OCCURRENCE OF ANTIBIOTIC RESIDUES IN POULTRY FEED AND CHICKEN MEAT WITH RISK CHARACTERIZATION

MD. AMIT KHAN

REGISTRATION NO. 19-10317

DEPARTMENT OF AGRICULTURAL CHEMISTRY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA -1207

DECEMBER, 2021

OCCURRENCE OF ANTIBIOTIC RESIDUES IN POULTRY FEED AND CHICKEN MEAT WITH RISK CHARACTERIZATION

BY

MD. AMIT KHAN

REGISTRATION NO. : 19-10317

Email: amitkhan151@gmail.com
Contact No. 01935-630228

A Thesis
Submitted to the Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University, Dhaka
In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (MS)

IN

AGRICULTURAL CHEMISTRY

SEMESTER: JULY-DECEMBER, 2021

Approved by:

Prof. Dr. Mohammed Ariful Islam Department of Agricultural Chemistry SAU, Dhaka **Supervisor**	**Dr. Md. Nurul Huda Bhuiyan** Principal Scientific Officer Institute of Food Science and Technology (IFST), BCSIR, Dhaka **Co-Supervisor**

Prof. Dr. Sheikh Shawkat Zamil
Chairman
Examination Committee
Department of Agricultural Chemistry

DEPARTMENT OF AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled **"OCCURRENCE OF ANTIBIOTIC RESIDUES IN POULTRY FEED AND CHICKEN MEAT WITH RISK CHARACTERIZATION"** submitted to the **Department of Agricultural Chemistry**, **Faculty of Agriculture**, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **Md. Amit Khan**, Registration No. **19-10317** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

December, 2021

Dhaka, Bangladesh

(Dr. Mohammed Ariful Islam)
Professor
Department of Agricultural Chemistry
SAU, Dhaka

Dedicated to
My
Beloved Parents

ACKNOWLEDGEMENTS

The author seems it a much privilege to express his enormous sense of gratitude to the Almighty Allah for there ever ending blessings for the successful completion of the research work.

The author wishes to express his gratitude and best regards to his respected Supervisor, ***Prof. Dr. Mohammed Ariful Islam****, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.*

The author wishes to express his earnest respect, sincere appreciation and enormous indebtedness to his reverend Co-supervisor, ***Dr. Md. Nurul Huda Bhuiyan****, Principal Scientific Officer, Institute of Food Science and Technology (IFST), BCSIR, Dhaka for his scholastic supervision, helpful commentary and unvarying inspiration throughout the research work and preparation of the thesis gratitude to Mohammad Tariqul Hasan, PSO (Principal Scientific Officer) and Maksuda Begum, SSO (Senior Scientific Officer) and all Lab Personnel who helped me during my research work in the Laboratory.*

The author feels to express his heartfelt thanks to the honorable Chairman, ***Prof. Dr. Sheikh Shawkat Zamil,*** *Department of Agricultural Chemistry along with all other teachers specially Prof. Dr. Md. Abdur Razzaque, Prof. Dr. Rokeya Begum, Prof. Dr. Md. Tajul Islam Chowdhury, Prof. Dr. Sirajul Islam Khan, Assoc. Prof. Abdul Kaium and Assist. Prof. Mahbuba Siddika and also all staff members of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.*

The author feels proud to express his deepest and endless gratitude to all of his course mates and friends to cooperate and help him during taking data from the field and preparation of the thesis. The author wishes to extend his special thanks to his lab mates, class mates and friends for their keen help as well as heartiest co-operation and encouragement.

The author expresses his heartfelt thanks to his beloved parents, Elder Sister and Brother and all other family members for their prayers, encouragement, constant inspiration and moral support for his higher study. May Almighty bless and protect them all.

