.w **(Baert *et al*., 2001**) and in quails (0.114 ± 0.003 $L.h^{-1}.kg^{-1}$) observed after i.v. administration of sulphamethoxazole @ 20$mg.kg^{-1}$ **(Lashev & Mihailov 1994)**. However, a low body clearance (0.02 ± 0.01 $L.h^{-1}$. Kg^{-1}) has been reported in fish, (**Wang *et al*., 2016**), and in sheep (0.038.75 ±3.61 $L.h^{-1}.kg^{-1}$) **(Youssef *et al*., 1981).** The results obtained for clearance in poultry birds in the present study, indicates that birds have more efficient ability to clear drug from circulation as compared to other species. Higher values for clearance in chickens compared to other species could be ascribed to a higher metabolic rate in the birds of lower body weight.

4.2.2 Pharmacokinetics of sulphadiazine following multiple (5) dose (24mg.kg^{-1}) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/ kg b.w) in poultry.

The plasma concentrations of sulphadiazine following first and last dose of multiple dose i.m. administration are depicted in **Table 4.9 and Table 4.10** respectively, and their mean plasma concentration depicted in **Table 4.11**.The semi logarithmic plasma concentration time curve for sulphadiazine following multiple (5) dose i.m. administration after first and last dose in poultry are respectively depicted in **Figure. 4.3** and **Figure 4.4**. The chromatogram of sulphadiazine obtained in plasma following multiple dose i.m. administration is depicted in **Figure 4.5**. Pharmacokinetic values describing the disposition kinetics of sulphadiazine after first and last dose following multiple (5) dose (24.0mg.kg^{-1}) i.m. administration in poultry are presented in **Table 4.12** and **Table 4.13** respectively,s and their mean are depicted in **Table 4.14**. The peak and trough plasma concentration of sulphadiazine with their means are depicted in **Table4.15.**

A one compartment model with first order rate of absorption adequately (R^2=0.98) described plasma concentration-time profile of sulphadiazine in poultry following multiple (5) dose (24.0mg.kg^{-1}) i.m. administration in the present study. The similar model has also reported by **O'Fallon *et al*. (2020)** in neonatal foals. In multiple dose therapy, the ultimate purpose is to achieve a steady drug effect or response with minimum side effects. The basic mathematical relationships applicable to single dose administration kinetics is also valid with slight modification for multiple dose kinetics.

In the present study, the peak plasma concentration of sulphadiazine was observed to be 25.12±1.236 and 25.38±0.678µg.mL^{-1} after the first and last dose, respectively, which were almost similar to peak concentration observed after single (22.95±0.72 µg.mL^{-1}) dose administration and were reported after 2hrs post-drug administration.

The rate constant of absorption phase (k_a) were 8.42±1.056 and 7.88±0.460 h^{-1} with absorption half-life ($t_{1/2ka}$) of 0.10±0.018and 0.09±0.006h after first and last dose, respectively, which is lower than that obtained in mandarian fish (5.7± 1.5 h) **(Wang *et al*., 2016)** and in grass carp (2.29 h) **(Ning Xu *et al*., 2020)**.

Table 4.9: Plasma concentration (μg.mL^{-1}) of sulphadiazine after first dose following multiple dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ±SE
0.08	16.1	14.54	16.23	12.11	15.11	14.51	17.54	15.23	14.56	15.10 ± 0.501
0.16	18.21	15.96	17.43	13.09	17.62	15.12	18.09	17.68	16.63	16.64 ± 0.560
0.25	20.12	18.14	18.67	14.12	18.1	16.54	19.91	18.07	17.23	17.87± 0.604041
0.5	21.7	22.71	19.07	16.43	19.84	18.39	21.32	20.03	19.01	19.83 ± 0.633
1	22.35	24.9	22.45	18.54	21.34	16.32	22.03	21.25	20.73	21.10 ± 0.819
2	25.84	28.63	16.11	26.76	24.02	26.12	28.43	25.45	24.78	25.12 ± 1.236
4	16.22	15.34	13.89	15.56	14.54	13.99	14.17	11.98	15.28	14.55 ± 0.416
8	10.21	8.31	6.44	5.13	5.99	6.51	7.32	5.89	6.03	6.87 ± 0.516
12	5.51	4.47	2.2	1.76	2.12	3.76	3.01	2.25	2.87	3.10 ± 0.416
24	0.25	0.198	0.24	0.54	0.58	0.44	0.43	0.32	0.31	0.36 ± 0.045
48	0.037	0.021	0.025	0.13	0.11	0.09	0.1	0.08	0.1	0.07 ± 0.013

Table 4.10: Plasma concentration (μg.mL^{-1}) of sulphadiazine after last dose following multiple dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ±SE
0.0	0.22	0.14	0.12	0.09	0.22	0.17	0.2	0.15	0.11	0.15 ± 0.015
0.08	15.54	14.54	16.03	14.65	15.11	14.51	15.98	15.23	14.56	15.12 ± 0.204
0.16	17.67	15.96	17.43	14.98	17.62	15.12	18.09	17.68	16.63	16.79 ± 0.393
0.25	21.56	19.79	18.31	15.51	18.1	16.54	19.91	19.04	17.23	18.44 ± 0.622
0.5	23.2	22.71	19.07	16.43	19.84	18.39	21.32	20.03	19.01	20.0 ± 0.712
1	25.5	24.9	22.45	18.54	22.01	20.23	22.03	21.25	20.73	21.96 ± 0.727
2	22.09	27.43	23.34	26.76	24.02	26.12	28.43	25.45	24.78	25.38 ± 0.678
4	17.23	14.98	13.89	15.56	14.54	13.99	14.17	11.98	13.76	14.45 ± 0.477
8	9.89	8.31	7.21	5.13	5.99	6.51	7.32	5.89	6.03	6.92 ± 0.487
12	4.98	3.54	2.2	1.76	2.12	3.76	3.01	2.25	2.87	2.94 ± 0.339
24	0.32	0.198	0.24	0.54	0.58	0.44	0.43	0.32	0.31	0.37 ± 0.043
48	0.05	0.13	0.03	0.11	0.089	0.1	0.06	0.07	0.12	0.08 ± 0.015

Table 4.11: Plasma concentration (μg.mL^{-1}) of sulphadiazine after first and last dose following multiple (5) dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	Mean±S.E.	
	First dose	Last dose
0.00	-	0.15 ± 0.015
0.08	15.10 ± 0.501	15.12 ± 0.204
0.16	16.64 ± 0.560	16.79 ± 0.393
0.25	17.87± 0.604041	18.44 ± 0.622
0.5	19.83 ± 0.633	20.0 ± 0.712
1.0	21.10 ± 0.819	21.96 ± 0.727
2	25.12 ± 1.236	25.38 ± 0.678
4	14.55 ± 0.416	14.45 ± 0.477
8	6.87 ± 0.516	6.92 ± 0.487
12	3.10 ± 0.416	2.94 ± 0.339
24	0.36 ± 0.045	0.37 ± 0.043
48	0.07 ± 0.013	0.08 ± 0.015

Table 4.12: **Pharmacokinetic parameters of sulfadiazine after first dose following multiple dose (24 mg .kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Unit	I	II	III	IV	V	VI	VII	VIII	IX	MEAN±SE
$t_{1/2ka}$	h	0.073	0.147	0.048	0.226	0.077	0.074	0.072	0.080	0.088	0.10±0.018
$t_{1/2k10}$	h	6.193	4.519	4.806	3.930	4.742	5.791	5.020	4.418	5.015	4.93±0.230
V/F	L.Kg^{-1}	0.794	0.668	0.923	0.727	0.827	0.927	0.777	0.815	0.834	0.81 ± 0.027
CL/F	L.Kg^{-1}.h^{-1}	0.088	0.102	0.133	0.128	0.120	0.110	0.107	0.128	0.115	0.11±0.004
T_{max}	h	0.474	0.753	0.325	0.988	0.469	0.472	0.447	0.471	0.523	0.54±0.066
C_{max}	µg.mL^{-1}	23.873	26.668	20.657	23.091	22.569	20.381	24.186	22.761	22.286	22.94 ± 0.631
AUC $_{0-t}$	µg.h.mL^{-1}	223.888	195.034	149.992	155.845	165.243	179.622	186.1	156.153	173.146	176.11 ± 7.791
AUC $_{0-\infty}$	µg.h.mL^{-1}	224.945	195.162	150.141	155.880	165.394	180.206	186.350	156.238	173.378	176.41 ± 7.878
AUMC	µg.h^2.mL^{-1}	2033.627	1313.955	1051.727	934.7703	1150.237	1525.026	1369.084	1013.973	1276.721	1296.56±111.400
MRT	h	9.040	6.732	7.004	5.996	6.954	8.4626	7.3468	6.489	7.363	7.26±0.317
A	µg.mL^{-1}	25.477	30.945	21.872	29.168	24.576	21.846	26.103	24.962	24.388	25.48±1.002
k_a	h^{-1}	9.459	4.695	14.236	3.064	8.893	9.342	9.6184	8.658	7.853	8.42 ± 1.056
k_{10}	h^{-1}	0.111	0.153	0.144	0.176	0.146	0.119	0.138	0.156	0.138	0.14±0.006

Table 4.13: **Pharmacokinetic parameters of sulfadiazine after last dose following multiple dose (24 mg .kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Unit	I	II	III	IV	V	VI	VII	VIII	IX	MEAN±SE
$t_{1/2ka}$	h	0.085	0.130	0.068	0.098	0.081	0.101	0.085	0.077	0.086	0.09±0.006
$t_{1/2k10}$	h	5.491	4.442	4.997	5.066	4.665	5.139	4.847	4.392	4.852	4.87 ±0.115
V/F	$L.Kg^{-1}$	0.747	0.682	0.847	0.870	0.814	0.836	0.759	0.811	0.840	0.80 ± 0.019
CL/F	$L.Kg^{-1}.h^{-1}$	0.094	0.106	0.117	0.119	0.121	0.112	0.108	0.128	0.120	0.12±0.003
T_{max}	h	0.522	0.684	0.428	0.572	0.485	0.587	0.504	0.461	0.510	0.52±0.025
C_{max}	$\mu g.mL^{-1}$	25.033	26.329	22.225	21.234	22.843	22.096	24.496	22.904	22.1171	23.25 ± 0.555
AUC $_{0-t}$	$\mu g.h.mL^{-1}$	201.427	183.201	163.883	161.443	160.494	170.231	178.076	152.518	161.029	170.25 ± 5.019
AUC $_{0-\infty}$	$\mu g.h.mL^{-1}$	211.829	187.777	170.062	167.862	165.250	177.337	184.134	156.120	166.529	176.32 ± 5.516
AUMC	$\mu g.h^2.mL^{-1}$	1704.311	1238.944	1242.919	1250.916	1131.749	1340.851	1310.278	1006.99	1186.429	1268.15±63.745
MRT	h	8.045	6.597	7.308	7.452	6.848	7.561	7.115	6.450	7.124	7.16±0.165
A	$\mu g.mL^{-1}$	27.162	30.182	23.913	23.422	24.988	24.401	26.800	25.078	24.219	25.57±0.711
k_a	h^{-1}	8.091	5.312	10.157	7.008	8.486	6.811	8.153	8.900	8.042	7.88 ± 0.460
k_{10}	h^{-1}	0.126	0.156	0.138	0.136	0.148	0.134	0.142	0.157	0.142	0.15 ± 0.001

Table 4.14: Pharmacokinetic parameters (Mean±SE) of sulfadiazine in plasma following multiple (5) dose (24 mg.kg^{-1}) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Parameters	Units	First dose	Last dose
$t_{1/2ka}$	h	0.10±0.018	0.09±0.006
$t_{1/2k10}$	h	4.93±0.230	4.87 ±0.115
V/F	L.Kg^{-1}	0.81 ± 0.027	0.80 ± 0.019
CL/F	L.Kg^{-1}.h^{-1}	0.11±0.004	0.12±0.003
T_{max}	h	0.54±0.066	0.52±0.025
C_{max}	µg.mL^{-1}	22.94 ± 0.631	23.25 ± 0.555
AUC $_{0-t}$	µg.h.mL^{-1}	176.11 ± 7.791	170.25 ± 5.019
AUC $_{0-\infty}$	µg.h.mL^{-1}	176.41 ± 7.878	176.32 ± 5.516
AUMC	µg.h^2.mL^{-1}	1296.56±111.400	1268.15±63.745
MRT	h	7.26±0.317	7.16±0.165
A	µg.mL^{-1}	25.48±1.002	25.57±0.711
k_a	h^{-1}	8.42 ± 1.056	7.88 ± 0.460
k_{10}	h^{-1}	0.14±0.006	0.15 ± 0.001

Table 4.15: Peak and trough plasma (Mean±Se) concentration (ug.mL^{-1}) of sulphadiazine following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (days)	Peak	Trough
1	25.12±1.231	0.07±0.015
2	24.13±0.965	0.09±0.020
3	22.87±1.014	0.11±0.020
4	26.54±0.876	0.06±0.021
5	25.75±0.76	0.054±0.012

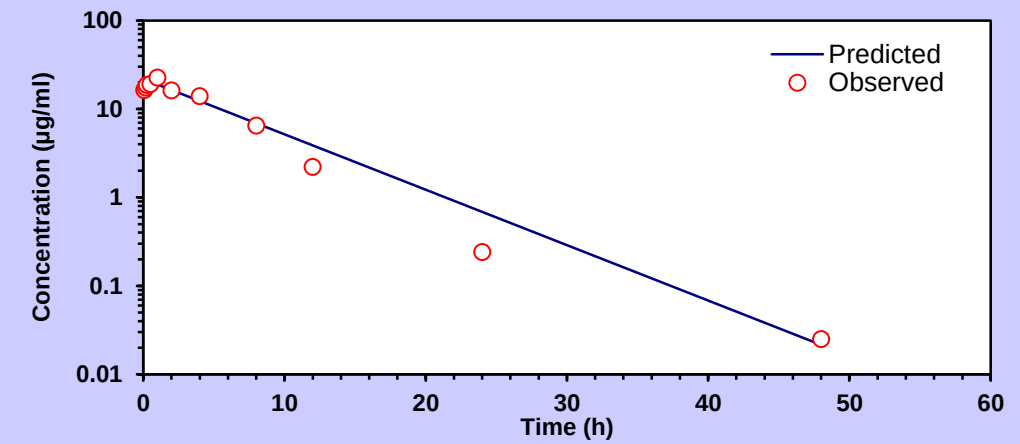


Figure 4.3: **Semi log plot of plasma concentration time profile of sulphadiazine after first dose following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

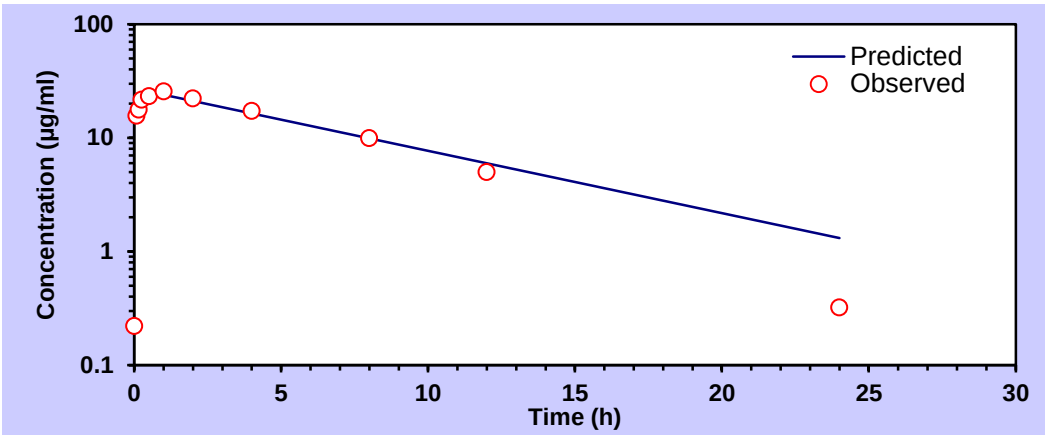


Figure 4.4: Semi log plot of plasma concentration time profile of sulphadiazine after last dose following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

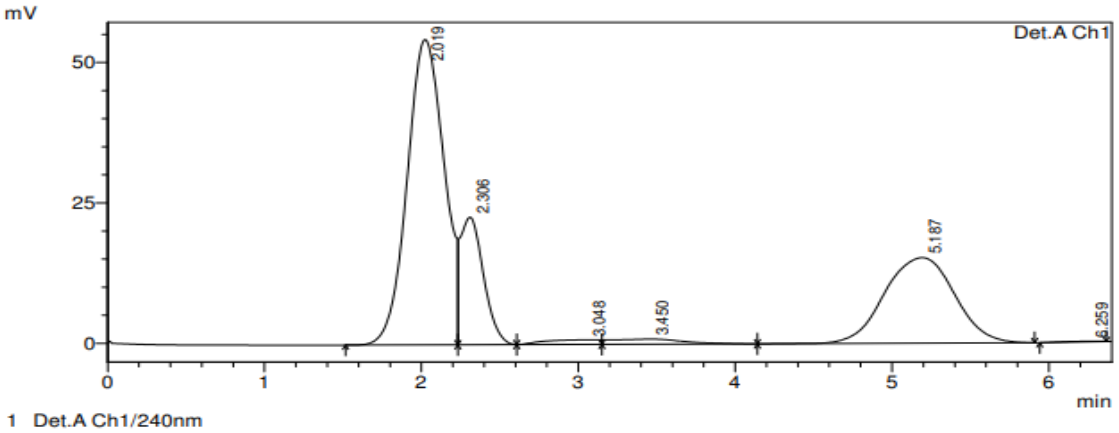


Figure 4.5: Chromatogram of sulphadiazine obtained in plasma following multiple (5) dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mgTMP/kg b.w) in poultry.

The elimination rate constant (k_{10}) obtained in the present study after first and last dose were 0.14±0.006 and 0.15 ± 0.001 h^{-1}, respectively, these findings can be corroborated to that obtained (0.16 ± 0.03 h^{-1}) in equines after i.v. administration of sulphadiazine @ 25mg.kg^{-1} b.w (**Van Duijkeren *et al.*, 1994),** and to the value (0.173±0.18 h^{-1}) obtained by **Batiaz *et al.*, (2005)** in sheep.

In our study, the elimination half-life ($t_{1/2k10}$) of 4.93±0.230 and 4.87 ±0.115h were obtained for sulphadiazine following first and last dose, respectively, after multiple (5) dose (24 mg.kg^{-1}) i.m. administration in poultry. These findings can be corroborated to that found (4.10h) in sheep (**Batiazs *et al.*, 2005**) and that obtained (3.71h) in broiler chicken (**Baert *et al.*, 2003**). The values reported in present study were lower (17.64h) than that reported in grass carp by **Ning Xu *et al.* (2020)** and to that obtained (25.9± 4.5h) in mandarian fish (**Wang *et al.*, 2016**).

The values of C_{max} in the present study were 22.94 ± 0.631 µg.ml^{-1} and 23.25 ± 0.555µg.ml^{-1} after first and last dose, respectively, which is quite similar to that observed in fish (27.94± 4.25 µg.mL^{-1}) when a combination of SDZ/TMP was given orally @ 120mg.kg^{-1} b.w (**Wang *et al.*, 2016),** and in chicken (29.5 ±8.3 µg.mL^{-1}) when sulphadiazine and trimethoprim combination given orally @ 30mg.kg^{-1} bw (**Baert *et al.*, 2001).** C_{max} value which was obtained (24.79 µg.mL^{-1}) by **Ning Xu *et al.* (2020)** in grass carps after oral administration of sulphadiazine @ 50 mg.kg^{-1} can also be corroborated to the value obtained in the present study. However, a higher C_{max} value (40.6 µg.mL^{-1}) as compared to our study has been reported in sheep plasma after i.v. administration of sulphadiazine and trimethoprim combination @ 30mg.kg^{-1} b.w by **Batiazs *et al.* (2005)** and in neonatal foals (37.8 ± 13.4 µg.ml^{-1}) after oral administration of sulphadiazine/trimethoprim suspension (24 mg.kg^{-1}); (**O'Fallon *et al.*, 2020).**

The values of T_{max} in the present study observed were 0.54±0.066 h and 0.52±0.025 h after first and last dose, respectively, which is lower than that observed in fish (16.0± 3.1h) (**Wang *et al.*, 2016**). However, value of T_{max} observed in the present study was similar to that reported in pigs (2.19 h) by **Baert *et al.* (2001)**, but is longer than that obtained in chickens (1.64 h) (**Baert *et al.*, 2003)**.