The Author

OCCURRENCE OF ANTIBIOTIC RESIDUES IN POULTRY FEED AND CHICKEN MEAT WITH RISK CHARACTERIZATION

ABSTRACT

The present study was conducted to determine the occurrence of antibiotic residues in poultry feed and chicken meat with risk characterization. To perform this experiment, 5 samples of layer muscle, 5 samples of layer liver, 5 samples of broiler muscle, 5 samples of broiler liver, 7 samples of local breed chicken muscle (control) and three samples of local breed liver (control) were used. So, total 30 chicken samples and 10 feed samples (5 samples of layer feed and 5 samples of broiler feed) were collected from different poultry farm of Sirajganj and Gazipur district, Bangladesh during the period from March 2020 to June, 2021. To detect Tetracycline and Oxytetracycline residues, the collected chicken and feed samples were analyzed using High Performance Liquid Chromatography (HPLC) with fluorescence detector. In this study, 2 Layer muscle samples, 3 layer liver samples, 2 Broiler muscle samples and 2 Broiler liver samples were Tetracycline and Oxytetracycline positive whereas control muscle and liver samples were negative. Among the collected feed samples, 3 layer feed samples and 2 broiler feed samples were Tetracycline and Oxytetracycline positive but the rest of the samples showed no residue. Results showed that no chicken sample as well as no feed sample in the present study exceeded the maximum residue limit (MRL) of Tetracycline and Oxytetracycline.

LIST OF CONTENTS

LIST OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
LSD	=	Least Significant Difference
m^2	=	Meter squares
ml	=	MiliLitre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celceous
%	=	Percentage
NaOH	=	Sodium hydroxide
GM	=	Geometric mean
mg	=	Miligram
P	=	Phosphorus
K	=	Potassium
Ca	=	Calcium
L	=	Litre
µg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization

CHAPTER I

INTRODUCTION

Antibiotics are low to medium molecular weight compounds exhibiting a variety of chemical and biological properties. This is mainly employed for chemotherapeutic and prophylactic purposes and also used as feed additives to promote growth and improve feed efficiency (Swatantra *et al*., 2014). The most recent couple of many years, antibiotics have been largely used in animal cultivating for their prophylactic and therapeutic purposes (Thiele- Bruhn, 2003). Group of antimicrobials that are used in food animals include β-lactams (penicillin, ampicillin), tetracyclines (chlortetracycline, oxytetracycline), sulfonamides (sulfadiazine, sulfamethoxazole, sulfadimidine, sulfadimethoxine), macrolides (erythromycin, spiramycin), aminoglylosides (gentamycin, neomycin, streptomycin), quinolones (enrofloxacin, ciprofloxacin), miscellaneous (chloramphenicol, colistin, novobiocin) (Vragovic *et al*., 2012). Among them tetracyclines are commonly used antibiotics in Bangladesh as well as in the world. Tetracyclines are either naturally occurring product of fungi or semisynthetic derivatives of such products. They are effective against Gram positive and Gram negative bacteria, widely use in diseases or growth promoters and usually given to poultry via feed or water. They are effective against both Gram positive and Gram negative bacteria and in poultry the most common route of administration is oral in feed or water (Goetting *et al*., 2011 and Alaboudi *et al*., 2013).

Antibiotics are necessary to prevent and control infectious disease in poultry production. They are additionally utilized illegally as feed enhancements to animate creature development and profitability (Kantiani *et al*., 2010 and Laxminarayan *et al*., 2013). The abuse and inaccurate utilization of antibiotics convey the danger of their residues presence in palatable tissues of the chicken which can cause toxics and sensitivities in excessively hypersensitive consumer. (Marazuela, 2009). The abuse of antibiotics may also trigger the

development of resistant strains of bacteria, thus declining the efficiency of antibiotics used for animal treatment, leading to the treatment failure of livestock and affecting negatively the animal welfare (Laxminarayan *et al*., 2013).

The safe and effective use of antibiotic in animal production has received considerable attention in most of the countries in the world. However, the antibiotic residues from milk and meat may persist for longer period after treatment, when administration of antibiotics in label and extra label fashion and also not following of withholding period after treatment. The extra-label drug use may take the form of increased dose, increased frequency of treatment and use in an unapproved species, by an unapproved route of inoculation or in short, by any means not explicitly described on the drug product label (McEwen *et al*., 1992). The spread of antibiotic resistance is an issue of significant concern around the world. Consequently, the antibiotic residues are being considered as public health hazards (Haller *et al*., 2002). Since there is a concern about the transfer of antibiotic-resistant genes from animal flora to human pathogens. Several antibiotic residues in chicken have been detected in several places of Bangladesh where those studies mostly reported the frequency of detection. (Sarker *et al*., 2018). However, regarding the feed contamination with antibiotics is not well reported in Bangladesh. Whereas feed could be a potential source of antibiotics as they are enriched with protein (animal muscle and bone).So it necessary to understand the actual scenario of antibiotics in not only chicken but also in feed to evaluate risk of the general population of Bangladesh.