The volume of distribution (V/F) of sulphadiazine was estimated to be 0.81 ± 0.027L.kg^{-1} and 0.80 ± 0.019 L.kg^{-1} after first and last dose, respectively, in the

present study which was similar to that obtained (0.83 ± 0.16 $L.kg^{-1}$) in pigs (**Nouws *et al.*, 2011**), whereas a lower value (2.34± 0.78 $L.kg^{-1}$) was reported in mandarian fish by **Wang *et al.* (2016)**.

Clearance (CL/F) of sulphadiazine observed in the present study was 0.11±0.004 $L.h^{-1}.kg^{-1}$ and 0.12±0.003$L.h^{-1}.kg^{-1}$ after first and last dose, respectively, which is quite comparable to that obtained (0.12 $L.h^{-1}.kg^{-1}$) in sheep (**Batiazs *et al.*, 2005**) and to that reported (0.14 ±0.04 $L.h^{-1}.kg^{-1}$) in pigs by **Baert*et al.* (2001)**. However the present study values were found higher than that reported (0.06 ±0.01 $L.h^{-1}.kg^{-1}$) in mandarian fish (**Wang *et al.*, 2016)**.

The mean area under curve (AUC) after first and last dose were 176.11 ± 7.878 and 170.25 ± 5.019 $\mu g.h.ml^{-1,}$ respectively,which can be compared with that reported (146 µg. h. ml^{-1}) in sheep **(Batiazs *et al.*, 2005)** but was lower than that found (564.19µg. h. ml^{-1}) in ostrich **(Abu-Basha *et al.*, 2008).**

4.2.3. Tissue residue study of sulphadiazine ($\mu g.g^{-1}$) following single dose (24 $mg.kg^{-1}$) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The tissue residue concentration of sulphadiazine following single dose i.m. administration in poultry was depicted in Table **4.16.** The chromatogram obtained in tissue sample following single dose i.m. administration is depicted in Figure **4.6.**

The mean residual concentration of sulphadiazine detected at 24hrs post administration of drug were 0.20 ±0.027, 0.28±0.048 and 0.12 ±0.015 $\mu g.g^{-1}$ in liver, kidney and muscles, respectively. After 24hr of drug administration, the maximum residual concentration of sulphadiazine was found to be 0.28±0.048$\mu g.g^{-1}$ in kidney. The above results are in agreement with the findings (0.369±0.064 $\mu g.g^{-1}$) in broiler chicken (**Roncada *et al.*, 2011)** and in pigs (0.23 ± 0.045 $\mu g.g^{-1}$); **(Garwaki *et al.*, 1996).** In the present study the tissue concentration observed after 48hr of drug administration in liver, kidney and muscles were 0.035 ±0.007 $\mu g.g^{-1}$, 0.037±0.009 $\mu g.g^{-1}$ and 0.02±0 $\mu g.g^{-1}$ respectively. These concentrations were below the MRL (0.10 $\mu g.g^{-1}$). In the present study sulphadiazine residual concentration was not detected in intestine. No residual concentration in tissues were found 72hrs post administration of drug.

Table 4.16: Residual concentration (µg.g^{-1}) of sulphadiazine in various tissues after single dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry (n = 3).

Time	Poultry no	Tissues Residual concentration (µg.g^{-1})		
		Liver	kidney	muscles
Birds slaughtered after 24h following single dose drug administration	1	0.244	0.365	0.13
	2	0.198	0.26	0.10
	3	0.15	0.20	-
	Mean±SE	0.20±0.027	0.28±0.048	0.12±0.015
Birds slaughtered after 48h following single dose drug administration	4	0.05	0.05	0.02
	5	0.032	0.043	-
	6	0.023	0.02	-
	Mean±SE	0.035±0.007	0.037±0.009	0.02±0
Birds slaughtered after 72h following single dose drug administration	7	-	--	-
	8	-	--	-
	9	-	--	--

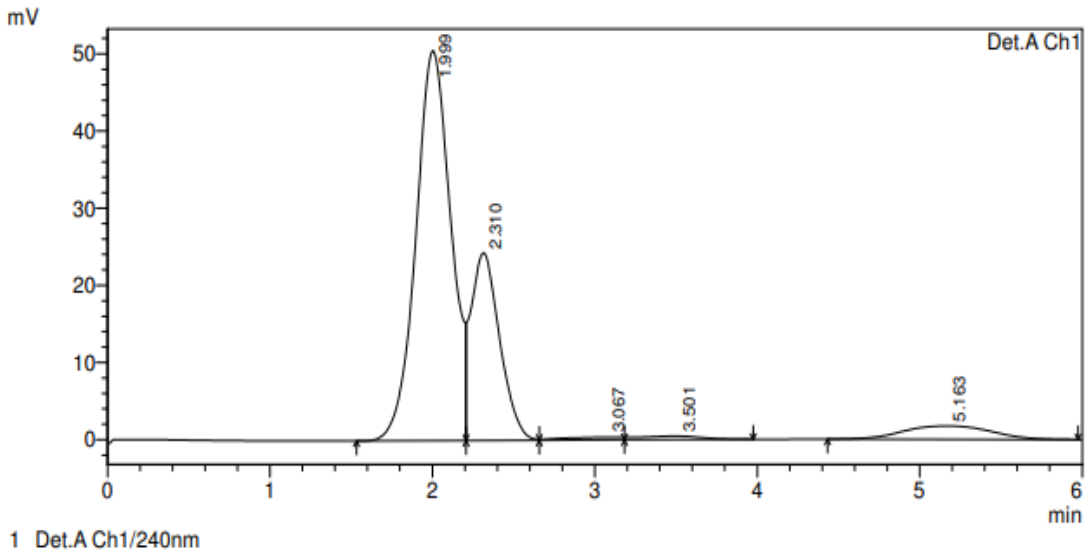


Figure 4.6: **Chromatogram of sulphadiazine obtained in tissue following single dose (24mg.kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

4.2.4 Tissue residue study of sulphadiazine (µg.g^{-1}) following multiple (5) dose (24 mg.kg^{-1} b.w) i.m administration of SDZ /TMP combination (20 mg SDZ + 4 mg TMP/ kg b.w) in poultry.

The tissue residue study was conducted in 9 birds. **Table 4.17** depicts the tissue residue concentration of sulphadiazine following multiple dose i.m administration in poultry. **Figure 4.7** depicts the chromatogram obtained in tissue sample following multiple dose administration. The mean residual concentration of sulphadiazine detected at 48hrs post administration of drug were 0.57±0.067, 0.78±0.044 and 0.23±0.034 µg.g^{-1} in liver, kidney and muscles, respectively. After 48 hr of drug administration, the maximum residual concentration of sulphadiazine was found (in kidney0.78±0.044 µg.g^{-1}), which was higher than that obtained (0.23±0.045 µg.g^{-1}) in pigs **(Garwaki *et al*., 1996)** fed on a diet containing TMP/SDZ formulation in the amount of 6 mg.kg^{-1} b.w (TMP) and 30 mg.kg^{-1} b.w (SDZ) for 5 consecutive days individually twice a day. However **Tzivara *et al*. (2013)** have reported a lower value (0.1079 µg.g^{-1}) of residual tissue concentration in kidney of pigs fed a premix 40%, containing 66.7 g TMP and 333.3 g SDZ per kg feed for 5 consecutive days.

In the present study, the tissue concentration of sulphadiazine after 72hr of its administration were 0.21±0.014, 0.43±0.028 and 0.12±0.018 µg.g^{-1} in liver, kidney and muscles, respectively, which was higher than that reported by **Roncada *et al.* (2011)** in liver **(**0.081±0.006) and kidney (0.154±0.056) tissues of broiler chicken when fed with a combination of SDZ and TMP at 300 mg. kg^{-1} and at 60 mg. kg^{-1} of the diet, respectively for 5 consecutive days. The tissue residues obtained after 72h of drug administration were above the MRL in the present study. The tissue residual concentration at 96hrs post administration of sulphadiazine were 0.05±0.005, 0.07±0.008 and 0.02±0.008 µg.g^{-1} in liver, kidney and muscles, respectively, which was lower than MRL (0.10 µg.g^{-1}). In the present study, based on the findings, it can be concluded that pre slaughter withdrawal period should not be less than 5 days.

As per several reports, the MRL for sulphonamides reported 100 ug.kg^{-1} in muscle, fat, liver and kidneys for all food-producing animals. Sulphadiazine concentrations decreased rapidly during the following six days and reached levels lower than the MRLs in all target tissues. The LOD for all tissues calculated was 0.025 µg.g^{-1}.

Table 4.17: Residual concentration (µg.g^{-1}) of sulphadiazine in various tissue following multiple (5) dose (24 mg.kg^{-1}) i.m. administration of the SDZ /TMP combination (20mg SDZ + 4 mg TMP/kg b.w) in poultry (n=3).

Time	Poultry no.	Tissues Residual concentration (ug/g)		
		Liver	Kidney	Muscles
Birds slaughtered after 48h following multiple dose drug administration	1	0.44	0.76	0.29
	2	0.66	0.87	0.23
	3	0.62	0.72	0.17
	Mean±SE	0.57±0.067	0.78±0.044	0.23±0.045
Birds slaughtered after 72h following multiple dose drug administration	4	0.24	0.46	0.15
	5	0.19	0.47	0.14
	6	0.22	0.38	0.09
	Mean±SE	0.21±0.014	0.43±0.028	0.12±0.018
Birds slaughtered after 96h following multiple dose drug administration	7	0.06	0.09	0.04
	8	0.05	0.07	0.02
	9	0.04	0.06	0.01
	Mean±SE	0.05±0.005	0.07±0.008	0.02±0.008

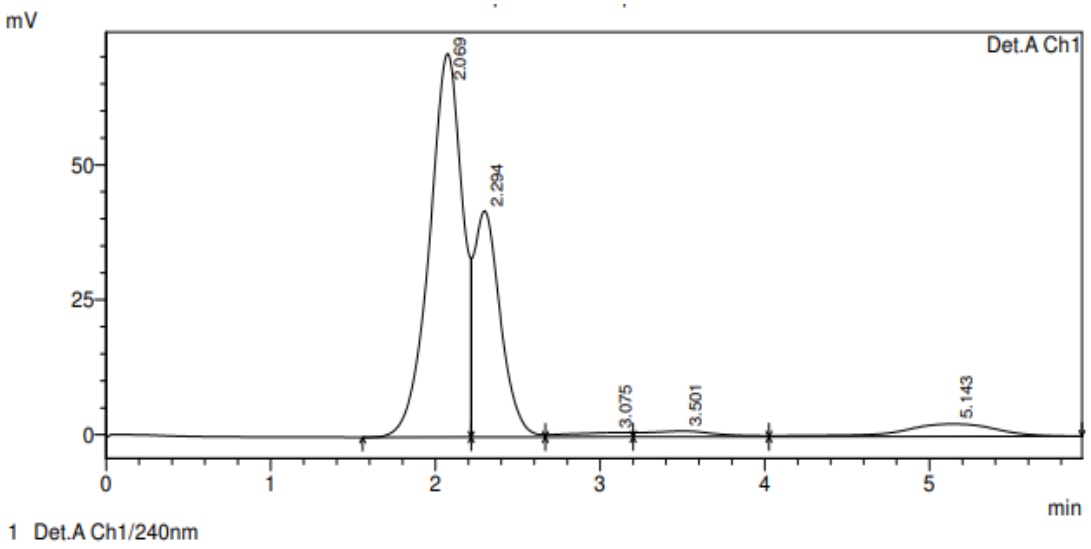


Figure 4.7: Chromatogram of sulphadiazine obtained in tissue following multiple (5) dose (24 mg.kg^{-1} bw) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg bw) in poultry.

4.3 Pharmacokinetics of trimethoprim

4.3.1 Pharmacokinetics of trimethoprim following single dose (24mg.kg^{-1} b.w) i.m administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/ kg b.w) in poultry.

The plasma concentration-time profile of trimethoprim is depicted in **Table 4.18**. The semi logarithmic plasma concentration-time profile of trimethoprim is depicted in **Figure 4.8**. The pharmacokinetic parameters describing the disposition kinetics of trimethoprim following single dose (24.0mg.kg^{-1}) i.m. administration are presented in **Table 4.19.** The chromatogram of trimethoprim obtained in plasma following single dose i.m. administration is depicted in **Figure 4.9.** A one-compartment model adequately (R^2=0.99) described plasma concentration-time profile of trimethoprim in poultry following single dose i.m. administration. Similar findings have been reported in broiler chicken **(Baert *et al*., 2003)**, in mandarian fish (**Wang *et al*., 2016)** and in elephants **(Page *et al*., 1991)**. The mean trimethoprim concentration in our study ranged from 0.09±0.017 to 1.27±0.056µg.ml^{-1}.

The rate constant of absorption phase (k_a) observed in the present study was 3.04 ±0.208h^{-1} with an absorption half-life ($t_{1/2ka}$) of 0.23 ±0.016h, which is comparable to that reported (0.34h) in pigs (**Baert *et al*., 2001),** but higher than that observed (2.4±1.0h) in fish by **Wang *et al*. (2016).** The value of A and MRT observed in the present study was 1.62 ±0.092 µg.ml^{-1} and 6.88 ±0.905h, respectively.

The values of C_{max} observed in the present study was 1.28 ±0.041 µg.mL^{-1}, which can be correlated to that reported (1.72 ± 0.36 µg.mL^{-1}) in horses after a single oral dose of TMP/SDZ combination (5 mg.kg^{-1}TMP and 25 mg.kg^{-1}SDZ) @30mg.kg^{-1} **(Van Duijkeren *et al*., 1994)** and to that observed (1.06±0.08 µg.ml^{-1}) in japanese quails **(Lashev and Mihailov, 1994).**

T_{max} observed (1.03 ±0.058 h) in the present study, was quite similar to that found (1.42h) in broiler chicken **(Baert *et al*., 2003).**

Table 4.18: Plasma concentration (Mean±SE) (µg.ml^{-1}) of trimethoprim after single dose (24 mg.kg^{-1}) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
0.08	0.56	0.32	0.42	0.34	0.41	0.61	0.34	0.30	0.47	0.44 ±0.047
0.16	0.63	0.52	0.67	0.57	0.54	0.72	0.62	0.65	0.71	0.60±0.031
0.25	0.87	0.74	0.86	0.73	0.61	0.87	0.75	0.72	0.81	0.78±0.043
0.5	1.04	1.12	0.97	1.01	1.05	1.03	1.06	0.99	0.96	1.03±0.020
1	1.44	1.21	1.21	1.14	1.17	1.45	1.35	1.48	1.32	1.27±0.056
2	1.31	1.33	1.43	1.1	1.01	0.98	1.05	1.23	1.15	1.19±0.076
4	0.87	0.97	0.74	0.85	0.98	0.56	0.68	0.95	0.84	0.82±0.064
8	0.43	0.51	0.32	0.65	0.52	0.24	0.33	0.64	0.51	0.445±0.060
12	0.22	0.23	0.098	0.32	0.31	0.089	0.076	0.35	0.24	0.21±0.040
24	0.09	0.1	0.05	0.04	0.15	0.06	0.03	0.12	0.13	0.09±0.017

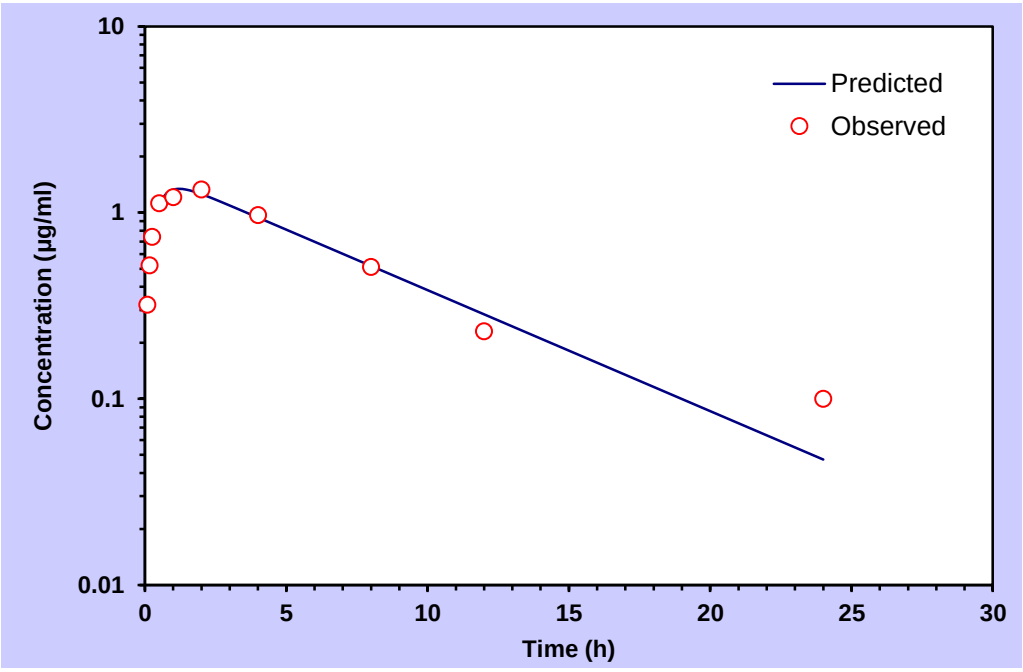


Figure 4.8: Semi log plot of plasma concentration time profile of trimethoprim after single dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Table 4.19: **Pharmacokinetic parameters (Mean±SE) of trimethoprim in plasma following single dose (24 mg.kg^{-1} bw) i.m administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.234	0.281	0.283	0.200	0.212	0.189	0.193	0.246	0.187	0.23 ±0.016
$t_{1/2k10}$	h	4.062	4.631	3.201	6.395	6.265	2.667	4.561	6.381	4.621	4.53 ±0.631
V/F	L.Kg^{-1}	2.408	2.486	2.324	3.081	3.063	2.522	2.583	2.781	3.067	2.64 ±0.137
CL/F	L.Kg^{-1}.h^{-1}	0.410	0.372	0.503	0.333	0.338	0.655	0.567	0.374	0.402	0.43 ±0.050
T_{max}	h	1.023	1.210	1.088	1.032	1.072	0.778	0.983	0.878	1.043	1.03 ±0.058
C_{max}	µg.ml^{-1}	1.394	1.341	1.359	1.160	1.159	1.295	1.234	1.187	1.312	1.28 ±0.041
AUC $_{0-t}$	µg.h/ml	9.560	10.432	7.898	11.061	10.943	6.088	8.876	11.073	7.954	9.33 ±0.804
AUC $_{0-\infty}$	µg.h/ml	9.732	10.747	7.946	11.979	11.801	6.101	8.935	10.834	9.823	9.71 ±0.944
AUMC	µg.h^2/ml	60.330	76.180	39.948	113.995	110.292	25.148	60.045	112.43	60.421	70.98±14.831
MRT	h	6.198	7.088	5.0273	9.515	9.345	4.121	5.87	7.521	6.612	6.88 ±0.905
A	µg.ml^{-1}	1.762	1.712	1.887	1.340	1.351	1.706	1.567	1.487	1.602	1.62 ±0.092
k_a	h^{-1}	2.957	2.464	2.4435	3.465	3.265	3.657	2.645	3.387	2.861	3.04 ±0.208
k_{10}	h^{-1}	0.170	0.149	0.216	0.108	0.110	0.259	0.163	0.212	0.168	0.17 ±0.024

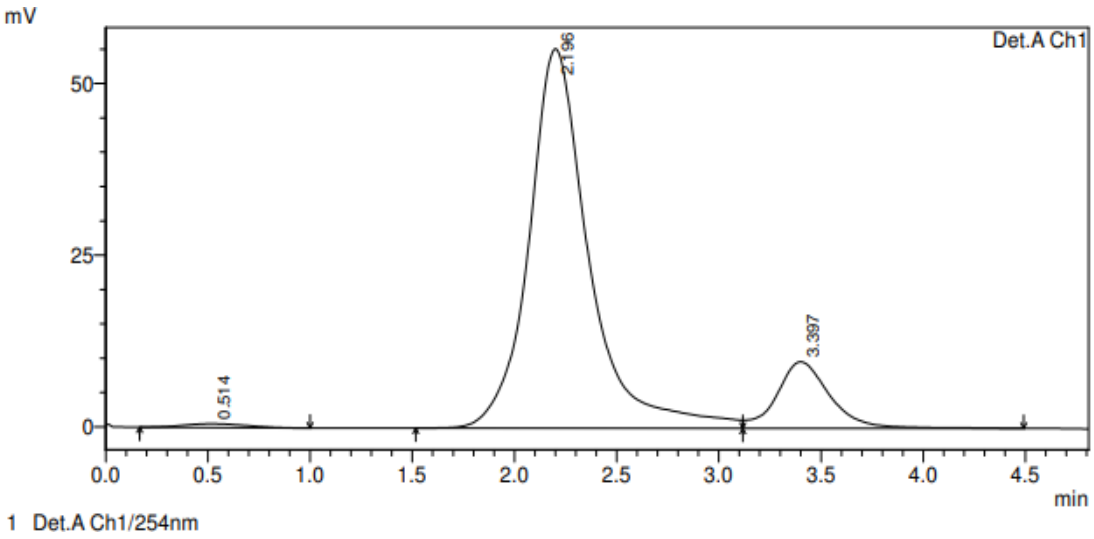


Figure 4.9: **Chromatogram of trimethoprim obtained in plasma following single dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