The drug resistance has gained its importance due to the transmission of antibiotic resistance factor to other enteric organisms which have posed a serious public health concern. In general, harmful effects of drug and chemical residues on health, which may be mutagens, carcinogenic, teratogenic,

reduction in reproductive performance, drug allergy and acute toxicity or poisoning in human (Swatantra *et al*., 2014).

Annually more than 50 billion chickens are raised as a source of protein food, while the present meat production in Bangladesh can meet only 68% of the national demand (Rahman *et al*., 2017). To meet the current need, farmers and stakeholders rely greatly on intensive poultry farming which brings usage of vaccines, vitamins and minerals and mostly antibiotics (Khan *et al*., 2018). Among the different forms of administration (oral, parenteral, or topical), it was reported that antibiotic residues exceeding the standards are usually encountered when administered through injection (Katz and Brady, 2000). Therefore, various authors have reported that antibiotic residues in food are likely to induce and accelerate the development of antibiotic resistance in bacteria, promote the transfer of antibiotic-resistant bacteria to humans, cause allergies (penicillin), and induce other more severe pathologies, such as cancers (sulfamethazine, oxytetracycline, and furazolidone), anaphylactic shock, nephropathy (gentamicin), bone marrow toxicity, mutagenic effects, and reproductive disorders (chloramphenicol) in humans (Bacanli and Basaran, 2019).

Therefore, the World Health Organization (WHO), the American Medical Association, and the American Public Health Association have urged a ban on growth-promoting antibiotics (Graham *et al*., 2007) and established standards to limit this phenomenon (Kempe and Verachtert, 2000). Consequently, drugs or antibiotic residues in food above the maximum level globally recognized by various public authorities are illegal (Kempe and Verachtert, 2000 and Milhaud and Pinault, 2001). Furthermore, observation of the waiting or withdrawal time and physicochemical analyses is mandatory to ensure that the antibiotics used or their analogs do not exceed the maximum residue limit (MRL) before the food is marketed (Arsene *et al*., 2022).

The present study is therefore aimed, to determine the antibiotics residues in muscle, liver and poultry feed samples of chicken, using both a microbial growth inhibition assay and high performance thin layer chromatography. The microbial assay was employed to prescreen the possible antibiotic-containing meat and feed samples. The study also conducted to justify authentic information on use of various antibiotics and their residues in muscle, liver and feed samples of chickens available in Bangladesh with the following objectives:

1. To determine the level of antibiotic residues in poultry feed and chicken meat.
2. To identify the risk of exposure to general population.

CHAPTER II

REVIEW OF LITERATURE

Available research reports on the occurrence of antibiotic residues in poultry feed and chicken meat with risk characterization are not sufficient in Bangladesh. However, a few relevant reports on this aspect in home and abroad are reviewed here under the following sequence.

Antibiotics are used largely for three purposes in animals; therapeutic use to treat sick animals, prophylactic use to prevent infection in animals and as growth promoters to improve feed utilization and production (Maraschiello *et al.,* 2001; Dipeolu and Alonge, 2002). For their growth promoting properties, they are routinely used at sub-therapeutic levels as animal feed additives (Okerman *et al.,* 1998). The mechanism of action of antimicrobial agent as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005; Niewold, 2007).

The term "Antimicrobial Growth Promoter" (AGP) is used to describe any medicine that destroys or inhibit bacteria and is administered at a low, sub therapeutic dose for the purpose of performance enhancement. Antimicrobial growth promoters are used to "help the animals to digest their food more efficiently, get maximum benefit from it and allow them to develop in to strong and healthy individuals". As prevention of diseases, enhancement of growth and feed efficacy are crucial to vital animal husbandry business, the use of AGP is increasing day-by-day (Ellin Doyle, 2001).

The specific physiological basis of the growth promoting effects of antibiotics is unknown, but is hypothesized to involve a nutrient sparing effect in the gut and selective suppression of species of bacteria and clinical expression of infection, i.e., disease prophylaxis (McEwen and Fedorka-Cray, 2002).

Existence of antibiotic residues in food stuff can pose hazards to human health. Among them are sensitivity to antibiotics, allergic reactions and imbalance of intestinal microflora, bacterial resistance to antibiotics in microorganisms and losses in the food industry (Cunha, 2001; Kirbis, 2006; Lolo *et al.*, 2006). So, quality control of food stuff regarding to antibiotic residues, is necessary (Salehzadeh *et al.*, 2007).