The elimination rate constant (k_{10}) observed in the present study was 0.17 ±0.024h^{-1} with an elimination half-life ($t_{1/2k10}$) of 4.53 ±0.631h, which was higher to that obtained in alpacas (0.74 ± 0.1h) after i.v. administration of sulphamethaxazole (80 mg.mL^{-1}) and TMP (16 mg.mL^{-1}) solution @ 15 mg.kg^{-1} b.w (**Chakwenya *et al.*, 2002)**. The half life calculated in the present study is comparable to that reported in buffalo calves (3.08h) when TMP given i.v. @ 10mg.kg^{-1}bw **(Iqbal *et al.*, 1994)**. The present study findings can also be corroborated to that obtained (3.30h) in ostrich, when TMP was given in combination with SDZ @ 30mg.kg^{-1} bw in the ratio of 5:1**(Abu-Basha *et al.*, 2008)**. However, the present study findings were lower to that obtained (17.1±3.4h) in fish when a combination of SDZ/TMP given orally @ 120 mg.kg^{-1} b.w (100mg of SDZ + 20mg of TMP/ kg b.w) **(Wang *et al.*, 2016).**

In the present study, the mean area under curve (AUC) observed was 9.71 ±0.944 h.µg.mL^{-1} which can be corroborated to the value (9.12 ± 4.80 h.µg.ml^{-1}) reported in alpacas after intragastric administration of trimethoprim @ 15mg.kg^{-1} bw **(Chakwenya *et al.*, 2002)**. However, higher value (3.66 h.µg.mL^{-1}) has been reported in japanese quails **(Lashev and Mihailov, 1994).**

The volume of distribution (V/F) of trimethoprim in the present study was estimated to be 2.64 ±0.137L.kg^{-1} which can be correlated to that reported (3.16 L.kg^{-1}) in broiler chicken **(Baert *et al.*, 2003)** and that obtained (2.81 L.kg^{-1}) in sheep **(Batiazs *et al.*, 2005).**

In the present study, clearance of drug (CL/F) estimated was 0.43 ±0.050 L.h^{-1}.kg^{-1}. A similar (0.54± 0.13 L.h^{-1}.kg^{-1}) (CL/F) value was observed in pigs (**Baert *et al.*, 2001),** whereas, higher value of clearance was obtained (3.31± 0.51 L.h^{-1}.kg^{-1}) in sheep **(Batiazs *et al.*, 2005)**.

4.3.2 Pharmacokinetics of trimethoprim following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The plasma concentration of trimethoprim after first and last dose following multiple (5) dose (24 mg.kg^{-1}) i.m. administration in poultry depicted in **Table 4.20** and **Table 4.21,** respectively and their means are depicted in **Table 4.22**.The semi-

logarithmic plasma concentration time curve for trimethoprim following first and last dose after multiple (5) dose i.m. administration in poultry is depicted in **Figure 4.10** and **4.11** respectively. The chromatogram of trimethoprim obtained in plasma following multiple dose i.m. administration is depicted in **Figure 4.12.** Pharmacokinetic values describing the disposition kinetics of trimethoprim after first and last dose following multiple (5) dose (24.0mg.kg^{-1}) i.m. administration in poultry are presented in **Table 4.23** and **Table 4.24,** respectively, and their means are depicted in Table **4.25**. The peak and trough plasma concentration of trimethoprim with their means are depicted in **Table 4.26**.

A one compartment model with first order rate of absorption adequately (R^2 = 0.99) described plasma concentration-time profile of trimethoprim in poultry following multiple (5) dose (24.0mg.kg^{-1}) i.m. administration. The similar model has also reported by **Baert *et al.* (2001)** in pigs and **O' Fallon *et al.* (2020)** in neonatal foals.

The peak plasma concentration of trimethoprim was observed to be 1.23±0.044 and 1.29±0.006µg.ml^{-1} at 1 and 2hr after the first and last dose, respectively. Thereafter, the plasma drug concentration decreased slowly to a minimum of 0.07±0.011 and 0.06±0.006 µg.ml^{-1} after first and last dose, respectively.

In the present study the rate constant of absorption phase (k_a) observed were 3.46 ± 0.329 and 4.34±0.318 h^{-1} after first and last dose, respectively, which were higher (0.29 ±0.07 h^{-1}) than that observed in fishes (**Wang *et al.*, 2016**), but is similar (3.29 ± 1.32 h^{-1}) to that found in neonatal foals (**O'Fallon *et al.*, 2020**). The absorption half-life ($t_{1/2ka}$) of 0.22 ± 0.023 and 0.17±0.010 h after first and last dose, respectively calculated in the present study was lower than that (2.4±1.0h) reported in fishes (**Wang *et al.*, 2016).**

The elimination rate constant of trimethoprim (k_{10}) after first and last dose observed in the present study were 0.18±0.019 and 0.12±0.007 h^{-1}, which can be compared to that reported (0.12 ± 0.039h^{-1}) in neonatal foals when a combination of sulphadiazine–trimethoprim suspension (333 mg SDZ/67 mg TMP) was administered orally @ 24 mg.kg^{-1} every 12 hr for 10 days **(O'Fallon *et al.*, 2020).** Our present study findings can also be corroborated to the values obtained (0.277h^{-1}) in hens

(**Queralt and Castells, 1985)**. However the value in present study was lower than that obtained (0.344 ±0.021 h^{-1}) in calves following administration of combination of SDZ/TMP given i.v. @ 30mg.kg^{-1}b.w (**Shoaf *et al.*, 1986).**

The values of C_{max} and T_{max} were 1.23 ± 0.018µg.ml^{-1} and 0.95 ± 0.071h after first and 1.28±0.009 µg.$ml^{-1}$and 0.86±0.035 h after the last dose, respectively. C_{max} obtained in this study was similar (1.13 µg.mL^{-1}) to that found in broiler chicken **(Baert *et al.*, 2003**) and also to that observed (1.16 µg.mL^{-1}) in warren hens (**Queralt and Castells, 1985)**. However, the value of C_{max} obtained in the present study was higher than that reported (0.71 µg.ml^{-1}) in ostrich with T_{max} of 2.07 h (**Basha *et al.*, 2008).**

Mean residence time (MRT) provides estimate regarding the persistence time of drug in the body. MRT observed in the present study was 6.40 ± 0.715h and 8.50±0.552h after first and last dose, respectively, following multiple dose administration of drug, which was higher (4.63h) than that obtained in sheep **(Batiazs *et al.*, 2005)**. MRT which observed (3.9 ±0.1 h) in horses (**Peck *et al.*, 2002),** after i.v administration of a bolus of sulfamethoxazole (12.5 mg/kg) and trimethoprim (2.5 mg/kg) was comparatively lower than our findings.

In the present study, elimination half-life ($t_{1/2k10}$) of trimethoprim observed were 4.22 ± 0.497 and 5.73±0.389h, following multiple dose i.m administration after first and last dose, respectively, was higher than that found in Japanese quail (2.36 ±0.62h) after oral administration of sulphamethoxazole and trimethoprim combination @ 60 mg.kg^{-1} b.w (**Lashev and Mihailov, 1994**). However, the present study findings were lower than that observed (17.1±3.4 h) in fishes (**Wang *et al.*, 2016).** These differences in the elimination half lives may be due to species difference and different route of administration and hepatic metabolism of drugs.

The mean area under curve (AUC) after first and last dose observed in the present study were 8.72 ± 0.80 and 11.70±0.665 µg.h.mL^{-1}, respectively, which was lower (21.1 ± 5.3µg.h.mL^{-1}) than that found in neonatal foals (**O' Fallon *et al.*, 2020)** but was similar (11.17± 2.74µg. h. ml^{-1}) to that found in horses **(Van Duijkeren *et al.*, 1994**). However, the findings of the present study was higher than that observed (3.63µg.h.mL^{-1}) in quails (**Lashev and Mihailov, 1994)**.

Table 4.20: Plasma concentration (Mean±SE) (μg.ml^{-1}) of trimethoprim after first dose following multiple dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
0.08	0.46	0.32	0.38	0.34	0.41	0.61	0.46	0.58	0.38	0.43 ±0.033
0.16	0.63	0.52	0.56	0.57	0.54	0.72	0.65	0.81	0.58	0.62±0.031
0.25	0.92	0.74	0.71	0.73	0.61	0.87	0.98	0.99	0.71	0.81±0.045
0.5	1.04	0.98	0.88	1.01	1.05	1.03	1.11	1.23	0.98	1.03±0.032
1	1.13	1.08	1.18	1.14	1.17	1.45	1.32	1.43	1.18	1.23±0.044
2	1.26	1.33	1.36	1.1	1.01	0.98	1.01	0.76	1.11	1.10±0.063
4	0.87	0.82	0.74	0.85	0.98	0.56	0.52	0.55	0.62	0.72±0.055
8	0.5	0.51	0.32	0.65	0.52	0.24	0.31	0.35	0.33	0.41±0.044
12	0.22	0.23	0.098	0.32	0.31	0.12	0.13	0.21	0.17	0.20±0.026
24	0.09	0.1	0.05	0.04	0.15	0.07	0.04	0.05	0.07	0.07±0.011

Table 4.21: Plasma concentration (Mean±SE) (µg.ml^{-1}) of trimethoprim after last dose following multiple dose (24 mg.kg^{-1}) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
0.0	0.05	0.04	0.05	0.07	0.03	0.05	0.04	0.04	0.03	0.04± 0.004
0.08	0.32	0.41	0.54	0.62	0.56	0.45	0.52	0.56	0.42	0.49±0.031
0.16	0.65	0.71	0.76	0.87	0.68	0.66	0.71	0.82	0.67	0.73±0.025
0.25	0.92	0.89	1.06	0.99	0.83	0.87	0.88	0.9	0.89	0.91±0.023
0.5	1.01	1.1	1.12	1.14	0.99	1.16	1.08	1.03	1.19	1.09±0.023
1	1.14	1.16	1.21	1.23	1.18	1.22	1.19	1.21	1.27	1.20±0.012
2	1.25	1.32	1.28	1.29	1.31	1.28	1.29	1.29	1.3	1.29 ± 0.006
4	0.97	0.83	0.9	0.93	1	0.85	0.83	0.86	0.85	0.89±0.020
8	0.53	0.49	0.61	0.73	0.83	0.46	0.45	0.44	0.5	0.56±0.045
12	0.21	0.31	0.41	0.42	0.31	0.21	0.22	0.23	0.26	0.29±0.027
24	0.04	0.07	0.06	0.04	0.1	0.04	0.07	0.06	0.05	0.06±0.006

Table 4.23: **Pharmacokinetic parameters (Mean±SE) of trimethoprim in plasma after first dose following multiple dose (24 mg.kg^{-1} bw) i.m administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.172	0.271	0.372	0.200	0.212	0.187	0.175	0.129	0.231	0.216±0.023
$t_{1/2k10}$	h	5.324	4.800	2.953	6.395	6.265	2.719	2.918	3.072	3.546	4.22 ± 0.497
V/F	L.Kg^{-1}	2.895	2.719	2.257	3.08	3.063	2.541	2.645	2.740	2.801	2.75 ± 0.085
CL/F	L.Kg^{-1}.h^{-1}	0.376	0.392	0.529	0.333	0.338	0.647	0.628	0.618	0.547	0.49± 0.043
T_{max}	h	0.881	1.191	1.274	1.032	1.072	0.775	0.756	0.617	0.974	0.95 ± 0.071
C_{max}	µg.ml^{-1}	1.231	1.238	1.313	1.160	1.159	1.291	1.263	1.269	1.180	1.23 ± 0.018
AUC $_{0-t}$	µg.h.mL^{-1}	10.128	9.850	7.518	11.061	10.943	6.162	6.344	6.440	7.234	8.41 ± 0.685
AUC $_{0-\infty}$	µg.h.mL^{-1}	10.610	10.188	7.549	11.979	11.801	6.176	6.367	6.471	7.305	8.72 ± 0.801
AUMC	µg.h^2.mL^{-1}	84.135	74.552	36.234	113.994	110.292	25.904	28.419	29.896	39.823	60.36±11.946
MRT	h	7.929	7.317	4.799	9.515	9.345	4.193	4.463	4.620	5.450	6.40 ± 0.715
A	µg.ml^{-1}	1.427	1.559	2.027	1.340	1.351	1.690	1.608	1.523	1.527	1.56 ± 0.069
k_a	h^{-1}	4.021	2.555	1.859	3.465	3.265	3.704	3.958	5.348	2.997	3.46 ± 0.329
k_{10}	h^{-1}	0.130	0.144	0.234	0.108	0.110	0.254	0.237	0.225	0.195	0.18 ± 0.019

Table 4.22: Plasma concentration (µg.ml^{-1}) of trimethoprim after first and last dose following multiple (5) dose (24 mg.kg^{-1} bw) i.m administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (h)	Mean±S.E.	
	First dose	Last dose
0.00	-	0.04± 0.004
0.08	0.43 ±0.033	0.49±0.031
0.16	0.62±0.031	0.73±0.025
0.25	0.81±0.045	0.91±0.023
0.5	1.03±0.032	1.09±0.023
1.0	1.23±0.044	1.20±0.012
2	1.10±0.063	1.29 ± 0.006
4	0.72±0.055	0.89±0.020
8	0.41±0.044	0.56±0.045
12	0.20±0.026	0.29±0.027
24	0.07±0.011	0.06±0.006

Table 4.24: **Pharmacokinetic parameters (Mean±SE) of trimethoprim in plasma after last dose following multiple dose (24 mg.kg^{-1} bw) i.m administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.199	0.174	0.125	0.112	0.177	0.193	0.170	0.144	0.194	0.165±0.010
$t_{1/2k10}$	h	5.313	5.344	6.730	7.437	7.481	4.526	4.843	5.136	4.717	5.73±0.389
V/F	L.Kg^{-1}	2.802	2.818	2.927	2.945	2.896	2.652	2.790	2.870	2.607	2.81±0.039
CL/F	L.Kg^{-1}.h^{-1}	0.365	0.365	0.301	0.274	0.268	0.406	0.399	0.387	0.383	0.35±0.017
T_{max}	h	0.979	0.889	0.734	0.689	0.979	0.919	0.851	0.765	0.933	0.86±0.035
C_{max}	µg.ml^{-1}	1.256	1.264	1.266	1.273	1.261	1.310	1.268	1.256	1.337	1.28±0.009
AUC $_{0-t}$	µg.h/ml	10.445	10.438	12.125	12.992	13.253	9.588	9.680	9.909	10.119	10.95±0.480
AUC $_{0-\infty}$	µg.h/ml	10.941	10.942	13.267	14.572	14.905	9.849	10.015	10.326	10.439	11.70±0.665
AUMC	µg.h^2/ml	87.009	87.123	131.221	158.732	164.688	67.066	72.440	78.673	73.976	102.33±12.840
MRT	h	7.952	7.962	9.890	10.892	11.048	6.809	7.232	7.618	7.086	8.50±0.552
A	µg.ml^{-1}	1.482	1.466	1.392	1.378	1.414	1.575	1.485	1.433	1.599	1.47±0.025
k_a	h^{-1}	3.482	3.977	5.528	6.178	3.913	3.580	4.076	4.801	3.564	4.34±0.318
k_{10}	h^{-1}	0.130	0.129	0.102	0.093	0.092	0.153	0.143	0.134	0.146	0.12±0.007

Table 4.25: Pharmacokinetic parameters (Mean±S.E.) of trimethoprim in plasma following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Parameter	Units	First dose	Last dose
$t_{1/2ka}$	h	0.216 ± 0.023	0.165±0.010
$t_{1/2k10}$	h	4.22 ± 0.497	5.73±0.389
V/F	L.Kg^{-1}	2.75 ± 0.085	2.81±0.039
CL/F	L.Kg^{-1}.h^{-1}	0.49± 0.043	0.35±0.017
T_{max}	h	0.95 ± 0.071	0.86±0.035
C_{max}	µg.ml^{-1}	1.23 ± 0.018	1.28±0.009
AUC $_{0-t}$	µg.h/ml	8.41 ± 0.685	10.95±0.480
AUC $_{0-\infty}$	µg.h/ml	8.72 ± 0.801	11.70±0.665
AUMC	µg.h^2/ml	60.36 ± 11.946	102.33±12.840
MRT	h	6.40 ± 0.715	8.50±0.552
A	µg.ml^{-1}	1.56 ± 0.069	1.47±0.025
k_a	h^{-1}	3.46 ± 0.329	4.34±0.318
k_{10}	h^{-1}	0.18 ± 0.019	0.12±0.007

Table 4.26: Peak and trough plasma concentration (µg.ml^{-1}) of trimethoprim following multiple (5) dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time (days)	Mean±S.E.	
	Peak	Trough
1	1.23±0.04	0.07±0.009
2	1.32±0.05	0.05±0.01
3	1.17±0.10	0.06±0.007
4	1.14±0.03	0.03±0.001
5	1.18±0.05	0.04±0.004

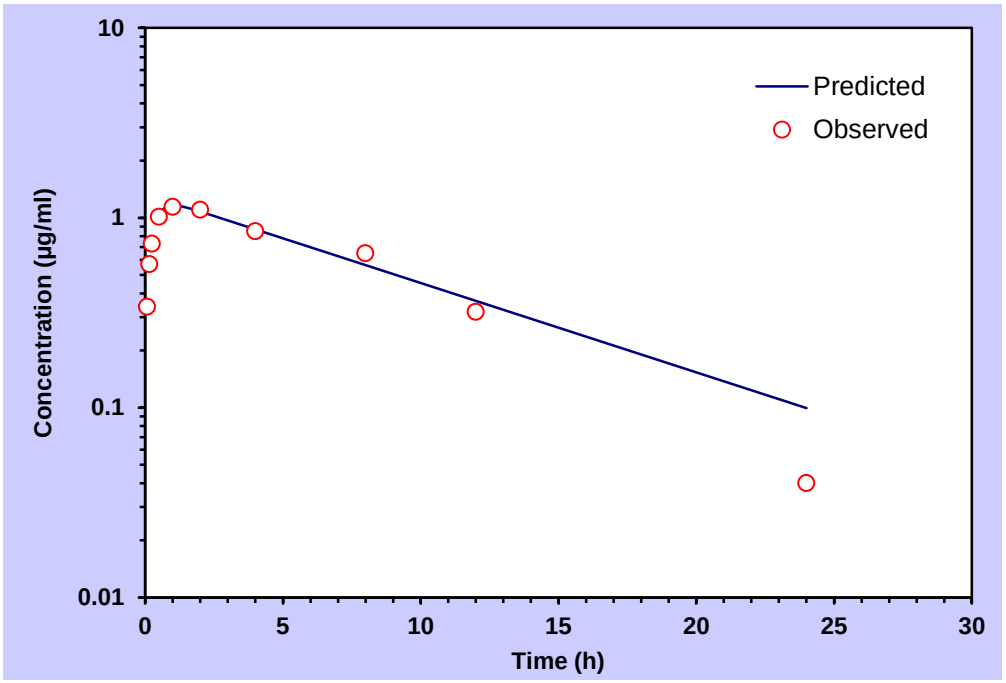


Figure 4.10: **Semi log plot of plasma concentration time profile of trimethoprim after first dose of multiple dose (24 mg.kg^{-1} bw) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry**.

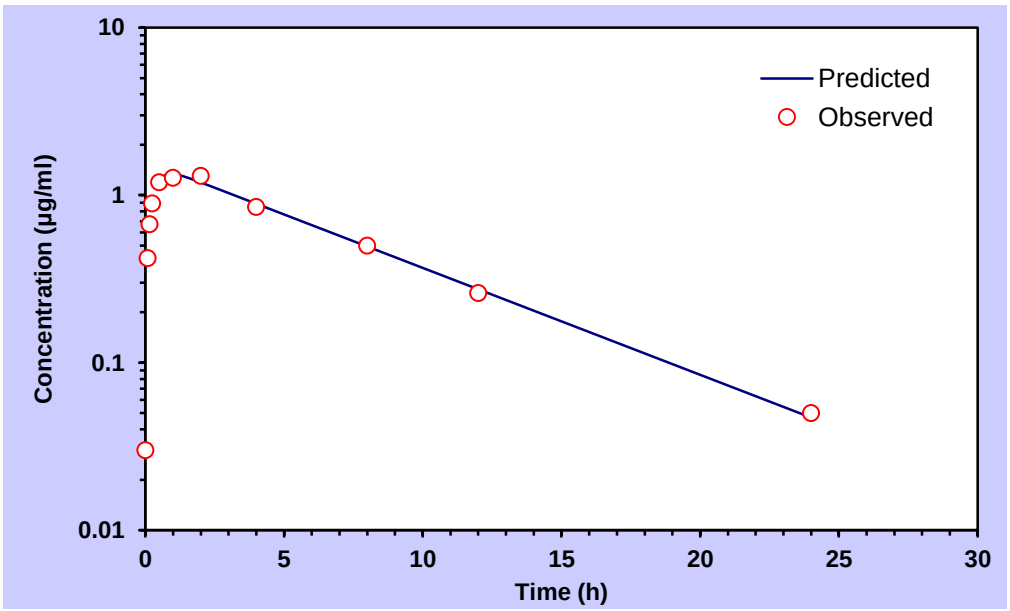


Figure 4.11: Semi log plasma plot of plasma concentration time profile of trimethoprim after last dose of multiple dose (24 mg.kg^{-1} bw) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

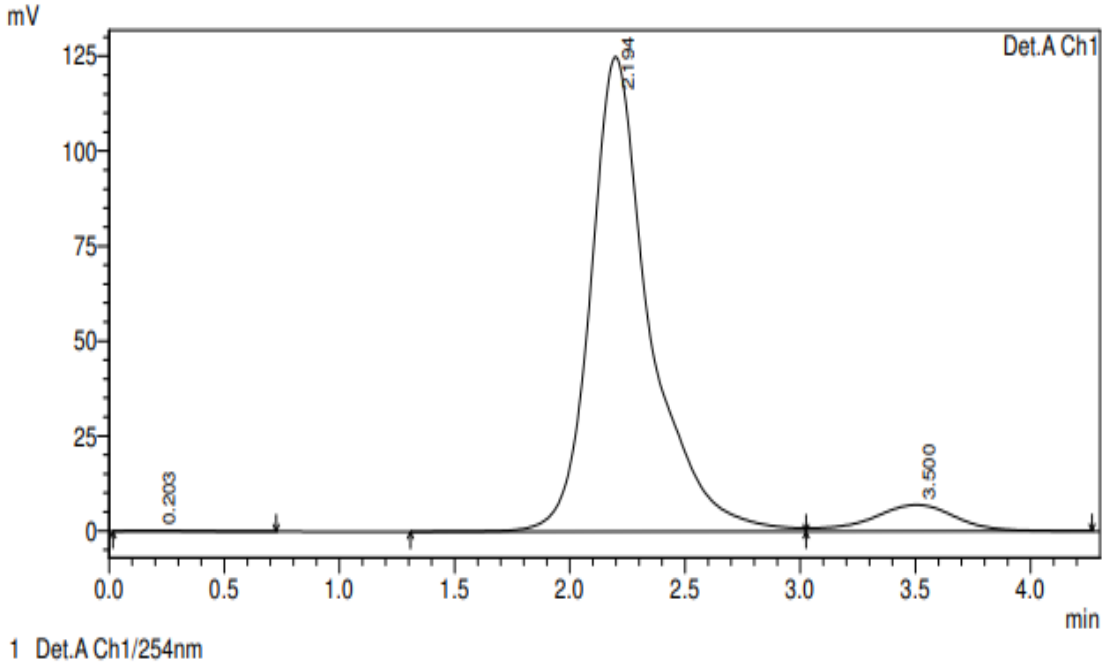


Figure 4.12: **Chromatogram of trimethoprim obtained in plasma following multiple (5) dose (24mg.kg^{-1} bw) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

The volume of distribution (V/F) following multiple dose i.m. administration after first (2.75 ± 0.085 L.kg^{-1}) and last dose (2.81±0.039 L.kg^{-1}), respectively, in the present study was similar to that observed (2.81 L.kg^{-1}) in sheep (**Batiazs *et al*., 2005)** and (2.33 ± 1.15 L.kg^{-1}) in alpacas (**Chakwenya *et al*., 2002).**

Clearance (CL/F) of trimethoprim in the present study were estimated to be 0.49 ± 0.043L.h^{-1}.kg^{-1} and 0.35±0.017 L.h^{-1} kg^{-1} after first and last dose, respectively, which was slightly lower (0.85±0.014 L.h^{-1}.kg^{-1}) than that observed in elephants after last dose **(Page *et al*., 1991)**, in broiler chicken (0.93 L.h^{-1}.kg^{-1}) **(Baert *et al*., 2003)** and also to that observed (0.720.00 L.h^{-1}.kg^{-1}) in neonatal calves (**Shoaf *et al*., 1986).** However the present study value was higher than that observed (0.05 ± 0.01 L.h^{-1}.kg^{-1}) in fishes (**Wang *et al*., 2016)**. Clearance (CL/F) of trimethoprim obtained in our study can be correlated to that obtained (0.54± 0.13 L.h^{-1}.kg^{-1}) in pigs **(Baert *et al*., 2001)**.

4.3.3 Tissue residue study of trimethoprim (µg.g^{-1}) following single dose (24 mg.kg^{-1} bw) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The tissue residue study was conducted in same 9 birds in which pharmacokinetic study was conducted. **Table 4.27** depicts the tissue residue concentration of trimethoprim in poultry following single dose i.m administration. **Figure 4.13** depicts the chromatogram obtained in tissue sample following single dose i.m administration of trimethoprim

The mean residual concentrations of trimethoprim detected at 24hrs post i.m. administration were 0.68 ±0.091, 0.52±0.043, 0.14±0.008 and 0.24±0.036 µg.g^{-1} in kidney, liver, muscles and intestine respectively. In the present study the maximum residual concentration of trimethoprim after 24h of drug administration was found in kidney tissue (0.68 ±0.091 µg.g^{-1}). Similarly, also highest residue concentration in kidney tissue in pigs have been reported **(Tzivara *et al*., 2013).**

The tissue concentration after 48hr of drug administration in kidney, liver and intestine was 0.08±0.0173, 0.02±0.005 and 0.003±0.003 µg.g^{-1} respectively, but in muscles no tissue residue could be detected after 48hrs. After 72hrs post administration of drug no residual concentration could be detected in any of the tissue. The highest concentration of trimethoprim was detected in kidney, however, it was much below MRL (0.05µg. g^{-1}).

4.3.4 Tissue residue of trimethoprim (µg.g^{-1}) following multiple (5) dose (24.0mg.kg^{-1} b.w) i.m administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The tissue residue study was conducted in same 9 birds in which multiple dose pharmacokinetic was conducted. **Table 4.28** depicts the tissue residue concentration of trimethoprim. **Figure 4.14** depicts the chromatogram obtained in tissue sample following multiple dose i.m. administration.

The mean residual concentration of trimethoprim detected at 48hrs post administration of drug were 1.19 ±0.101, 0.32 ±0.07, 0.91 ±0.134 and 0.41 ±0.055 µg.g^{-1}in kidney, intestine, liver and muscles, respectively. After 48 hr of drug administration, the maximum residual concentration of trimethoprim was found in kidney (1.19±0.101µg.g^{-1}).

Table 4.27: Residual concentration (μg.g^{-1}) of trimethoprim in various tissues after single dose (24 mg .kg^{-1} bw) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry (n = 3).

Time	Poultry no	Tissues Residual concentration (μg.g^{-1})			
		Kidney	Liver	Muscles	Intestine
Birds slaughtered after 24h following single dose drug administration	1	0.86	0.45	0.12	0.22
	2	0.65	0.52	0.14	0.31
	3	0.55	0.60	0.15	0.19
	Mean±SE	0.69±0.091	0.52±0.043	0.14±0.008	0.24±0.036
Birds slaughtered after 48h following single dose drug administration	4	0.11	0.02	-	0.01
	5	0.08	0.03	-	-
	6	0.05	0.02	-	-
	Mean±SE	0.08±0.017	0.02±0.005	-	0.003±0.003
Birds slaughtered after 72h following single dose drug administration	7	-	--	-	
	8	-	--	-	
	9	-	--	--	

Table 4.28: Residual concentration (μg.g^{-1}) of trimethoprim in various tissues after multiple (5) dose (24mg.kg^{-1} bw) i.m. administration of the SDZ/TMP combination at a total dose of 24 mg/kg (20 mg SDZ + 4 mg TMP/kg b.w) in poultry (n = 3).

Time	Poultry no	Tissues Residual concentration (μg.g^{-1})			
		Kidney	Liver	Muscles	Intestine
Birds slaughtered after 48h following multiple dose drug administration	1	1.12	0.72	0.5	0.21
	2	1.06	0.84	0.43	0.30
	3	1.39	1.17	0.31	0.45
	Mean±SE	1.19±0.101	0.91±0.134	0.41±0.055	0.32±0.07
Birds slaughtered after 72h following multiple dose drug administration	4	0.34	0.21	0.11	0.06
	5	0.25	0.12	0.08	0.05
	6	0.21	0.15	0.09	0.07
	Mean±SE	0.266±0.038	0.16±0.026	0.093±0.008	0.06±0.005
Birds slaughtered after 96h following multiple dose drug administration	7	0.04	0.03	0.02	-
	8	0.01	0.02	-	0.01
	9	0.03	0.02	--	-
	Mean±SE	0.0266±0.008	0.023±0.003	0.006±0.006	0.003±0.003

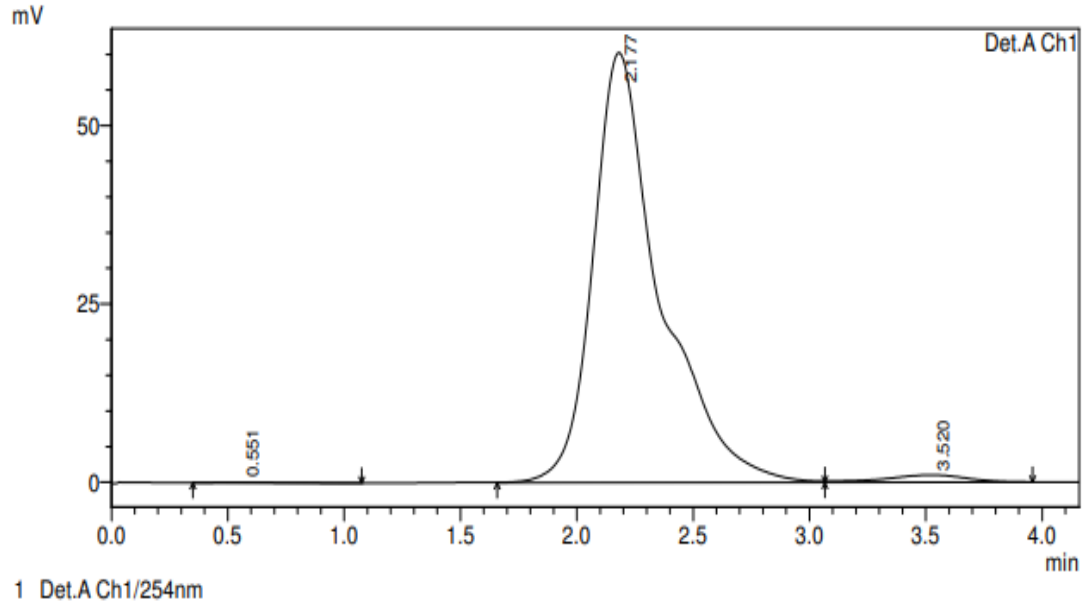


Figure 4.13: Chromatogram of trimethoprim obtained in tissue following single dose (24 mg. kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

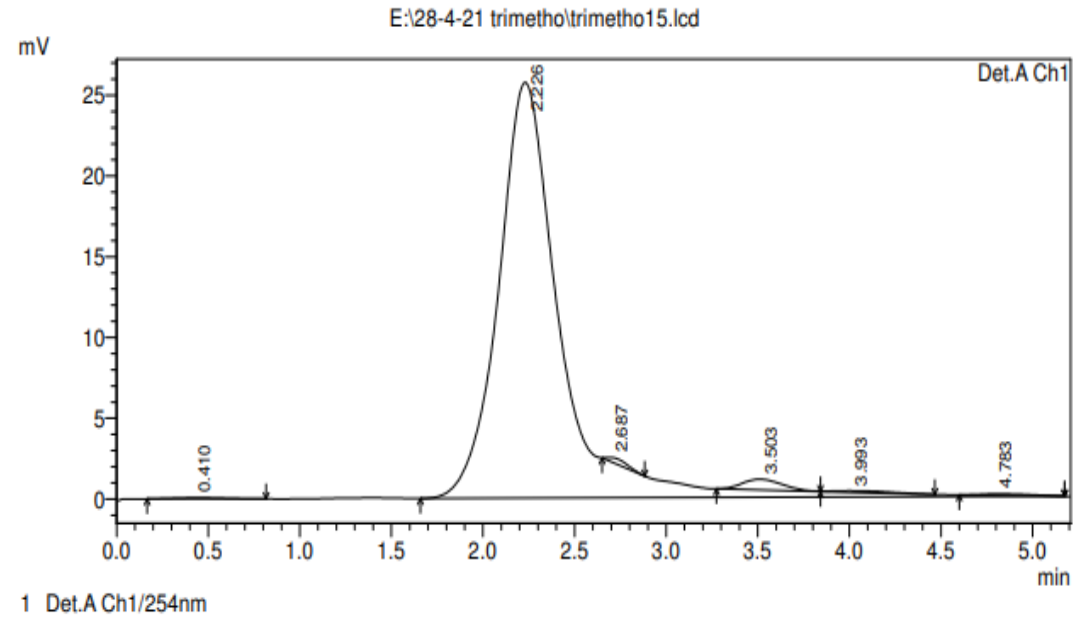


Figure 4.14: **Chromatogram of trimethoprim obtained in tissue following multiple (5) dose (24mg.kg^{-1} bw) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.**

Roncada *et al*. (2011) observed maximum residual concentration (0.249±0.039 $\mu g.g^{-1}$) in kidney tissue of broilers which were given feed containing 300 $mg.kg^{-1}$(SDZ) and 60 $mg.kg^{-1}$ (TMP). Similar findings have been reported in kidney tissue (1.29 $\mu g.g^{-1}$) of pigs **(Tzivara *et al*., 2013)**. Whereas **Garwaki et *al*. (1996)** reported maximum residual concentration (0.29 ±0.033 $\mu g.g^{-1}$) of trimethoprim in liver tissue fed with Trimethoprim/sulphadiazine (TMP/SDZ) formulation in the amount of 6 $mg.kg^{-1}$ b.w (TMP) and 30 $mg.kg^{-1}$ b.w (SDZ)**.** In the present study, the tissue concentration after 72hr of drug administration were 0.266 ± 0.038, 0.093 ±0.008, 0.16 ±0.026 and 0.06 ±0.005 $\mu g.g^{-1}$in kidney, muscle, liver and intestine, respectively. The tissue residual concentration at 96hr post administration of drug were 0.026±0.008, 0.006±0.006, 0.023 ± 0.003 and 0.003 ± 0.003 $\mu g.g^{-1}$ in kidney, muscle, liver and intestine, respectively. The results show that trimethoprim depletes rapidly in all tissues.

4.4 Dosage regimen of sulphadiazine-trimethoprim following single dose (24 $mg.kg^{-1}$ bw) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The objective of disposition kinetics is to obtain a suitable dosage regimen. Knowledge of interrelationship between pharmacodynamics and pharmacokinetics is important in choosing an appropriate drug and determining its optimal dosage regimen for treatment of various infections. The dosage regimen of sulphadiazine – trimethoprim following single dose i.m. administration is depicted in **Table 4.29.** A therapeutic concentration of 9.5 $\mu g.mL^{-1}$ and 0.5 $\mu g.mL^{-1}$ for sulphadiazine and trimethoprim, respectively (**Abu-Basha *et al*., 2008)** was considered for calculation of dosage regimen. Based on the pharmacokinetic data of SDZ/TMP following i.m. administration in poultry, the dosage regimen was calculated at dosing intervals of 6, 8 and 12 h. For sulphadiazine, a priming dose of 18.70 $mg.kg^{-1}$, 24.74 $mg.kg^{-1}$ and 43.32 $mg.kg^{-1}$ followed by the subsequent maintenance dose 10.72$mg.kg^{-1}$, 16.67 $mg.kg^{-1}$ and 35.25 $mg.kg^{-1}$ were calculated at the dosing interval of 6, 8 and 12h respectively. The minimum steady state concentration C_{ss}(min) were estimated as 16.55, 14.067 and11.62 $\mu g.ml^{-1}$ and the maximum steady state concentration C_{ss}(max) as 38.95, 43.40 and 62.76 $\mu g.ml^{-1}$ at 6,8 and 12h respectively. A priming dose (D) of 43.32 $mg.kg^{-1}$ and a maintenance dose of 35.25 $mg.kg^{-1}$ for every 12 hr were estimated to maintain the therapeutic concentration.

For trimethoprim, a priming dose of 3.64mg.kg^{-1}, 5.10 mg.kg^{-1} and 10.0 mg.kg^{-1} followed by the subsequent maintenance dose 2.3mg.kg^{-1} 3.7 mg.kg^{-1} and 8.7 mg.kg^{-1} were calculated at the dosing interval of 6, 8 and 12h respectively. The minimum steady state concentration C_{ss} (min) were estimated 0.78, 0.66 and 0.56 µg.mL^{-1} and the maximum steady state concentration C_{ss} (max) as 2.14, 2.60 and 4.36µg.mL^{-1} at 6, 8 and 12h respectively. A priming dose of 10.0mg.kg^{-1} and a maintenance dose of 8.7 mg.kg^{-1} for every 12 hr were estimated to maintain the therapeutic concentration.

Table 4.29: Dosage regimen of sulphadiazine-trimethoprim following single dose (24 mg.kg^{-1} bw) i.m. administration of SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Drug	Desired therapeutic concentration (µg.ml^{-1})	Dose interval (h)	Priming dose (mg.kg^{-1})	Maintenance dose (mg.kg^{-1})	Maximum steady state concentration, C^{ss}_{max} (µg.ml^{-1})	Minimum steady state concentration, C^{ss}_{min} (µg.ml^{-1})
Sulphadiazine	9.5	6	18.70	10.72	38.95	16.55
	9.5	8	24.74	16.67	43.40	14.067
	9.5	12	43.32	35.25	62.76	11.62
Trimethoprim	0.5	6	3.6	2.3	2.14	0.78
	0.5	8	5.10	3.7	2.60	0.66
	0.5	12	10.0	8.7	4.36	0.56

4.5 Effect on hematological and biochemical parameters of poultry following multiple (5) dose (24 mg.kg^{-1} bw) i.m. administration of sulphadiazine-trimethoprim combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

4.5.1 Haematological parameters

The hematological variables are useful in clinical diagnosis and are often used to detect physiological changes following different stress conditions. Hematological profile of poultry treated with sulphadiazine-trimethoprim, is shown in **Table 4.30**. No significant change was observed in values of PCV, hemoglobin, TEC and TLC as compared to control in group II (blood collected at 48h of drug administration) group III (blood collected at 72h of drug administration) and group IV (blood collected at

96h of drug administration). **Sampaio *et al*. (2016)** have also reported no significant changes in haematology of fish, when Sulfamethazine (SMZ) was given @ 422 $mg.kg^{-1}$ body weight, for a period of 11 days, via medicated feed. No significant changes were also observed in the study conducted by **Pach *et al*. (2021)** in mice fed with medicated diet containing 1,365 ppm of SDZ and 275 ppm of TMP, whereas **Saganuwan, 2006** reported a significant ($P < 0.05$) decrease in PCV when sulphadimidine was given @ 100 $mg.kg^{-1}$ body weight for a period of 7 days to mongrel dogs. **Saglam and Yonar (2009)** found a significant ($P<0.05$) decrease in the erythrocyte and leucocyte count in rainbow trout after sulfamerazine treatment at different dose rates of 100 $mg.kg^{-1}$, 200 $mg.kg^{-1}$ bw for 21days.