2.1 Antibiotic residues in chicken feed, meat and/or animal products

The poultry sub-sector of livestock sector is an essential part of fostering agricultural growth and reduces malnutrition for the people of Bangladesh (Hamid *et al*., 2017). It is an integral part of farming system in Bangladesh which created direct and indirect employment opportunity including support services near about six million people (Hamid *et al*., 2017; Raihan and Mahmud, 2008). Unfortunately, the research findings from Bangladesh showed presence of antibiotic residues and heavy metals in poultry feed, meat and eggs at public health concerning level (Bhuiyan *et al*., 2021, Bristy *et al*., 2019; Islam *et al*., 2019; Rashid *et al*., 2018).

Adulteration of poultry meat and products is a serious public health concern which have detrimental effects for human life and health. Poultry meat and poultry feed can be contaminated by variety of contaminates (Sarker *et al*., 2020; Altherwi *et al*., 2018). Among them principle contaminants are heavy metals, antibiotics, metabolites, microorganisms, mycotoxins, hormones, polychlorinated biphenyls, genetically modified organisms, nitrates and nitrites, toxic pigments, pesticide residues, dioxins, melamine and so one (Sarker *et al*., 2018; Hu *et al*., 2017).

Antibiotics are the chemical agents which were formulated for saving the life of people and livestock from infections of bacteria (Hyung *et al*., 2017). The imprudent use of antibiotics both for prophylactic and therapeutic purposes has considered as global concern due to its residual effect, and subsequent adverse health hazards of consumers (Ferdous *et al*., 2019; Chanda *et al*., 2014). More

importantly, in unauthorized veterinary practice, antibiotics are used illegally as growth promoter in the livestock and poultry instead of therapeutic (Altherwi *et al*., 2018; Talukder *et al*., 2017). Thus the wide spread use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which include; carcinogenicity, mutagenicity, bone marrow toxicity, allergy (Paul *et al*., 2020; Nisha, 2008) as well as appearance of a resistant strains of pathogenic bacteria (Hussein and Khalil, 2013).

In poultry, antibiotic usage had facilitated their efficient production and also enhanced the health and well-being of poultry by reducing the incidences of disease, but unfortunately, unauthorized use of these antibiotics, the failure to follow label directions or inappropriate withdrawal period of time before slaughtering of animals could lead to contamination of edible poultry tissue with antibiotic residues, with potential adverse effects on human health (Donoghue, 2003). Antibiotic residues in foods of animal origin are one of the sources of concern among the public and medical health professionals (Adams, 2001).

Alam *et al*. (2021) carried out to identify and quantify antibiotic residues, heavy metals and toxins in poultry feed and meat in the two selected poultry production belts of Bangladesh and reported that presence of harmful contaminants and residues in poultry feed and meat have serious public health consequence. A total of 94 broiler feed samples and 60 broiler meat samples were collected and tested by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Enzyme-linked Immunosorbent Assay (ELISA) for identification and quantification of the parameters. Antibiotic residues were detected in 18.89% of the feed samples, whereas, there were no toxin (Aflatoxin) positive samples. Among the antibiotic positive samples, Oxytetracycline (OTC) was found predominant and detected in 12.22% cases. The mean concentrations of Cadmium (Cd),

Lead (Pb), Chromium (Cr) were found as 0.04 mg/kg, 1.28 mg/kg and 2.55 mg/kg respectively in feed samples. In the case of meat samples, the mean concentration of OTC, Ciprofloxacin (CIP), and Tetracycline (TCL) residues were found 8.67 µg/kg, 7.18 µg/kg and 0.81 µg/kg accordingly. The highest mean concentration of Oxytetracycline (OTC) (10.15 µg/kg) was found in samples collected from local poultry sellers, whereas, the highest mean concentration of Tetracycline (TCL) (1.35 µg/kg) and Ciprofloxacin (CIP) (10.62 µg/kg) were observed in the samples obtained from local farm. The highest percentage of TCL and CIP (64% and 48% respectively) were found in samples collected from local farm. Chlortetracycline (CTC) was found predominant (70%) in samples collected from Contract farms. On the other hand, out of 60 meat samples, Cd and Cr were detected in only one meat sample with concentration of 56.41 mg/kg and 14.44 mg/kg respectively. Lead was not detected in any of the meat samples.