Table 4.30: Effect on hematological profile at different time intervals following multiple (5) dose (24 $mg.kg^{-1}$ bw) i.m. administration of sulphadiazine-trimethoprim combination (20 mg SDZ + 4 mg TMP / kg bw) in poultry (n=3)

Parameters	I (control)	II (48hr)	II (72hr)	III (96hr)
Hb($g.dL^{-1}$)	7.35±0.28	8.0±0.45	9.4±0.32	8.35±0.40
PCV(%)	22.05±0.83	24.6±1.06	28.2±0.95	25.05±0.93
TEC(10^6/ul)	2.95±0.26	2.84±0.04	2.23±0.30	2.91±0.24
TLC($10^{3/}$ul)	24.05±0.69	25.63±0.62	23.28±2.85	22.45±1.40

4.5.2 Biochemical parameters

Effect of multiple (5) dose ($24mg.kg^{-1}$ bw) i.m. administration of sulphadiazine-trimethoprim combination, on biochemical profile of poultry is depicted in **Table 4.31**. No significant change was observed in the estimated biochemical parameters i;e AST, ALT, Glucose, Cholesterol, Creatinine, Urea in group II, group III and group IV. These findings of present study were different from that of **Youssef *et al.* (1981)** who have reported significant increase in the blood glucose concentration in ewes, which reached its maximum level 2 h after injection and then gradually declined to return to the normal level within 24 h and serum concentrations of ketone bodies, total lipids and calcium were significantly reduced,

after intravenous administration of sulphadiazine at the therapeutic dose of 100 $mg.kg^{-1}$ body weight. Our findings can be correlated with that of **O' Fallon *et al.* (2020)** who also found no significant changes in biochemical parameters of foals given sulfadiazine–trimethoprim combination @ 24 $mg.kg^{-1}$ every 12 hr for 10 days. However, significant decrease in total bilirubin and alkaline phosphatase was observed in mongrel dogs, when given sulphadimidine given @ 100 $mg.kg^{-1}$ body weight for a period of 7 days (**Saganuwan, 2006**).

Table 4.31: Effect on biochemical profile at different time intervals following multiple (5) dose (24 $mg.kg^{-1}$ bw) i.m. administration of sulphadiazine-trimethoprim combination (20 mg SDZ + 4 mg TMP/ kg bw) in poultry (n=3)

Parameters	Group I (Control)	Group II (48hr)	Group III (72hr)	Group IV (96hr)
Total protein ($g.dL^{-1}$)	3.22±0.12	3.23 ± 0.078	3.11±0.06	3.55±0.25
Albumin ($g.dL^{-1}$)	1.96±0.04	2.01±0.060	1.97±0.05	1.98±0.07
Globulin ($g.dL^{-1}$)	1.13±0.09	1.34±0.06	1.3±0.04	1.45±0.33
Cholesterol ($mg.dL^{-1}$)	134.23±5.65	134.51±5.33	133.91±4.67	139.5±7.56
Glucose ($mg.dL^{-1}$)	220.25±18.54	217.44±13.37	202.98±10.58	211.6±13.6
Uric acid ($mg.dL^{-1}$)	3.0±0.69	3.21±0.72	3.03±0.66	2.59±0.79
Creatinine ($mg.dL^{-1}$)	0.97±0.06	1.04±0.08	1.03±0.03	0.95±0.09
AST(U/L)	145.63±5.88	142.75±5.39	142.44±6.75	145.4±7.68
ALT(U/L)	11.26±0.23	11.3±0.26	11.22±0.12	10.87±0.35

4.6 Comparison of pharmacokinetic parameters of sulphadiazine and trimethoprim after single dose (24.$mg.kg^{-1}$ b.w) i.m. administration of SDZ/TMP combination (20mg SDZ and 4mg TMP/kg b.w) in poultry.

To achieve the optimal ratio in blood, a combination of sulphadiazine/ trimethoprim (SDZ/TMP) is widely used in the dose ratio of 5:1 **(Prescott *et al.*, 2000; Spoo and Riviere, 2001; Batzias *et al.*, 2005).** In the present study, the combination of sulphadiazine-trimethoprim was administered in the same ratio of 5:1 at a dosage of 4 $mg.kg^{-1}$b.w TMP and 20 $mg.kg^{-1}$bw sulphadiazine for direct comparison of plasma concentrations**. Table 4.32** depicts the concentration ratio

(Mean) of SDZ vs TMP as a function of time after i.m. administration and **Table 4.33** represents pharmacokinetic parameters (Mean ± SE) describing the disposition of sulphadiazine and trimethoprim in poultry birds following i.m administration in a combined (20 mg SDZ + 4 mg TMP/kg b.w) preparation.

The ratio between the serum levels of SDZ and TMP observed in the present study was higher than 18. This ratio remained steady for 4 hr.

Following single i.m. dose, sulphadiazine was rapidly absorbed with a C_{max} of 22.95 ± 0.725 $\mu g.ml^{-1}$ achieved at 0.59 ± 0.088h (T_{max}). In the current study, trimethoprim concentration was measured in poultry plasma with a C_{max} of 1.28 ±0.041 $\mu g.ml^{-1}$ achieved at T_{max} 1.03 ±0.058h post injection. However, TMP could not be detected in sheep plasma following i.m administration **(Batzias *et al*., 2005)** and in calves' plasma following s.c. administration (**Shoaf *et al*., 1987).**

In the present study, following i.m. administration concentrations above 2 $\mu g.ml^{-1}$ (SDZ) and 0.1 $\mu g.ml^{-1}$ (TMP) were maintained for at least 12 h for both drugs, similar findings have been reported by **Baert *et al.* (2001)** in broiler chicken after oral administration of sulphadiazine–trimethoprim combination @ 30$mg.kg^{-1}$bw. The concentration of SDZ (2$\mu g.ml^{-1}$) and TMP (0.1$\mu g.ml^{-1}$) has been reported to be effective (**Shoaf *et al*., 1986**).

In our study, TMP concentration in plasma was not detectable after 24 h, whereas SDZ could be detected upto 48 h after i.m. administration of SDZ/TMP combination. Similar findings have been reported by **Van Duijkeren *et al.* (1994)**.

We observed a relatively slow absorption of sulphadiazine as compared to trimethoprim with maximum plasma concentrations reaching at 2h and between 0.25 and 1 h respectively, after administration of combined formulations. For both drugs, the decline in plasma concentrations after peak concentrations was less rapid as compared to the findings reported by **Baert *et al.* (2001)** in broiler chicken after i.v. administration of sulphadiazine-trimethoprim combination.

Table 4.32: Concentration (µg.ml^{-1}) ratios (mean) of SDZ vs. TMP as a function of time after single dose (24 mg.kg^{-1} b.w) i.m. administration of the SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Time(h)	Concentration (µg.ml^{-1})		
	Sulphadiazine	Trimethoprim	Ratio (SDZ/TMP)
0.08	14.54	0.44	33.045
0.16	16.11	0.6	26.85
0.25	17.22	0.78	22.076
0.5	19.56	1.03	18.99
1	20.77	1.27	16.35
2	24.72	1.19	20.77
4	15	0.82	18.29
8	6.95	0.44	15.79
12	2.96	0.21	14.09
24	0.37	0.09	4.11
48	0.06		

Table 4.33: Pharmacokinetic parameters (Mean ± SE) describing the disposition of sulphadiazine and trimethoprim after single dose (24 mg.kg^{-1} bw) i.m. administration of the SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

Parameters	Units	Mean±SE	
		Sulphadiazine	Trimethoprim
$t_{1/2ka}$	h	0.11±0.027	0.23 ±0.016
$t_{1/2k10}$	h	4.73±0.241	4.53 ±0.631
V/F	L.Kg^{-1}	0.85±0.044	2.64 ±0.137
CL/F	L.Kg^{-1}.h^{-1}	0.12±0.007	0.43 ±0.050
T_{max}	h	0.59±0.088	1.03 ±0.058
C_{max}	µg.ml^{-1}	22.95±0.725	1.28 ±0.041
AUC $_{0-t}$	µg.h/ml	170.91±6.996	9.33 ±0.804
AUC $_{0-\infty}$	µg.h/ml	171.12±7.026	9.71 ±0.944
AUMC	µg.h^2/ml	1208.89±89.218	70.98 ±14.831
MRT	h	7.00±0.315	6.88 ±0.905
A	µg.ml^{-1}	26.20±1.498	1.62 ±0.092
k_a	h^{-1}	8.02±1.338	3.04 ±0.208
k_{10}	h^{-1}	0.14±0.009	0.17 ±0.024

In the present study, a shorter elimination half life of TMP as compared to SDZ following single dose i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP / kg b.w) was observed which indicates the tendency of poultry to eliminate trimethoprim more rapidly than sulphadiazine. Similarly, lower elimination half life for trimethoprim than sulphadiazine also reported in broiler chicken (1.0 and 2.7 h) after i.v. administration of TMP/SDZ combination **(Loscher *et al*., 1990)** and in mandarin fish after oral administration of TMP/SDZ combination @ 120 $mg.kg^{-1}$ in the same ratio of 5:1 as used in the present study **(Wang *et al*., 2015).**

In the present study, the volume of distribution for TMP (2.64 ±0.137 $L.kg^{-1}$) was higher than SDZ (0.85 ± 0.055 $L.kg^{-1}$). Similar findings have been reported (0.43 and 2.21 $L.kg^{-1}$) in chicken **(Baert *et al*., 2003);** (0.96 and 3.3 $L.kg^{-1}$) **(Loscher *et al*., 1990)** and in pigs (0.55 and 2.02 $L.kg^{-1}$) **(Baert *et al*., 2001).**

The small volume observed for sulphadiazine was due to the chemical properties of the sulphadiazine molecule (hydrophilic, a weak acid, partly ionized under plasma pH conditions). Trimethoprim is a lipid-soluble weak organic base that is widely distributed and therefore, the concentration of trimethoprim in plasma is expected to be lower than those in tissues **(Prescott *et al*., 2000; Baggot, 2001).**

Clearance observed in the present study for SDZ (0.12±0.007 $L.Kg^{-1}.h^{-1}$) was lower than as compared to TMP (0.43 ±0.050 $L.Kg^{-1}.h^{-1}$). Similar findings have been observed by others, in broiler chicken (0.09 and 0.93 $L.Kg^{-1}.h^{-1}$) after oral administration of SDZ/TMP combination at a total dose of 33.33$mg.kg^{-1}$ SDZ and 6.67$mg.kg^{-1}$ TMP (**Baert *et al*., 2003),** in sheep (0.12 and 3.31$L.Kg^{-1}.h^{-1}$) after i.v. administration of SDZ/TMP combination given @ 30$mg.kg^{-1}$b.w**. (Batiazs *et al*., 2005).**

4.7 Pharmacokinetics of amprolium

4.7.1 Pharmacokinetics of amprolium following single dose (30$mg.kg^{-1}$) oral administration in poultry.

The plasma concentration-time profile following single dose (30.0$mg.kg^{-1}$) oral administration of amprolium in poultry is depicted in **Table 4.34**. **Figure 4.15,** represents the semi log plasma concentration-time profile of amprolium. The chromatogram of amprolium obtained in plasma following single dose oral administration is depicted in **Figure 4.16**.

Table 4.34: Plasma concentration (ug.ml^{-1}) of amprolium after single dose (30mg.kg^{-1}) oral

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ± SE
0.25	0.16	0.23	0.34	0.19	0.33	0.29	0.18	0.24	0.25	0.25±0.030
0.5	0.34	0.32	0.65	0.44	0.52	0.48	0.35	0.38	0.56	0.45±0.049
1	0.75	0.56	1.23	0.71	0.98	0.73	0.65	0.70	0.91	0.80±0.090
1.5	0.44	0.28	0.73	1.32	0.46	0.51	1.01	1.1	0.58	0.71±0.150
3	0.15	0.18	0.22	0.25	0.25	0.19	0.30	0.26	0.17	0.20±0.016
5	0.11	0.14	0.19	0.12	0.16	0.15	0.14	0.12	0.13	0.14±0.011
7	0.07	0.12	0.11	0.09	0.08	0.06	0.07	0.05	0.09	0.08±0.009
13	0.02	0.08	0.06	0.04	0.05	0.035	0.042	0.021	0.05	0.047±0.008

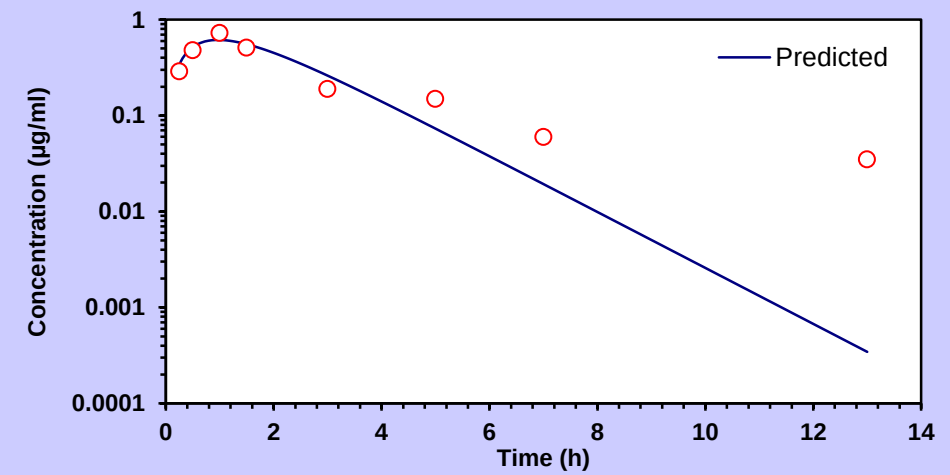


Figure 4.15: Semi logarithmic plot showing the plasma concentration –time profile of amprolium after single dose (30mg.kg^{-1}) oral administration in poultry.

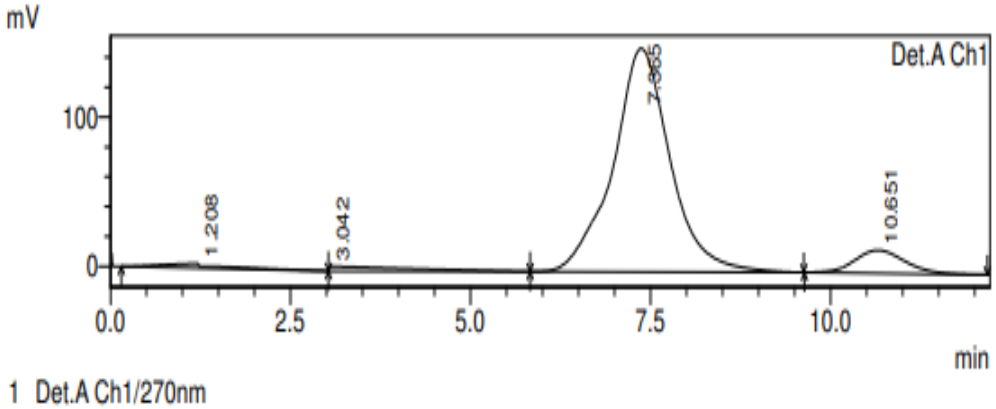


Figure 4.16: Chromatogram of amprolium obtained in plasma following single dose (30mg.kg^{-1}) oral administration.

The mean plasma concentration of amprolium in the present study ranged from 0.047±0.08 to 0.80±0.09 μg.ml^{-1}. The pharmacokinetic parameters describing the disposition kinetics of amprolium following single dose (30.0mg.kg^{-1}) oral administration are presented in **Table 4.35**. A one-compartment model adequately (R^2=0.96) described plasma concentration-time profile of amprolium in poultry following single dose oral administration.

The rate constant of absorption phase (k_a) was observed to be 1.25±0.207h^{-1} in the present study, which was higher than that reported (0.181±0.076 h^{-1}) (**Hamamoto *et al.*, 2000);** and 0.522 h^{-1} (**Alam *et al.*, 1987)** in chicken.

Absorption half-life ($t_{1/2ka}$) of 0.65±0.074h was observed in the present study, was lower than (5.328 ± 3.989h) that observed by **Alam *et al.* (1987)** in white leg horns. However **El-Sayed *et al.* (1995)** have reported a lower **(**0.17±0.09 h) value, in hubbard broiler chicken when amprolium was given orally @ 30mg.kg^{-1} b.w than our study.

A C_{max} (maximum plasma concentration) value of 0.69±0.052 μg.mL^{-1} observed in the present study was higher than that (0.224± 0.158 μg.ml^{-1}) obtained by **Hamamoto *et al.* (2000)** in white-leghorn hens when amprolium was given orally @ 13mg.kg^{-1} b.w in fasting conditions. However, the value of C_{max} obtained in our study was much lower than that (42.9 ±1.11 ug.mL^{-1}) reported in in hubbard broiler chicken when amprolium was given orally @ 30mg.kg^{-1} b.w (**El-Sayed *et al.*, 1995)**.

T_{max} observed in the present study was 1.079±0.055h, which is lower than (3.67 ±0.05h) that reported by **El-Sayed *et al.* (1995)** in chicken. The value of A (zero time blood drug concentration intercept of distribution phase) and MRT observed in the present study was 32.973±0.179 μg.mL^{-1} and 2.416±8.750h, respectively, following single oral dose administration of amprolium.

In the present study, the elimination rate constant (k_{10}) was 0.76±0.071h^{-1}, was higher than that reported in white leg horn birds (0.2517), when amprolium was given orally @20mg.kg^{-1} b.w (**Alam *et al.*, 1987)**. However, a higher value of elimination rate constant has been reported in chicken, when amprolium was given orally @ 26mg.kg^{-1} b.w **(Hamamoto *et al.*, 2000).**

Table 4.35: Pharmacokinetic parameters of amprolium in plasma following single dose (30mg.kg^{-1}) oral administration in poultry.

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.713	0.255	0.663	0.871	0.435	0.461	0.859	0.916	0.652	0.65±0.074
$t_{1/2k10}$	h	0.750	2.279	0.702	0.901	1.050	1.032	0.890	0.947	0.688	1.03±0.162
V/F	mL.kg^{-1}	21.169	56.237	12.359	13.287	22.679	25.482	14.682	15.744	15.834	21.941±4.541
CL/F	mL.kg^{-1} .h^{-1}	19.558	17.096	12.192	10.217	14.963	17.111	11.427	11.515	15.930	14.45±1.076
T_{max}	h	1.055	0.909	0.984	1.278	0.944	0.969	1.261	1.344	0.967	1.079±0.055
C_{max}	ug.ml^{-1}	0.534	0.404	0.919	0.844	0.709	0.613	0.765	0.712	0.715	0.691±0.052
AUC $_{0-t}$	µg.h.ml^{-1}	1.533	1.716	2.460	2.934	2.004	1.752	2.624	2.603	1.883	2.168±0.164
AUC $_{0-\infty}$	µg.h.ml^{-1}	1.533	1.754	2.460	2.936	2.004	1.753	2.625	2.605	1.883	2.172±0.163
AUMC	µg.h^2 .ml^{-1}	3.239	6.419	4.847	7.508	4.297	3.778	6.626	7.008	3.644	5.263±0.543
MRT	ug.ml^{-1}	0.600	29.117	2.258	2.129	67.073	43.071	58.170	58.421	35.912	32.973±0.179
A	h	3.658	2.112	2.143	2.155	2.557	1.970	2.524	2.690	1.935	2.416±8.750
k_a	h^{-1}	0.971	2.708	1.045	0.795	1.592	1.501	0.806	0.756	1.062	1.25±0.207
k_{10}	h^{-1}	0.923	0.304	0.986	0.768	0.659	0.671	0.778	0.731	1.006	0.76±0.071

The elimination half-life ($t_{1/2k10}$) in the present study was 1.03±0.162h, which was lower than (2.75 h) obtained by **Alam *et al*. (1987)** but higher than that (0.292h) obtained by **Hamamoto *et al*. (2000).**

The mean area under curve (AUC) calculated in our study was 2.168±0.164 $h.\mu g.mL^{-1}$ which was much lower than that obtained (167.06 $h.\mu g.mL^{-1}$) in white leg horns after oral administration of amprolium @ 20$mg.kg^{-1}$ b.w (**Alam *et al*., 1987).**

The volume of distribution (V/F) of amprolium in the present study was estimated to be 21.94±4.541 $L.kg^{-1}$ which was similar to that reported in chicken (24.70± 14.20 $L.kg^{-1}$) (**Hamamoto *et al*., 2000)**.