Bahmani *et al*., (2020) carried out a study for monitoring and risk assessment of tetracycline residues in foods of animal origin. A total of 450 samples consisting of red meat, poultry meat, aquatic product and raw milk were collected during winter 2016 and summer 2017. 22.2% (100/450) of collected meat and raw milk samples were found to be contaminated with antibiotic residues in the initial screening. According to the enzyme linked immunosorbent assay (ELISA) results, the mean tetracyclines (TCs) concentration of meat samples determined as follows: chicken (155.41 lg/kg) [turkey (138.68 lg/kg) [quail (130.7 lg/kg) [cow (108.92 lg/ kg) [calf (105.18 lg/kg) [goat (99.4 lg/kg) [sheep (95.22 lg/kg) [rainbow trout (35.62 lg/kg) [shrimp (31.80 lg/kg). The content of TCs in cow, goat and sheep milk samples were found to be ranged 45.6–163.5 lg/L, 72.4–101.1 lg/L and 65.5–98.9 lg/L, respectively. 5.7% (26/450) of samples had TCs confirmed the ELISA results using high performance liquid chromatography coupled with ultra-violet detection, although the concentration of TCs residues in samples was higher than that of ELISA.

Antibiotics are mainly employed for chemotherapeutic, prophylactic purposes and also used as feed additives to promote growth and improve feed efficiency. However, Jayalakshmi *et al*. (2017) conducted a study on antibiotic residues in animal products and its impact on environments and human health and reported that antibiotic residues in animal products may occur, when administration of drug in extra label fashion and not following of withholding period after treatment. Many of the administered drugs are not completely absorbed from gut and excreted through faeces and urine as either parent compound or its toxic metabolites. The antibiotic resistance in animal and human leads to poor response to treatment during illness. The antibiotic residues in animal product causes harmful effect on health and also interfere with the processing of animal food products.

Sarker *et al*. (2017) conducted a study with the objectives of screening of antibiotic residues in broiler chicken meat and liver collected from farms and local markets in Bangladesh. A total of 160 samples (breast, thigh muscle and liver) were collected from markets and farms from different region of Bangladesh. PBS buffer system with trichloracetic acid and diethyl ether based sample extraction was performed. For comparison the standard antibiotics; Ciprofloxacin (CIP), Enrofloxacin (ENR), Oxytetracycline (OTC), Amoxicillin (AMOX) and Doxycycline (DOX) were prepared by dissolving in methanol. Samples were pointed on TLC plates transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. Retention factor (Rf) was calculated after observing the chromatograms on UV light at 256 nm. Same Rf value of standard and sample considered similar compound. Liver sample were mostly positive for antibiotics residue followed by thigh muscles and breast muscle. The frequency of antibiotic residues was highest in liver followed by thigh muscles and breast muscle. Among the antibiotics CIP ranked top in all types of sample. In breast muscle highest antibiotic was CIP (39%) followed by DOX (26%), AMOX (24%), OTC (23%) and lowest was ENR (21%). In thigh muscle, 42, 29, 28, 27 and 24% sample was positive for CIP, OTC, DOX,

AMOX and ENR, respectively. Highest number of liver samples were shown positive result for all screened antibiotics (CIP-52%, OTC-46%, DOX-43%, AMOX-42% and ENR-36%). This study ascertained those antibiotics residues are present in chicken muscle and liver which causes serious health hazards to consumers.

Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro Municipality was done by Nonga *et al.* (2009). Seventy broiler chicken liver samples were collected for quantitative antimicrobial residue analysis by use of two parallel tests; agar well diffusion and Delvotest SP® assay. The results indicated that 70% of the farms are positive for antimicrobial residues. Ninety percent of the respondents had knowledge on antimicrobial withdrawal period. However, 95% of farmers slaughtered their chicken before withdrawal period because they were afraid of losses and unaware of the effects of antimicrobial residues in humans. They suggest that poultry farmers need to be educated on the possible effects associated with use of food with antimicrobial residues.

The assessment of antibiotic residues in broiler chicken collected from farms located in Tanzania showed that 70% of the farms were positive to antimicrobial residues (Nonga *et al.*, 2009).

Shahid *et al.* (2007) conducted a study to monitor the presence of antibiotic residue in poultry meat. Swab test on food animal employing *Bacillus subtilis* as test organism on nutrient agar was used. A total of 100 tissue samples (33 liver, 33 kidney and 34 muscles) were collected from local market of Rawalpindi and Islamabad. Thirteen liver (39.4%), 9(27.3%) kidney and 7(20.6%) muscle sample were detected positive for antibiotic residues.