Clearance of drug (CL/F) observed in the present study was 14.45±1.076 $L.h^{-1}.kg^{-1}$ which is higher than that reported (1.32±0.206$L.h^{-1}.kg^{-1}$) in chicken after i.v administration of amprolium @ 13$mg.kg^{-1}$ b.w (**Hamamoto *et al*., 2000)**; 0.198 $L.h^{-1}.kg^{-1}$ after oral administration of amprolium @ 20$mg.kg^{-1}$ b.w (**Alam *et al*., 1987)** and 0.033 $L.h^{-1}.kg^{-1}$ reported when amprolium was given orally @ 30$mg.kg^{-1}$ b.w (**El-Sayed *et al*., 1995).**

The differences in pharmacokinetic parameters between the results obtained in the present study and those reported in other studies were mainly due to the differences in plasma amprolium concentration and also the technique employed for analysis. In the present study, HPLC was used for analysis, while in other studies spectrophotometry was used with no chromato-graphic separation and a nonspecific thiochrome method (**Alam *et al*., 1987; El-Sayed *et al*., 1995**). Some studies reported that a separation process by chromatographic purification was needed to measure only amprolium concentrations without impurities in feed by spectrophotometrical method (**Davis, 1968; Severijnen *et al*., 1975; Kentzer *et al*., 1988**). In analytical methods of vitamin B1 (VB1), concentrations of VB1 in plasma analysed by a nonspecific spectrophotometric method after chromatographic separation, were approximately 4 times higher than concentrations of VB1 measured by HPLC (**Weber and Kewitz, 1985**). Therefore plasma concentrations measured by a spectrophotometric method tend to be higher than those determined by HPLC. Another possible cause of variation in the result of the present study and that reported by other workers could be due to difference in the breed and sex of chicken (**Alam *et al*., 1987; El-Sayed *et al*., 1995**).

4.7.2 Pharmacokinetics of amprolium following multiple (5) dose (30.0mg.kg^{-1}) oral administration in poultry.

The plasma concentrations of amprolium after first and last dose are depicted in **Table 4.36** and **Table 4.37,** respectively**,** and their means are depicted in **Table 4.38.** The semi log plasma concentration time curve for amprolium after first and last dose following multiple (5) dose oral administration in poultry is depicted in **Figure. 4.17** and **Figure 4.18**, respectively.

Pharmacokinetic values describing the disposition kinetics of amprolium after first and last dose following multiple (5) dose (30.0mg.kg^{-1}) oral administration in poultry are presented in **Table 4.39** and **Table 4.40,** respectively, and their means are depicted in **Table 4.41.** The peak and trough plasma concentration of amprolium with their means are depicted in **Table 4.42**.

A one compartment model with first order rate of absorption adequately (r=0.96) described plasma concentration-time profile of amprolium in poultry following multiple (5) dose (30.0mg.kg^{-1}) oral administration. The chromatogram of amprolium obtained in plasma following multiple dose oral administration is depicted in **Figure 4.19.**

The peak plasma concentration of amprolium was observed to be 0.94±0.083 and 1.01±0.04µg.mL^{-1} at 1hr after the first and last dose, respectively. Thereafter, the plasma drug concentration decreased slowly to a minimum of 0.039±0.003 after first dose and 0.021±0.004 µg.ml^{-1} last dose, respectively.

The rate constant of absorption phase (k_a) calculated in our study were 0.87±0.059h^{-1} and 1.001±0.095h^{-1} which is higher than 0.416±0.314h^{-1} observed in chicken when amprolium was give orally at a dose rate of 13mg.kg^{-1} b.w under fasting conditions (**Hamamoto *et al*., 2000).**

In the present study absorption half-life ($t_{1/2ka}$) of 0.68±0.039 h and 0.73± 0.052h were observed after first and last dose, respectively. However a higher value of absorption half-life (5.328±3.98h) has been reported in chicken after oral administration of amprolium at a dose rate of 26mg.kg^{-1} b.w under non-fasting conditions **(Hamamoto *et al*., 2000)**.

Table 4.36: Plasma concentration (µg.mL^{-1}) of amprolium after first dose following multiple dose (30mg.kg^{-1}) oral administration in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ±SE
0.25	0.15	0.25	0.45	0.16	0.32	0.28	0.19	0.26	0.32	0.26±0.031
0.5	0.33	0.72	0.64	0.53	0.47	0.35	0.45	0.61	0.52	0.51±0.043
1	0.72	1.5	0.98	0.75	0.81	1.01	0.72	0.87	1.11	0.94±0.083
1.5	0.58	1.64	0.44	0.46	1.23	0.73	0.51	0.47	0.76	0.75±0.13
3	0.25	0.15	0.16	0.16	0.3	0.25	0.19	0.21	0.33	0.22±0.021
5	0.18	0.11	0.12	0.12	0.18	0.16	0.13	0.14	0.21	0.15±0.011
7	0.12	0.08	0.09	0.07	0.11	0.06	0.05	0.095	0.11	0.08±0.008
13	0.06	0.035	0.042	0.027	0.03	0.041	0.027	0.048	0.043	0.039±0.003

Table 4.37: Plasma concentration (µg.mL^{-1}) of amprolium after last dose following multiple dose (30mg.kg^{-1}) oral administration in poultry.

Time (h)	I	II	III	IV	V	VI	VII	VIII	IX	Mean ±SE
0.0	0.04	0.01	0.02	0.03	0.02	0.01	0.02	0.03	0.02	0.02 ±0.0
0.25	0.14	0.25	0.35	0.23	0.22	0.25	0.15	0.25	0.31	0.28±0.04
0.5	0.23	0.67	0.62	0.55	0.45	0.33	0.43	0.63	0.52	0.55±0.05
1	0.62	1.43	0.89	0.82	0.82	1.12	0.73	0.86	1.12	1.01±0.04
1.5	0.54	1.67	0.43	1.11	1.24	0.73	0.98	0.45	0.77	0.98±0.07
3	0.26	0.24	0.2	0.31	0.29	0.26	0.19	0.2	0.34	0.25±0.03
5	0.17	0.15	0.12	0.16	0.17	0.15	0.12	0.13	0.22	0.13±0.01
7	0.11	0.09	0.07	0.06	0.09	0.07	0.08	0.09	0.11	0.05±0.002
13	0.05	0.035	0.034	0.027	0.03	0.041	0.01	0.03	0.043	0.021±0.004

Table 4.38: Plasma concentration (µg.ml^{-1}) of amprolium (Mean±S.E.) after first and last dose following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.

Time (h)	Mean±S.E.	
	First dose	Last dose
0.00	-	0.02 ±0.0
0.25	0.26±0.031	0.28±0.04
0.5	0.51±0.043	0.55±0.05
1	0.94±0.083	1.01±0.04
1.5	0.75±0.13	0.98±0.07
3	0.22±0.021	0.25±0.03
5	0.15±0.011	0.13±0.01
7	0.08±0.008	0.05±0.002
13	0.039±0.003	0.021±0.004

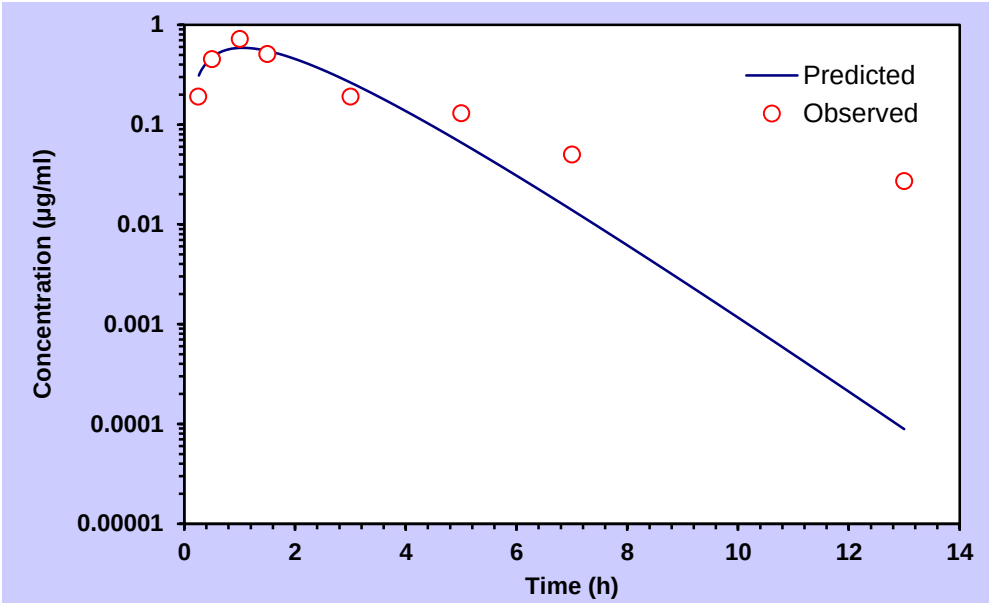


Figure 4.17: **Semi logarithmic plot of plasma concentration –time profile of amprolium after first dose following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.**

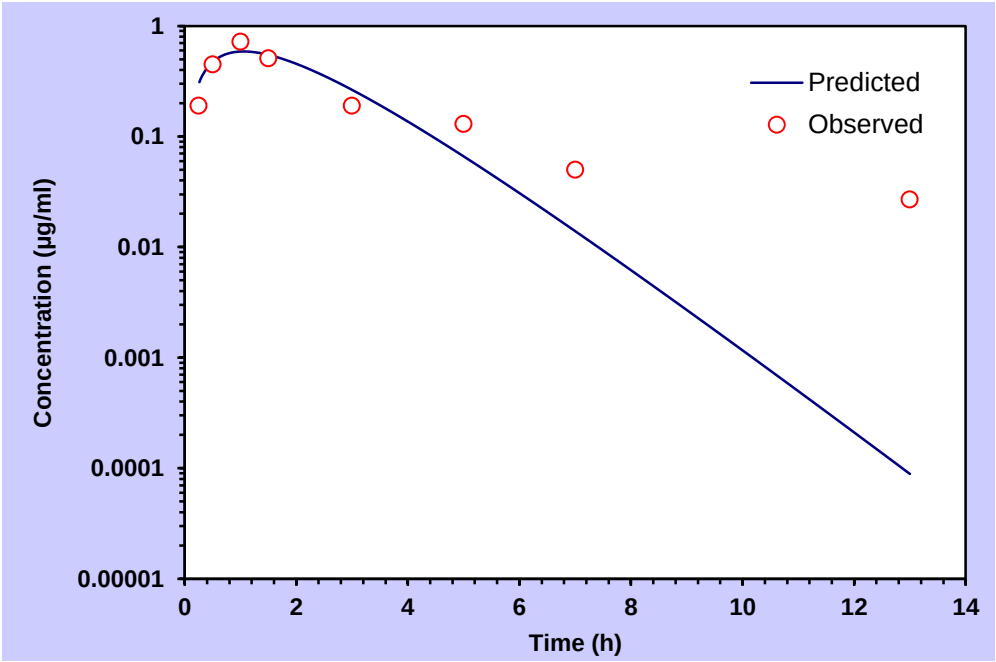


Figure 4.18: **Semilograthmic plot of plasma concentration –time profile of amprolium after last dose following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.**

Table 4.39: Pharmacokinetic parameters of amprolium in plasma after first dose following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.526	0.743	0.645	0.651	0.862	0.781	0.706	0.459	0.750	0.680±0.039
$t_{1/2k10}$	h	0.559	0.769	1.274	0.688	0.891	0.818	0.755	0.906	0.840	0.833±0.061
V/F	L.Kg^{-1}	14.582	8.804	25.904	18.945	12.627	15.060	19.409	21.657	13.591	16.731±1.638
CL/F	L.Kg^{-1}.h^{-1}	18.061	7.925	14.091	19.069	9.820	12.761	17.811	16.563	11.207	14.145±1.251
T_{max}	h	0.782	1.091	1.283	0.966	1.265	1.153	1.053	0.913	1.145	1.072±0.051
C_{max}	µg.ml^{-1}	0.780	1.275	0.576	0.598	0.888	0.749	0.587	0.688	0.858	0.778±0.068
AUC $_{0-t}$	µg.h/ml	1.660	3.784	2.125	1.573	3.053	2.350	1.684	1.811	2.676	2.302±0.235
AUC $_{0-\infty}$	µg.h/ml	1.660	3.785	2.128	1.573	3.054	2.350	1.684	1.811	2.676	2.202±0.257
AUMC	µg.h^2/ml	2.602	8.264	5.895	3.042	7.729	5.425	3.553	3.570	6.144	5.136±0.647
MRT	h	1.566	2.183	2.769	1.933	2.530	2.308	2.109	1.971	2.295	36.92 ± 10.04
A	µg.ml^{-1}	34.549	98.925	2.346	29.575	74.396	45.081	24.079	2.8125	20.577	2.185±0.110
k_a	h^{-1}	1.317	0.932	1.073	1.063	0.803	0.886	0.980	1.507	0.923	1.054±0.070
k_{10}	h^{-1}	1.238	0.900	0.543	1.006	0.777	0.847	0.917	0.764	0.824	0.87±0.059

Table 4.40: Pharmacokinetic parameters of amprolium in plasma after last dose following multiple dose (30mg.kg^{-1}) oral administration in poultry.

Parameters	Units	I	II	III	IV	V	VI	VII	VIII	IX	Mean±SE
$t_{1/2ka}$	h	0.760	0.786	0.438	0.834	0.859	0.784	0.877	0.490	0.752	0.731±0.052
$t_{1/2k10}$	h	1.288	0.812	0.784	0.873	0.896	0.823	0.904	0.796	0.862	0.893±0.051
V/F	L.Kg^{-1}	27.580	8.890	20.164	13.132	12.680	14.553	16.514	20.094	13.647	16.361±1.843
CL/F	L.Kg^{-1}.h^{-1}	14.831	7.586	17.816	10.424	9.809	12.248	12.662	17.483	10.961	12.647±1.157
T_{max}	h	1.411	1.153	0.834	1.231	1.265	1.159	1.284	0.893	1.1614	1.155±0.061
C_{max}	µg.ml^{-1}	0.509	1.261	0.711	0.859	0.888	0.776	0.678	0.686	0.864	0.804±0.069
AUC $_{0-t}$	µg.h/ml	2.018	3.953	1.683	2.877	3.057	2.448	2.368	1.715	2.736	2.539±0.239
AUC $_{0-\infty}$	µg.h/ml	2.022	3.954	1.683	2.877	3.058	2.449	2.369	1.715	2.736	2.540±0.239
AUMC	µg.h^2/ml	5.980	9.120	2.972	7.090	7.745	5.681	6.089	3.186	6.377	6.027±0.659
MRT	h	2.956	2.306	1.765	2.463	2.532	2.319	2.570	1.857	2.330	38.591±11.684
A	µg.ml^{-1}	2.652	105.922	3.377	51.473	57.942	43.147	61.798	3.886	17.118	2.344±0.120
k_a	h^{-1}	0.911	0.881	1.579	0.830	0.806	0.883	0.789	1.412	0.921	2.344±0.120
k_{10}	h^{-1}	0.537	0.853	0.883	0.793	0.773	0.841	0.766	0.870	0.803	1.001±0.095

Table 4.41: Pharmacokinetic parameters of amprolium in plasma following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.

Parameters	Units	Mean±Se	
		First dose	Last dose
$t_{1/2ka}$	h	0.680±0.039	0.731± 0.052
$t_{1/2k10}$	h	0.833±0.061	0.893±0.051
V/F	L.Kg^{-1}	16.731±1.638	16.361±1.843
CL/F	L.Kg^{-1}.h^{-1}	14.145±1.251	12.647±1.157
T_{max}	h	1.072±0.051	1.155±0.061
C_{max}	µg.ml^{-1}	0.780±0.068	0.804±0.069
AUC $_{0-t}$	µg.h/ml	2.302±0.235	2.539±0.239
AUC $_{0-\infty}$	µg.h/ml	2.202±0.257	2.540±0.239
AUMC	µg.h^2/ml	5.136±0.647	6.027±0.659
A	h	36.92 ± 10.04	38.591 ±11.684
MRT	µg.ml^{-1}	2.185±0.110	2.344±0.120
k_{10}	h^{-1}	1.05±0.070	2.344±0.120
k_a	h^{-1}	0.87±0.059	1.001±0.095

Table 4.42. Peak and trough plasma concentration (µg.ml^{-1}) of amprolium following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.

Time (days)	Peak	Trough
1	0.99±0.04	0.032±0.0
2	1.11±0.04	0.04±0.01
3	1.22±0.03	0.05±0.01
4	1.25±0.05	0.023±0.01
5	1.01±0.03	0.030±0.01

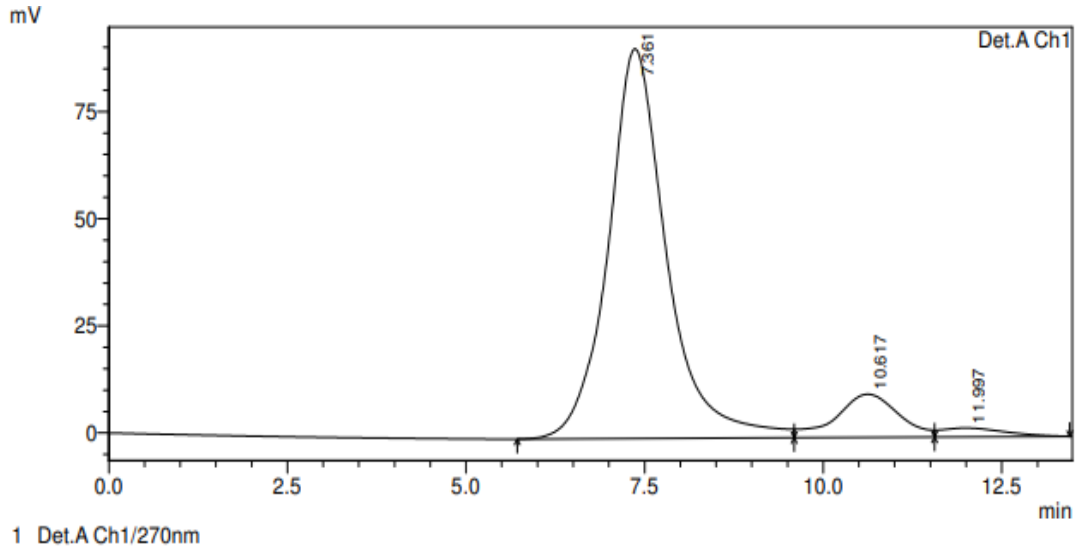


Figure 4.19: **Chromatogram of amprolium obtained in plasma following multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.**

In the present study, the values of C_{max} were 0.78±0.068 µg.ml^{-1} and 0.804±0.069 after first dose and last dose, respectively, which is slightly higher than that reported (0.224± 0.158 µg.mL^{-1}) in white-leghorn hens when amprolium was given orally at a dose of 13mg.kg^{-1} b.w in fasting conditions **(Hamamoto *et al.*, 2000)**.

The value of C_{max} obtained in our study is much lower than that reported (42.9 ±1.11ug.mL^{-1}) by **El-Sayed *et al.* (1995).** T_{max} obtained in the present study was 1.072±0.051 h and 1.155±0.061h after the last dose, respectively. Whereas other workers have reported a higher value (3.67 ±0.05h) of T_{max} in achieving peak plasma concentration in white leg horns (**El-Sayed *et al.*, 1995).**

The elimination rate constant (k_{10}) after first and last dose were 1.05±0.070h^{-1} and 2.34±0.120h^{-1}, which was similar to that (2.887±1.26 h^{-1}) obtained in chickens when amprolium was given orally @ 26mg.kg^{-1} b.w in non-fasting condition **(Hamamoto *et al.*, 2000)**. However, elimination rate constant observed in the present study was higher than (0.2517 h^{-1}) that reported in white leg horns given amprolium orally at a dose rate of 20mg/kg b.w (**Alam *et al.*, 1987)**

Elimination half-life ($t_{1/2k10}$) observed in the present study were found to be 0.833±0.061h and 0.893±0.051h, respectively, after first and last dose following

multiple dose (30mg.kg^{-1} b.w) oral administration of amprolium, which was lower than that reported (4.89 ±0.3 h) in chicken (**El-Sayed *et al*., 1995).**

The mean area under curve (AUC) after first and last dose were 2.302±0.235 and 2.539±0.239 μg.h.mL^{-1} respectively. This value is slightly higher than observed in chicken (0.648 ±0.537μg.h.mL^{-1}) when amprolium was give orally @13mg.kg^{-1} b.w under fasting conditions(**Hamamoto *et al*., 2000).** However, **Alam *et al*. (1987)** have reported a higher (167.06 h.μg.mL^{-1}) value in white leg horns.