Another study conducted in Iran has screened enrofloxacin residue in chicken muscles collected from 90 broilers farms in Tahran. The study findings showed that 24% of the farms showed residues above the MRLs (Salehzadeh *et al.*, 2007).

Antibiotic residues in chicken meat samples have been reported by many research studies. Antibiotic residues were reported in broilers commercialized in Nigeria, with a high incidence rate (33.1%) (Kabir *et al.*, 2004).

Alhendi *et al*. (2000) studied drug residues in broiler chickens fed with antibiotics in ration. Daily oral administration of two dose levels of 1 and 2 mg/kg body mass of ampicillin (groups A1 and A2), 50 and 100 mg/kg body weight of oxytetracycline (groups O1 and O2) and 50 and 100 mg/kg body mass sulphadimidine (groups S1 and S2), in broiler feed resulted in an immediate increase in concentrations of antibiotics in plasma and tissues from day 1 until day 40 of the treatment. At day 40 a range of 0.61 to 1.94, 0.24 to 2.25, 1.30 to 6.70 µg/g or µg/ml of A, O and S, respectively was found in tissues or plasma. Withdrawal of medicated feed resulted in a rapid decline in tissue concentration parallel to that of plasma, and withdrawal times were 5 days for oxytetracycline and sulphadimidine and 6 days for ampicillin.

Al-Ghamdi *et al.* (2000) collected chicken muscle, liver and egg samples from 33 broiler and 5 layer farms in the eastern province of Saudi Arabia. Antibiotic-residue positive samples were identified in the products of 23 (69.7%) broiler and 3 (60%) layer poultry farms. All the antibiotic-residue positive broiler farms were positive for at least one tetracycline compound in raw muscle (87%) and liver (100%) respectively, while 73.9% broiler farms were positive for 2 or more tetracyclines in these two tissues. Furthermore, 82.6% of the antibiotic-residue-positive farms had mean concentrations of at least one tetracycline compound in excess of the permissible maximum residue limit (MRL) in raw muscle and liver. This study confirmed widespread misuse of tetracycline agents including multiple uses of drugs belonging to the same pharmacological group and lack of implementation of recommended withdrawal times.

2.2 Toxicity of antibiotic residues

Veterinary Antibiotics (VAs) are commonly being used by the farmers and veterinary practices, that may lead to drug residues from food animals to human, and subsequently adverse health hazards may develop to the consumer (Chanda *et al*., 2014). Significant level of exposure of antibiotic residues from animal food products to individual may modulate immunological responses and can detrimentally affect intestinal microbiota in susceptible individual (Ramatla *et al*., 2017).

About 60% of an ingested dose of oxytetracycline is absorbed from the gastrointestinal tract and widely spread in the body (Doyle, 2006; Mund *et al*., 2017). In recent years, many studies have shown that antibiotics administered to poultry and livestock were accumulated in liver, kidney, muscle and bones exceeding the Maximum Residual Limits (MRL) (Sarker *et al*., 2016).

The residues of antibiotics or its metabolites in meat and other foods of animal origin may cause adverse effects on consumers' health. The presence of residues and its associated harmful health effects on humans makes the control of antibiotics residues an important measure in ensuring consumer protection. Other important effects mainly due to the presence of residual antibiotics consist in allergic reactions or the selection of resistant bacteria that could be transferred to humans through the food chain (Butaye *et al.*, 2001). In addition, the consumption of trace levels of antibiotics residues in foods from animal origin may have consequences on the indigenous intestinal microflora which constitutes an essential component of human physiology. This flora acts as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria (Vollard and Clasener, 1994) and has an important role for food digestion. So, the ingestion of trace levels of antimicrobials in foods must take into account potentially harmful effects on the human gut flora (Cerniglia and Kotarski, 1999).

A number of possible adverse health effects of antibiotics residues have been suggested. These include the following:

1. Allergic/Anaphylactic reactions to residues.
2. Chronic toxic effects occurring with prolonged exposure to low levels of antibiotics.
3. Development of antibiotic-resistant bacteria in treated animals. These bacteria might then cause difficult-to-treat human infections.
4. Disruption of normal human flora in the intestine. Antibiotics might reduce total numbers of these bacteria or selectively kill some important species.

Antibiotics are among the essential veterinary medicine compounds associated with animal feed and food animal production. Arsene *et al*. (2022) reported that the use of antibiotics for the treatment of bacterial infections is almost unavoidable, with less need to demonstrate their importance. Although banned as a growth factor for a few years, their use in animals can add residues in foodstuffs, presenting several environmental, technological, animal health, and consumer health risks. With regard to human health risks, antibiotic residues induce and accelerate antibiotic resistance development, promote the transfer of antibiotic-resistant bacteria to humans, cause allergies (penicillin), and induce other severe pathologies, such as cancers (sulfamethazine, oxytetracycline, and furazolidone), anaphylactic shock, nephropathy (gentamicin), bone marrow toxicity, mutagenic effects, and reproductive disorders (chloramphenicol). Antibiotic resistance, which has excessively increased over the years, is one of the adverse consequences of this phenomenon, constituting a severe public health issue, thus requiring the regulation of antibiotics in all areas, including animal breeding.

Mubito *et al*. (2014) designed a structured questionnaire for poultry farmers and pharmaceutical outlets/shops to obtain information on antibiotic usage, awareness of withdrawal periods and public health concerns on drug residues.

Of 100 layer's chicken production farmers interviewed in this study, the average flock size was 560. All interviewed participants apply antibiotics through oral route of drug administration. Of these participants, 93% treat their chicken in accordance with directives from veterinary drug vendors, 4% follow the manufacturer's instructions and 3% depends on their own experience. The survey found that most frequently used antibiotic drugs belong to the group of tetracycline and sulfonamides. All interviewed poultry farmers were aware of drug withdrawal period but none of them declared to observe this requirement because they fear investment losses. Authors concluded that there is high risk of exposure to unacceptable levels of drug residues from poultry products, as a result of failure to observe antibiotic withdrawal periods.

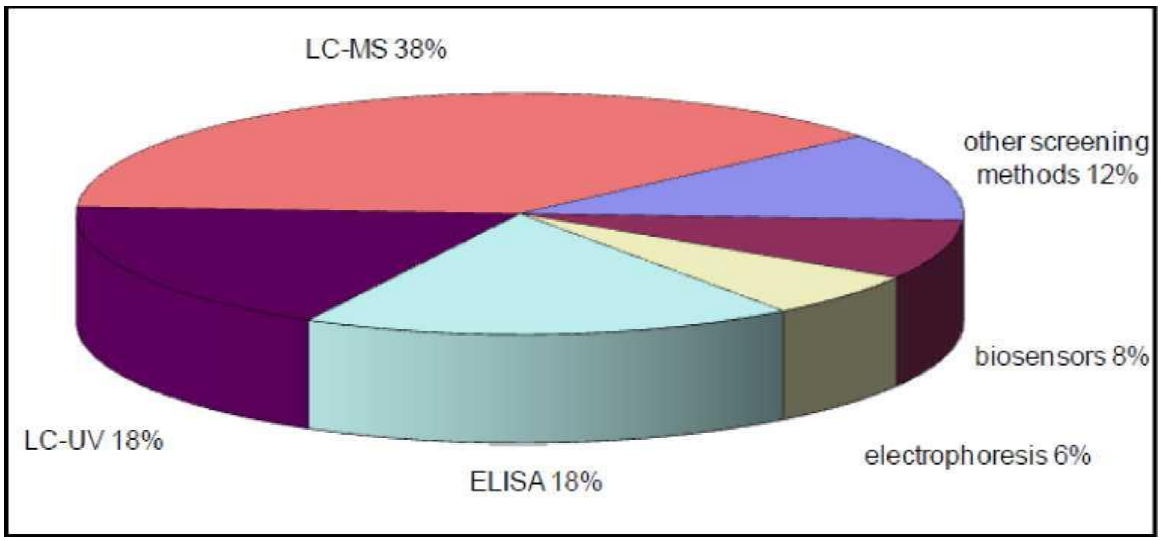
Annan-Prah *et al*. (2012) investigated the contributory factors for antibiotic uses and abuses as well as the public health implications thereof by means of questionnaires, interviews and on-the-spot inspections. Their results indicated that 31 drugs - antibiotics, coccidiostats and an anthelminthic were being overly used to cover up husbandry and hygiene lapses and to make economic gains in terms of their constant use as growth promoters during the birds" lifetime. Taking advantage of the situation, manufacturers have produced and marketed poultry feed supplements loaded with tylosin, chloramphenicol, tetracycline and neomycin, antibiotics that have been banned in feed for food-producing animals. From market-ready eggs from these farms, antibiotic residues to which *E. coli, S. aureus* and *B. subtilis* were susceptible to were detected. Antibiotic residues were generally more concentrated in the albumen than in the yolk.