The volume of distribution (V/F), in the present study was estimated to be 16.731±1.638 L.kg^{-1} and 16.361±1.843 L.kg^{-1} after first and last dose, respectively following multiple dose oral administration of amprolium which was higher than reported (12.03±9.73L.kg^{-1}) in chicken following oral administration of amprolium @ 13mg.kg^{-1} b.w (**Hamamoto *et al*., 2000)**

In the present study, the clearance (CL/F) of amprolium were estimated to be 14.145±1.251 L.h^{-1}.kg^{-1}, after first and 12.647±1.157 L.h^{-1}.kg^{-1} the last dose, respectively. The body clearance reported by other workers in chicken is lower (1.32±0.206 L.h^{-1}.kg^{-1}) than present study values (**Hamamoto *et al*., 2000);** 0.198 L.h^{-1}.kg^{-1} (**Alam *et al*., 1987);** 0.033 L.h^{-1}.kg^{-1} (**El-Sayed *et al*., 1995).**

4.7.3 Tissue residue study of amprolium (μg.g^{-1}) following single dose (30.0mg.kg^{-1}) oral administration in poultry.

The tissue residue study was conducted in 9 birds. **Table 4.43** depicts the tissue residue concentration of amprolium following single dose oral administration (30mg.kg^{-1} b.w). **Figure 4.20** depicts the chromatogram obtained in tissue sample following single dose administration of amprolium in poultry. The mean residual concentration of amprolium detected at 24hrs post administration of drug were 0.53±0.01, 0.29±0.01 and 0.155±0.035 in liver, kidney and muscles, respectively. The maximum residual concentration of amprolium was found in liver (0.53±0.01μg.g^{-1}). A higher residual concentration (1.41 μg.g^{-1}) in liver of chicken has been observed following oral administration of amprolium @80mg.kg^{-1} body weight (**Takahashi *et al*., 1994).** In the present study, the tissue concentration declined after 48hr of drug administration in liver, kidney and muscle 0.075±0.015, 0.045±0.005, 0.025±0.015

µg.g^{-1}, respectively. No residual concentration was found 72hrs post administration of drug. Food and Drug Administration have established a maximum residue limits (MRL) of amprolium in chicken muscle (0.5 µg.g^{-1}) (**Kim *et al.*, 2012).** The residual concentration found in our study was below the MRL.

4.7.4 Tissue residue study of amprolium (µg.g^{-1}) following multiple (5) dose (30.0mg.kg^{-1}) oral administration in poultry.

The tissue residue study was conducted in 9 birds. **Table 4.44** depicts the tissue residue concentration of amprolium following multiple (5) dose (30mg.kg^{-1} b.w). oral administration. **Figure 4.21** depicts the chromatogram obtained in tissue sample following multiple oral dose administration of amprolium. The mean residual concentration of amprolium detected at 48hrs post administration of drug were 0.62±0.04, 0.26±0.02 and 0.16±0.02 µg.g^{-1} in liver, kidney and muscles, respectively. After 48 hr of drug administration, the maximum residual concentration (0.62±0.04 µg.g^{-1}) of amprolium was found in liver. **Petz *et al*. (1980),** have also reported a higher value (0.8 µg.g^{-1}) of amprolium in liver when amprolium was given in a ration @ 0.0125 %.

In the present study, the tissue residual concentration after 72hr observed in liver (0.15±0.008 µg.g^{-1}), kidney (0.08±0.008 µg.g^{-1}) and muscle (0.05±0.005 µg.g^{-1}), were below the LOQs observed in our study which were 0.22 and 0.25 µg.g^{-1} for muscles and liver, respectively, and were below the tolerance level (0.5ug.g^{-1} for muscles and 0.3 ug.g^{-1} for liver). Whereas, **Hormazabal and Yndestad (1996)** found higher value (5ng.g^{-1}) of LOQ in chicken meat. After 96hrs of administration of drug, the tissue residual concentration in liver, kidney and muscles were 0.03±0.005, 0.03±0.005 and 0.01±0.003 µg.g^{-1} respectively. Comparison of tissue residue concentration following single dose and multiple dose administration shows accumulation of drug in the tissue following multiple dose administration. The tissue concentration following single dose administration depleted after 72hr of administration of drug, whereas following multiple dose administration the drug concentration depletion after 96hr of administration. Consumer safety needs to be assessed for all pharmacologically active substances which are intended for use in food producing animals in accordance with Council Regulation (EEC) No 2377/90 (EEC, 1990; EC, 2009).

Table 4.43: Residual concentration of amprolium in various tissues after following single dose (30mg.kg^{-1}) oral administration in poultry (n=3)

Time of bird slaughter (hours)	Poultry no	Tissues Residual concentration (µg.g^{-1})		
		Liver	Kidney	Muscles
Birds slaughtered after 24h following single dose drug administration	1	0.56	0.30	0.19
	2	0.55	0.28	0.12
	3	0.49	0.29	0.14
	Mean±SE	0.53±0.01	0.29±0.01	0.155±0.035
Birds slaughtered after 48h following single dose drug administration	4	0.09	0.05	0.04
	5	0.06	0.04	0.01
	6	0.074	0.046	0.024
	Mean±SE	0.075±0.015	0.045±0.005	0.025±0.015
Birds slaughtered after 72h following single dose drug administration	5	-	-	-
	6	-	-	-
	7	--	-	-
	Mean±SE	-	-	-

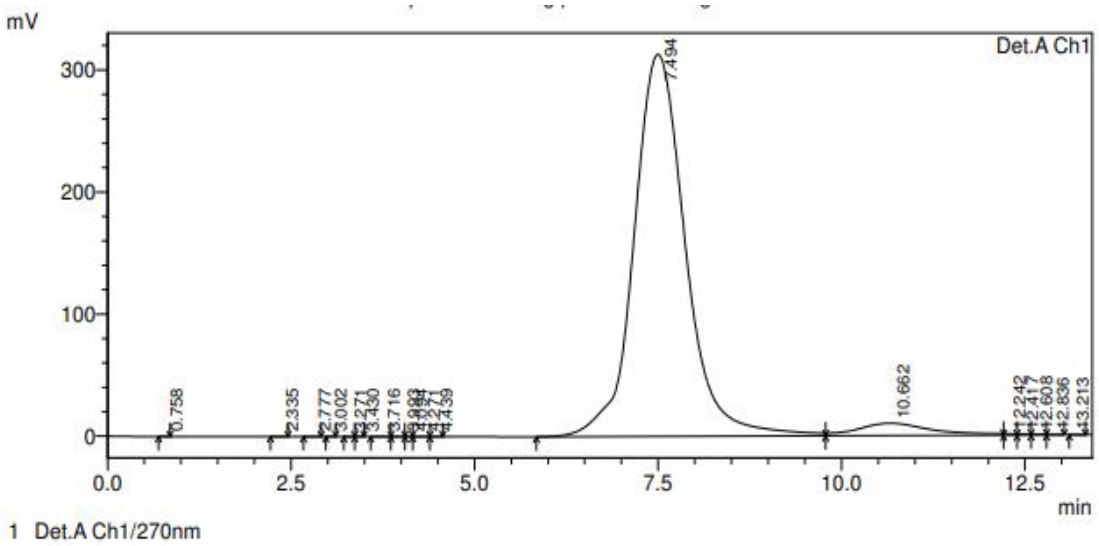


Figure 4.20: Chromatogram of amprolium obtained in tissue following single dose (30mg.kg^{-1}) oral administration in poultry.

Table 4.44: Residual concentration of amprolium in various tissues after following multiple (5 days) dose (30mg.kg^{-1}) oral administration in poultry. (n=3)

Time of bird slaughter post drug administration (hours)	Poultry no	Tissues Residual concentration (µg.g^{-1})		
		Liver	Kidney	Muscles
Birds slaughtered after 48h following multiple dose drug administration	1	0.70	0.32	0.19
	2	0.54	0.25	0.12
	3	0.62	0.22	0.17
	Mean±SE	0.62±0.04	0.26±0.02	0.16±0.02
Birds slaughtered after 72h following multiple dose drug administration	4	0.17	0.10	0.05
	5	0.15	0.07	0.04
	6	0.14	0.08	0.06
	Mean±SE	0.15±0.008	0.08±0.008	0.05±0.005
Birds slaughtered after 96h following multiple dose drug administration	7	0.04	0.02	0.01
	8	0.03	0.03	0.02
	9	0.02	0.04	0.01
	Mean±SE	0.03±0.005	0.03±0.005	0.01±0.003

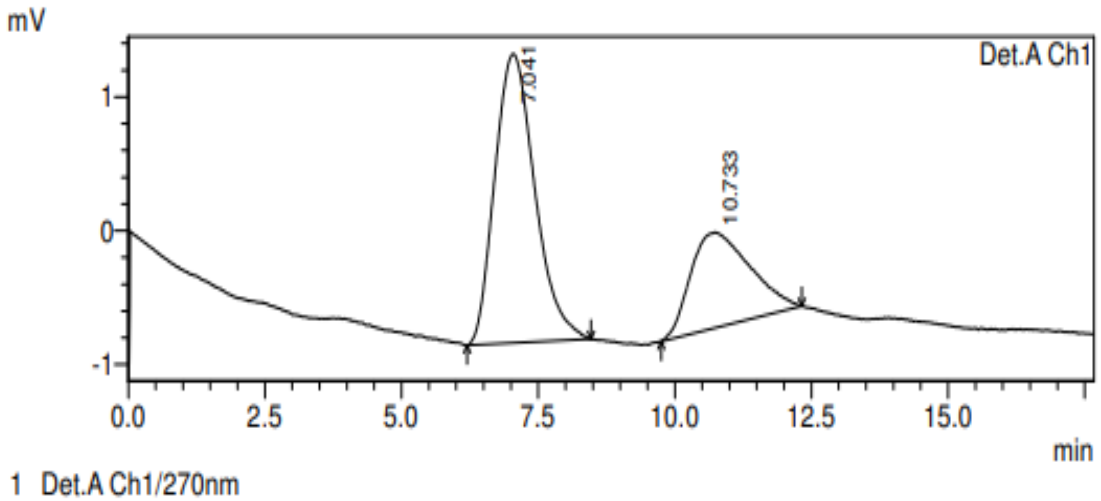


Figure 4.21: **Chromatogram of amprolium obtained in tissue following multiple (5) dose (30mg.kg^{-1} b.w) oral administration.**

4.7.5 Dosage regimen of amprolium following single dose (30mg.kg^{-1}) oral administration in poultry.

The oral dosage regimen of amprolium following single dose (30mg.kg^{-1}) oral administration is depicted in **Table 4.45.** The desired therapeutic concentration of amprolium to calculate the dosage regimen for oral was 0.20µg.ml^{-1}. Based on the pharmacokinetic data, an oral dosage regimen with a priming dose of 28.4 mg.kg^{-1} C^{ss}_{min} of 0.21 µg.ml^{-1} and C^{ss}_{max} of 0.61 µg.ml^{-1}, respectively followed by maintenance dose of 28.3 mg.kg^{-1} was calculated at 12 h interval.

4.7.6 Effect on hematological and biochemical parameters of poultry following multiple (5) dose (30mg.kg^{-1}) oral administration of amprolium.

Hematological parameters

Hematological profile of poultry treated with amprolium, is shown in **Table 4.46**. No significant change was observed in value of PCV, hemoglobin, TEC and TLC as compared to control in group II group III and group IV, these findings can be correlated with findings of **Ghasemi-Sadabadi *et al.* (2020)**.

Biochemical parameters

Effect of multiple (5) dose (30mg.kg^{-1}) administration of amprolium, on biochemical profile of poultry is depicted in **Table 4.47.** No significant ($p<0.05$) changes were observed in the estimated biochemical parameters i.e. AST, ALT, Glucose, Cholesterol, Creatinine, Urea of group II, groupIII and group as compared to control. These findings can be correlated with that of **Salih *et al.* (2019)** who have reported no significant changes in biochemical parameters of broiler chickens Ross 308. Similar finding was found by **Ghasemi-Sadabadi *et al.* (2020)** supplemented a basal diet with 125 ppm of amprolium.

Table 4.45: Dosage regimen of amprolium following single dose (30mg.kg^{-1}) oral administration in poultry.

Desired therapeutic concentration (µg.ml^{-1})	Dose interval (h)	Priming dose (mg.kg^{-1})	Maintenance dose (mg.kg^{-1})	Minimum steady state concentration, C^{ss}_{min} (µg.ml^{-1})	Maximum steady state concentration, C^{ss}_{max} (µg.ml^{-1})
0.2	12	28.4	28.3	0.21	0.61

Table 4.46: Effect of amprolium on hematological profile at different time intervals following multiple (5) dose (30.0 mg/kg) oral administration in poultry (n=3)

Parameters	I (control)	II (48hr)	II (72hr)	III (96hr)
Hb (g dl^{-1})	7.35±0.28	8.80±0.45	10.4±0.32	8.35±0.40
PCV (%)	22.05±0.83	25.6±1.04	31.2±0.95	25.50±0.62
TEC (×10^6/ul)	2.95±0.26	2.90±0.04	2.30±0.30	2.90±0.24
TLC(×10^3/ul)	24.05±0.69	29.23±0.56	22.28±3.55	20.45±1.40

Table 4.47: Effect of amprolium on biochemical profile at different time intervals following multiple (5) dose (30.0mg/kg) oral administration in poultry (n=3).

Parameters	Group I (Control)	Group II (48hr)	Group III (72hr)	Group IV (96hr)
Total protein (g dl^{-1})	3.22±0.12	3.08±0.10	3.11±0.36	3.65±0.25
Albumin (g dl^{-1})	1.96±0.04	1.89±0.05	2.01±0.10	1.98±0.07
Globulin (g dl^{-1})	1.13±0.09	1.32±0.13	1.41±0.16	1.48±0.33
Cholesterol (mg dl^{-1})	134.23±5.65	129.8±6.58	144.6±8.6	139.5±7.56
Glucose (mg dl^{-1})	220.25±18.54	210.9±17.04	198.8±12.6	208.6±16.6
Uric acid (mg dl^{-1})	3±0.69	2.89±0.84	3.56±1.13	2.57±0.97
Creatinine (mg dl^{-1})	0.97±0.06	1.11±0.40	0.89±0.52	0.95±0.09
AST (U/L)	145.63±5.88	158.3±9.34	139.7±13.2	150.4±7.68
ALT (U/L)	11.26±0.23	10.67±0.84	11.32±1.20	9.87±0.35

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

5.1 Pharmacokinetics of sulphadiazine in poultry

This experiment was designed to study pharmacokinetics of sulphadiazine following single and multiple dose i.m. administration of SDZ/TMP combination @ 24 $mg.kg^{-1}$ b.w (20 mg SDZ + 4 mg TMP / kg b.w) in poultry. One-compartment model adequately described plasma concentration-time profile of sulphadiazine in poultry following single and multiple dose i.m. administration.

In the present study, the mean plasma concentrations of sulphadiazine following single dose i.m. administration in poultry ranged from 0.08 ± 0.021$\mu g.mL^{-1}$ to 24.06 ± 1.308$\mu g.mL^{-1}$

The peak plasma concentration (24.06 ± 1.308$\mu g.mL^{-1}$) of the drug was attained at 2h post single i.m. administration and a mean plasma concentration of 0.08 ± 0. $\mu g.ml^{-1}$ was detected up to 48h. The volume of distribution (V/F), clearance (CL/F), area under curve(AUC) and elimination half life ($t_{1/2k10}$) of sulphadiazine were estimated to be 0.85±0.044.kg^{-1}, 0.12±0.007 $L.h^{-1}.kg^{-1}$, 170.91±6.996 $h.\mu g.mL^{-1}$ and 4.73±0.241h, respectively, following single dose i.m. administration in poultry.

The peak plasma concentration after first (25.126 ± 1.236$\mu g.mL^{-1}$) and last (25.38 ± 0.678 $\mu g.mL^{-1}$) dose of sulphadiazine was obtained at 2h following multiple (5) dose (24$mg.kg^{-1}$) i.m. administration in poultry. In the present study, the elimination rate constant (k_{10}) after first and last dose were 0.142±0.006 h^{-1} and 0.15 ± 0.001h^{-1}, respectively and elimination half-life ($t_{1/2k10}$) in the present study after first and last dose were 4.93±0.230h and 4.87 ±0.115h, respectively. The volume of distribution (V/F) estimated to be 0.81 ± 0.027$L.kg^{-1}$ and 0.80 ± 0.019$L.kg^{-1}$ after first and last dose respectively, and clearance of drug (CL/F) of sulphadiazine was 0.11±0.004 $L.h^{-1}.kg^{-1}$ after first dose and 0.12±0.003$L.h^{-1}.kg^{-1}$ after the last dose. The mean area under curve (AUC) after first and last dose were 176.113 ± 7.791and 170.256 ± 5.019$h.\mu g.\ mL^{-1}$ respectively. The values of C_{max} and T_{max} were 22.94 ±

0.631 µg.mL^{-1} and 0.54±0.066 h after first dose and 23.25 ± 0.555 µg.mL^{-1} and 0.52±0.025h after the last dose, respectively.

5.1.1 Tissue residue of sulphadiazine (µg.g^{-1}) following single and multiple (5) dose (24mg.kg^{-1}) i.m. administration of SDZ /TMP combination (20 mg SDZ + 4 mg TMP / kg b.w) in poultry.

After single dose study, 24h post administration, the maximum residual concentration of sulphadiazine was found to be 0.28±0.048, 0.20±0.0270 and 0.12±0.015 µg.g^{-1} in kidney, liver and muscle, respectively. After 48h of post administration tissue concentration was found to be 0.037±0.009, 0.02±0 and 0.035±0.007µg.g^{-1} in kidney, muscles and liver, respectively. Drug could not be detected in liver, muscles and kidney after 72h of administration.

After multiple dose study, 48h post administration, the maximum residual concentration of sulphadiazine was found to be 0.57±0.067, 0.78±0.044 and 0.23±0.045 µg.g^{-1} in liver, kidney and muscles, respectively. After 72h post administration, residual concentrations found to be 0.21±0.014, 0.43±0.028 and 0.12±0.018 µg.g^{-1} in liver, kidney and muscles, respectively. The residual concentrations further declined after 96h post administration as 0.05±0.005, 0.07±0.008 and 0.02±0.008 µg.g^{-1} in liver, kidney and muscles, respectively. A withdrawal period based on the guidelines of EMEA/CVMP/036/95 (1996) is being proposed. a withdrawal period of 5days is recommended for sulphadiazine-trimethoprim in broiler birds.

5.2 Pharmacokinetics of trimethoprim in poultry

This experiment was designed to study the pharmacokinetics of trimethoprim following single and multiple dose i.m. administration of SDZ /TMP combination @ 24 mg.kg^{-1} b.w (20 mg SDZ + 4 mg TMP / kg b.w) in poultry. One-compartment model adequately described plasma concentration-time profile of trimethoprim in poultry following single and multiple dose i.m. administration.

In the present study, the mean plasma concentrations of trimethoprim following single dose (24 mg.kg^{-1}) i.m. administration in poultry ranged from 0.09±0.0170µg.ml^{-1} to 1.27±0.056µg.ml^{-1}

The peak plasma concentration (1.27±0.056μg.ml^{-1}) of the drug was attained at 1h post single i.m. administration and a mean plasma concentration of 0.09±0.0170μg.ml^{-1} was detected up to 24h. The V/F, CL/F, mean area under curve (AUC) and elimination half life ($t_{1/2k10}$) of trimethoprim were estimated to be 2.64±0.137L.kg^{-1} and 0.43±0.050L.h^{-1}.kg^{-1}, 9.33 ±0.804 h.μg.mL^{-1} and 4.53 ±0.631h, respectively, following single dose i.m. administration in poultry.

The peak plasma concentration after first (1.23±0.044μg.ml^{-1}) and last (1.29 ± 0.006 μg.mL^{-1}) dose of trimethoprim was obtained, respectively at 1 and 2h following multiple (5) dose (24mg. kg^{-1}) i.m. administration in poultry. In the present study, the elimination rate constant (k_{10}) after first and last dose were 0.18 ± 0.019 h^{-1} and 0.12±0.007h^{-1}, respectively, and elimination half-life ($t_{1/2k10}$) in the present study after first and last dose were 4.22 ± 0.497h and 5.73±0.389h, respectively. The volume of distribution (V/F) estimated to be 2.75 ± 0.085L.kg^{-1} and 2.81±0.039L.kg^{-1} after first and last dose respectively, and clearance of drug (CL/F) was 0.49 ± 0.043 L.h^{-1}.kg^{-1} after first dose and 0.35±0.017L.h^{-1}.kg^{-1} after the last dose. The mean area under curve (AUC) after first and last dose were 8.41 ± 0.685 and 10.95±0.480 h.μg. mL^{-1}, respectively. The values of C_{max} and T_{max} were 1.23±0.018μg.mL^{-1} and 0.95± 0.071 h after first dose and 1.28±0.009μg.mL^{-1} and 0.86±0.035h after the last dose, respectively.

5.2.1 Tissue residue of trimethoprim (μg.g^{-1}) following single and multiple (one dose per day for five days) dose (24mg.kg^{-1}) i.m. administration of SDZ /TMP combination (20 mg SDZ + 4 mg TMP/kg) bw in poultry.

After 24h post administration of single dose of trimethoprim the maximum residual concentration of trimethoprim was found to be 0.69±0.091, 0.52±0.043 and 0.14±0.008μg.g^{-1} in kidney, liver and muscle, respectively and 0.08±0.0173μg.g^{-1} in kidney, 0.02±0.005 μg.g^{-1} in liver, respectively after 48h post administration and in muscles no residue was found. Drug could not be detected in liver, muscles, kidney and intestine after 72h of administration.

Following multiple dose, after 48h post administration, the maximum residual concentration of trimethoprim was found to be 0.91 ±0.134, 1.19 ±0.101and 0.41

±0.055 and 0.32±0.07 μg.g^{-1} in liver, kidney, muscles and intestine, respectively, and 0.16 ±0.026, 0.266 ± 0.038 and 0.093 ±0.008 and 0.06±0.005μg.g^{-1} in liver, kidney muscles and intestine, respectively, 72h post administration. The residual concentrations was found to be declined after 96h post administration as 0.023±0.003, 0.026±0.008 and 0.006±0.006 μg.g^{-1} in liver, kidney and muscles, respectively.

5.3 Comparison of pharmacokinetic parameters of sulphadiazine and trimethoprim after single dose (24.mg.kg^{-1} b.w) i.m. administration of SDZ/TMP combination (20mg SDZ and 4mg TMP/kg b.w) in poultry.

In the present study, the combination of sulphadiazine-trimethoprim was administered in the same ratio of 5:1 at a dosage of 4 mg.kg^{-1}b.w TMP and 20 mg.kg^{-1} b.w sulphadiazine for direct comparison of plasma concentrations. Following single i.m. dose, sulphadiazine was rapidly absorbed with a C_{max} of 22.95 ± 0.725 μg.ml^{-1} achieved at 0.59 ± 0.088h (T_{max}). In the current study, trimethoprim concentration was measured in poultry plasma with a C_{max} of 1.28 ±0.041 μg.ml^{-1} achieved at 1.03 ±0.058h post injection. The volume of distribution for TMP (2.64 ±0.137 L.kg^{-1}) was higher than SDZ (0.85 ± 0.055 L.kg^{-1}). Clearance observed in the present study for SDZ (0.12±0.007L.Kg^{-1}.h^{-1}) is lower than as compared to TMP (0.43 ±0.050 L.Kg^{-1}.h^{-1}.

5.4 Dosage regimen of sulphadiazine-trimethoprim following single dose (24 mg.kg^{-1} bw) i.m. administration of SDZ/TMP combination (20 mg SDZ + 4 mg TMP/kg b.w) in poultry.

The dosage regimen of sulphadiazine –trimethoprim following single dose i.m. administration was calculated with therapeutic concentration of 9.5 μg.mL^{-1} and 0.5 μg.mL^{-1} for sulphadiazine and trimethoprim, respectively. For sulphadiazine a priming dose (D) of 43.32 mg.kg^{-1} and a maintenance dose of 35.25 mg.kg^{-1} for every 12 h were estimated to maintain the therapeutic concentration. The minimum steady state concentration C_{ss} (min) and the maximum steady state concentration C_{ss} (max) were estimated as 11.62 was 62.67 μg.mL^{-1}, respectively.

For trimethoprim a priming dose of 10.0mg.kg^{-1} and a maintenance dose of 8.7 mg.kg^{-1} for every 12 h were estimated to maintain the therapeutic concentration. The minimum steady state concentration C_{ss} (min) and the maximum steady state concentration C_{ss} (max) were estimated as 0.56 was 4.36μg.mL^{-1}, respectively.

5.5 Effect on hematological and biochemical parameters of poultry following multiple (5) dose (24mg.kg^{-1}) i.m. administration of combination of SDZ /TMP (20 mg SDZ + 4 mg TMP / kg b.w)

In the present study following multiple (5) dose (24mg.kg^{-1}) i.m. administration of SDZ /TMP combination (20 mg SDZ + 4 mg TMP / kg b.w), no significant change could be observed for sulphadiazine and trimethoprim in the estimated haematological and biochemical parameters, in any of the treated groups as compared to control one.

5.6 Pharmacokinetics of amprolium in poultry

This experiment was designed to study pharmacokinetics of amprolium following single and multiple dose oral administration of amprolium @ 30mg.kg^{-1} b.w in poultry. One-compartment model adequately described plasma concentration-time profile of amprolium in poultry following single and multiple dose oral administration.

In the present study, the mean plasma concentration of amprolium following single dose (30 mg.kg^{-1}) oral administration in poultry ranged from 0.047±0.008 μg.ml^{-1} to 0.80±0.09μg.ml^{-1}

The peak plasma concentration (0.80±0.09μg.ml^{-1}) of the drug was attained at 1h post single oral administration and a mean plasma concentration of 0.047±0.008 μg.ml^{-1} was detected up to 13h. The V/F, CL/F, AUC and elimination half life ($t_{1/2k10}$) of amprolium were estimated to be 21.941±4.54 L.kg^{-1} and 14.460±1.292L.h^{-1}.kg^{-1}, 2.168±0.164h.μg.mL^{-1} and 1.027±0.162h, respectively, following single dose oral administration in poultry.

The peak plasma concentration after first (0.94±0.083μg.ml^{-1}) and last (1.01±0.04 μg.ml^{-1}) dose of amprolium was obtained, respectively at 1h following multiple (5) dose (@30mg. kg^{-1}) oral administration in poultry. In the present study, the elimination rate constant (k_{10}) after first and last dose were 1.054±0.070 h^{-1} and 2.344±0.120 h^{-1} respectively and elimination half-life ($t_{1/2k10}$) after first and last dose were 0.833±0.061h and 0.893±0.051h, respectively. The volume of distribution (V/F)

estimated to be 16.731±1.638L.kg^{-1} and 16.361±1.843L.kg^{-1} after first and last dose respectively, and clearance of drug (CL/F) was 14.145±1.251 L.h^{-1}.kg^{-1} after first dose and 12.647±1.157L.h^{-1}. kg^{-1} after the last dose. The mean area under curve (AUC) after first and last dose were 2.302±0.235 and 2.539±0.239 h.µg. mL^{-1}, respectively. The values of C_{max} and T_{max} were 0.778±0.068µg.mL^{-1} and 1.072±0.051h after first dose and 0.804±0.069µg.mL^{-1} and 1.155±0.061h after the last dose, respectively.

5.6.1 Tissue residue of amprolium (µg.g^{-1}) following single and multiple (5) dose (30mg.kg^{-1}) oral administration in poultry.

Following single dose after 24h post administration, the maximum residual concentration of amprolium was found to be 0.29±0.01, 0.53±0.01 and 0.155±0.035 µg.g^{-1} in kidney, liver and muscle, respectively. After 48h of post administration tissue concentration was found to be 0.045±0.005 µg.g^{-1} in kidney, 0.075±0.015 µg.g^{-1} in liver, respectively and 0.025±0.015 µg.g^{-1} was found in muscles. Drug could not be detected in liver, muscles and kidney after 72h of administration.

Following multiple dose after 48h post administration, the maximum residual concentration of amprolium was found to be 0.62±0.04, 0.26±0.02 and 0.16±0.02 µg.g^{-1} in liver, kidney and muscles, respectively. After 72h of post administration tissue concentration was found to be 0.15±0.008, 0.08±0.008 and 0.05±0.005µg.g^{-1} in liver, kidney and muscles, respectively. The residual concentrations further declined after 96h post administration as 0.03±0.005, 0.03±0.005 and 0.01±0.003µg.g^{-1} in liver, kidney and muscles, respectively.

5.6.2 Dosage regimen of amprolium following single dose (30mg.kg^{-1}) oral administration in poultry.

The desired therapeutic concentration of amprolium to calculate the dosage regimen for oral was 0.20µg.ml^{-1}. Based on the pharmacokinetic data, an oral dosage regimen with a priming dose of 28.4 mg.kg^{-1} C^{ss}_{min} of 0.21 µg.mL^{-1} and C^{ss}_{max} of 0.61 µg.ml^{-1}, respectively followed by maintenance dose of 28.3 mg.kg^{-1} was calculated at 12 h interval.

5.6.3 Effect on hematological and biochemical parameters of poultry following multiple (5) dose (30mg kg^{-1}) oral administration of amprolium.

In the present study following multiple (5) dose (30mg. kg^{-1}) oral administration of amprolium no significant change could be observed for in the estimated haematological and biochemical parameters in any of the groups as compared to control.

The following conclusions were drawn from the present investigation:

1 The disposition kinetics of sulphadiazine and trimethoprim following single and multiple dose i.m. and amprolium following single and multiple oral dose, administration followed a one- compartment model with first order rate constant.

2 The ratio between the serum levels of SDZ and TMP was higher than 18:1. This ratio remained steady for 4 hr.

3 After i.m. administration concentrations above 2µg.ml^{-1} (SDZ) and 0.1µg.ml^{-1} (TMP) were maintained for at least 12 h for both the drugs.

4 Sulphadiazine and trimethoprim accumulated in liver, muscle and kidney after multiple (5) dose administration with higher accumulation in kidney.

5 Amprolium is being accumulated in liver, muscle and kidney after multiple (5) dose administration with higher accumulation in liver.

6 An i.m. dosage regimen of sulphadiazine with a priming dose (D) of 43.32 mg.kg^{-1} and a maintenance dose of 35.25 mg.kg^{-1} for every 12 hr were estimated to maintain the therapeutic concentration (C^{ss}_{min} of 11.62 µg.ml^{-1}, C^{ss}_{max} of 62.76µg.ml^{-1}).

7 An i.m. dosage regimen of trimethoprim with a priming dose of 10.0mg.kg^{-1} (C^{ss}_{min} of 0.56 µg.ml^{-1}, C^{ss}_{max} of 4.36µg.ml^{-1}) followed by a maintenance dose of 8.7 mg.kg^{-1} at 12 h interval is recommended.

8 An oral dosage regimen of amprolium with a priming dose of 28.4mg.kg^{-1} (C^{ss}_{min} of 0.21 µg.ml^{-1}, C^{ss}_{max} of 0.61µg.ml^{-1}) followed by a maintenance dose of 28.3 mg.kg^{-1} at 12 h interval is recommended

9 The LOD and LOQ for all the three drugs was 0.025µg.ml^{-1} and 0.075 µg.ml^{-1}, respectively.

10 LOQs observed for amprolium which were 0.22 and 0.25 µg.g^{-1} for muscles and liver and were below the MRL (0.5ug.g^{-1} for muscles and 0.3 ug.g^{-1} for liver).

11 Based on the scientific residues guidelines of EMEA, a withdrawal period of 5 days is recommended for all the three drugs.

12 The LOD of SDZ for all the tissues was 0.025 µg.mL^{-1} and for TMP was 0.020 µg.mL^{-1}.

13 LOQ for SDZ and TMP for all the tissues of chicken corresponding was 0.05 µg.g^{-1} and 0.025 µg.g^{-1}, respectively.

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2	M.V.Sc.	GBPUA&T	2011	8.458
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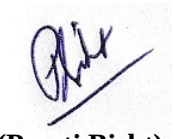

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ABSTRACT

The present study was undertaken to investigate the pharmacokinetics and tissue residue study of sulphadiazine-trimethoprim ,when given intramuscular (i.m) in a combination (20mg.kg^{-1} sulphadiazine and 4mg.kg^{-1} trimethoprim) @ 24.0mg.kg^{-1} and amprolium given orally @ 30.0mg.kg^{-1} in poultry following single and multiple (5) dose administration.

In the present study Biotrim®, a combination of sulphadiazine-trimethoprim in the ratio of 5:1 (sulphadiazine 400mg and trimethoprim 80mg) was given @24.0mg.kg^{-1} b.w i.m as a single dose to nine birds and as a multiple dose to nine birds for 5 consecutive days. Amprolium 20% was used and given @30.0mg.kg^{-1} b.w orally as a single dose to nine birds and as a multiple dose to nine birds for 5 consecutive days.

The concentration of sulphadiazine-trimethoprim and amprolium in plasma and tissue of these animals was analysed by HPLC. The initial peak plasma concentration of 24.06 and 1.27µg.ml^{-1} for sulphadiazine and trimethoprim was detected in poultry following single dose i.m. administration and 0.94µg.ml^{-1} for amprolium following single dose oral adminimistration, respectively. The volume of distribution, clearance, mean area under curve (AUC) and elimination half- life calculated was 0.85L.kg^{-1}, 0.12L.h^{-1}.kg^{-1}, 171.12h.µg.mL^{-1} and 4.73h and 2.64 L.kg^{-1},0.43 L.h^{-1}.kg^{-1}, 9.71 h.µg.mL^{-1}and 4.53h for sulphadiazine (SDZ) and trimethoprim (TMP) respectively, following single dose i.m administration of SDZ/TMP combination. For amprolium, the volume of distribution, clearance, mean area under curve (AUC) and elimination half- life following single dose oral administration calculated was 21.941 L.kg^{-1},14.446 L.h^{-1}.kg^{-1},2.172 h.µg.mL^{-1} and 1.027h, respectively.

In multiple dose study, volume of distribution, clearance, mean area under curve and elimination half-life calculated for sulphadiazine was 0.81 L.kg^{-1}, 0.11 L.h^{-1}.kg^{-1},176.41 h.µg.ml^{-1} and 4.93h after first dose and 0.80 L.kg^{-1}, 0.12L.kg^{-1}, 176.32 h.µg.ml^{-1} and 4.87 h after last dose, respectively. For TMP volume of distribution, clearance, mean area under curve and elimination half-life calculated as 2.75L.kg^{-1}, 0.49L.h^{-1}.kg^{-1},8.72h.µg.ml^{-1} and 4.22h, respectively, after first dose and 2.81L.kg^{-1},0.35L.h^{-1}.kg^{-1},11.70h.µg.ml^{-1} and 5.73h, respectively after last. For amprolium volume of distribution, clearance, mean area under curve and elimination half-life calculated was 16.731L.kg^{-1}, 14.145L.h^{-1}.kg^{-1}, 2.202h.µg.ml^{-1} and 0.833±0.061h, respectively after first dose, 0.893L.kg^{-1},12.647L.h^{-1}.kg^{-1},2.540h.µg.ml^{-1}and 16.361h, respectively after last dose. According to the results obtained in the pharmacokinetic study an individualized dosage regimen were calculated. An i.m. dosage regimen of sulphadiazine with a priming dose of 43.32 mg.kg^{-1} and a maintenance dose of 35.25 mg.kg^{-1} for every 12 hr were estimated to maintain the therapeutic concentration. An i.m. dosage regimen of trimethoprim with a priming dose of 10.0mg.kg^{-1} followed by a maintenance dose of 8.7 mg.kg^{-1} at 12 h interval is recommended. For amprolium an oral dosage regimen with a priming dose of 28.4mg.kg^{-1} followed by a maintenance dose of 28.3 mg.kg^{-1} at 12 h interval is recommended. Following single dose i.m administration highest residual concentration of sulphadiazine and trimethoprim were observed in kidney (0.28 µg.g^{-1} for SDZ and 0.69µg.g^{-1} for TMP) followed by liver at 24hrs post administration of SDZ/TMP combination. No drug residue was found after 72h of post administration of SDZ/TMP combination. In case of multiple dose i.m administration of SDZ/TMP combination, residue of SDZ and TMP were present in kidney, liver and muscle upto 96hr post administration with highest concentration in kidney (0.78µg.g^{-1} for sdz and1.19 µg.g^{-1} for tmp). For amprolium following single dose oral administration residue were found in liver, kidney, muscles with highest concentration found in liver (0.53 µg.g^{-1}) at 24hr post administration. In case of multiple dose oral administration of amprolium, highest concentration found in liver (0.62µg.g^{-1}) at 48hr post administration. Tissue residue were found in liver, kidney and muscles upto 96 hr post administration of amprolium.

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पृष्ठ संख्या	:	129	सलाहकार	:	डॉ. ए.एच. अहमद

सारांश

वर्तमान अध्ययन में ब्रॉयलर पक्षियों में सल्फाडायाजीन–ट्राइमेथोप्रिम संयुक्त रूप से 5:1 के अनुपात में (24 मिलिग्राम/किलोग्राम) अन्तः मांसपेशीय माध्यम से एवम एम्प्रोलियम (30 मिलिग्राम/किलोग्राम) मौखिक माध्यम से एकल एवम विभिन्न (5 दिन) खुराकों में दिया गया व फार्माकोकाइनेटिनस एवं दवाओं की अवशेष मात्रा का अध्ययन किया गया।

नौ (9) अलग–अलग पक्षियों में एकल व विभिन्न खुराकों को दिया गया। रक्त जल में दवाओं की मात्रा का अध्ययन एवम ऊतक नमूनों का अध्ययन एच.पी.एल.सी. द्वारा विश्लेषण के अधीन किया गया।

सल्फाडायाजीन–ट्राइमेथोप्रिम (अन्तः मांसपेशीय) एवम एम्प्रोलियम (मौखिक) के एकल खुराक देने के पश्चात् वितरण आयतन, क्लीयरैन्स, ए.यू.सी. एवं एलीमिनेशन हाफ लाईफ क्रमशः 0.80, 2.64, 21.941 ली./किलोग्राम, 0.12, 0.43, 14.446 ली./घंटा × कि.,171.12, 9.71, 2.172 घंटा × माइक्रोग्राम/मिली. एवम 4.73, 4.50, 1.027 घंटा पाया गया।

विभिन्न (5) खुराक अध्ययन में सल्फाडायाजीन, ट्राइमेथोप्रिम एवम एम्प्रोलियम की पहली खुराक के बाद वितरण आयतन, क्लीयेरेन्स, ए.चू.सी. एवं एलीमिनेशन हाफ लाईक क्रमशः 0.81, 2.75, 16.731 ली./कि., 0.11, 0.47, 12.647 ली./घंटा × कि., 176.41, 8.72, 2.54 घंटा × माईक्रोग्राम/मिली., 4.93, 4.22, 8.43 घंटा एवम आखरी खुराक के बाद क्रमशः 0.80, 2.81, 0.893 ली./कि., 0.122, 0.35, 12.647 ली./घंटा कि., 176. 32, 11.70, 2.540 घंटा × माइक्रोग्राम/मिलीग्राम, 4.87, 5.73 एवम 16.361 घंटा पायी गयी।

एकल खुराक सल्फाडायाजीन, ट्राइमेथोप्रिम (अन्तः मांसपेशीय) एवम एम्प्रोलियम (मौखिक माध्यम) के लिए प्राईमिंग खुराक क्रमशः 43.32, 10.0, 28.4 मिलिग्राम/कि. एवम मेटीनेज खुराक क्रमशः 35.25, 8.7 एवम 28.3 मिलिग्राम/कि. पाया एवम अनुमोदित किया गया।

एकल खुराक अन्तः मांसपेशीय के 24 घंटे बाद सल्फाडायाजीन एवम ट्राइमेथोप्रिम की अधिकतम अवशेष मात्रा गुर्दे में 0.28 माईक्रोग्राम/ग्राम (सल्फाडायाजीन), 0.69 माईक्रोग्राम/ग्राम (ट्राइमेथोप्रिम) पायी गयी। 72 घंटे बाद कोई अवशेष नही पाया गया।

विभिन्न (5) खुराक के 48 घंटे बाद गुर्दो में सल्फाडायाजीन (0.78 माईक्रोग्राम/ग्राम) एवम ट्राइमेथेप्रिम (1.19 माईक्रोग्राम/ग्राम) की उच्चतम मात्रा पायी गयी। एम्प्रोलियम के लिए एकल खुराक (मौखिक) 24 घंटे बाद एवं विभिन्न खुराक के 48 घंटे पश्चात् उच्चतम अवशेष मात्रा यकृत में क्रमशः 0.53 माइक्रोग्राम/ग्राम एवं 0.62 माइक्रोग्राम/ग्राम पायी गयी।

(ए.एच. अहमद)
सलाहकार

(प्रीति बिष्ट)
लेखिका