2.3 Determination of antibiotics residue by chromatographic methods

The identity and quantity of the residue in a suspected sample cannot be determined with a screening test. Hence the decision about the compliance of a sample cannot be based on a screening result. Therefore there is a need for specific chromatographic or other confirmatory methods.

At the beginning of the twentieth century, the Russian botanist Mikhail Tswett invented and named chromatography. He separated plant pigments by passing solution mixtures through a glass column packed with fine particles of calcium carbonate. The separation of those pigments appeared as colored bands on the column. Tswett named his separation method for the two Greek words "chroma" and "graphein" which mean "color" and "to write," respectively (Skoog *et al.*, 1998). In the past six decades, chromatography has been extensively applied to all branches of science. The 1952 Nobel Prize in chemistry was awarded to A. J. P. Martin and R. L. M. Synge for their contributions to chromatographic separations, which tremendously impacted chemistry-related sciences. More impressively between 1937 and 1972, a total of 12 Nobel Prizes were based on work in which chromatography was a key tool.

In all chromatographic separations, the sample is carried by the mobile phase, which may be a gas, a liquid, or a supercritical fluid. The mobile phase is then percolated through an immiscible stationary phase that is fixed on a solid substrate. When the sample passes through the stationary phase, species are retained to varying degrees as a result of the physicochemical interaction between the sample species and the stationary phase. The separation of species appears in the form of bands or zones resulting from various retentions. Chemical information can thus be analyzed qualitatively and/or quantitatively on the basis of these separated zones.



Distribution of the analytical methods used for the determination of antibiotics in food (Chafer-Pericas *et al.,* 2010)

High Performance Thin layer chromatography (HPTLC) is a sensitive and reliable method for monitoring low amounts of different biological and chemicals. HPTLC allows the qualitative and quantitative detection of multi-residues in meat. The plates are sprayed with an appropriate chromogenic reagent or viewed under UV light for visualisation of compounds. Detection by fluorescence is also applied. Quantification is achieved by measuring the relative intensity of the spot verses that of the internal standard by scanning densitometry. Modern HPTLC has been automatised at a high level. Determination of drug residues in food is an important application of HPTLC. Milk and meat samples (beef, pork, fish, poultry, etc) can be subjected to analysis of multiclass, multiresidue screening.

Jammoul and Darra (2019) conducted a study on harmful effects on consumer health due to antibiotic residue in chicken. This study aims at screening the antibiotic residues from 80 chicken samples collected from farms located in different regions of Lebanon. An optimized multi-class method for identification and quantification of 30 antibiotics from four different chemical classes (sulfonamides, tetracyclines, quinolones, and beta-lactams) has been developed by using liquid chromatography–mass spectrometry. The evaluation

of antibiotics residues in 80 chicken muscles samples has shown that 77.5% of samples were at least contaminated with antibiotics residues, out of which 53.75% were exposed to co-occurrence of multidrug residues. The screening of the four antibiotics families has shown that ciprofloxacin (quinolones) represents the highest occurrence percentage (32.5%), followed by amoxicillin (β-lactams) (22.5%) and then tetracyclines (17.5%). Means of sarafloxacin, amoxicillin, and penicillin G residues levels were above the Maximum Residue Limit (MRL) recommended limit according to the European Union EC. This study revealed that chicken samples collected from Lebanese farms contain antibiotic residues. Guidelines for prudent use of antimicrobials agents for chicken should be adopted to reduce the prevalence of resistant Salmonella in chicken.

Boix *et al*. (2014) did a qualitative screening of 116 veterinary drugs in feed by liquid chromatography–high resolution mass spectrometry. All compounds tested were detected at 0.02 mg kg^{-1}, based on the presence of the accurate-mass deprotonated molecule. The procedure was applied to 22 feed samples, where trimethoprim, robenidine, or α and β nandrolone were detected and identified.

Wageh *et al*. (2013) did monitoring on antibiotic residues in food of African scenario and they have reported that tetracyclines were the most predominantly prescribed antibiotics in Africa, and of all antibiotic-associated residues, tetracyclines represent 41% of cases, followed by β-lactams at 18%.

Omeiza *et al*. (2012) performed a survey on chloramphenicol residues in eggs using thin layer chromatography and found that 11 out of 144 eggs samples were containing chloramphenicol residues in excess of MRL recommended guidelines.

Lopes *et al*. (2012) developed and validated a methodology to qualitatively screening veterinary drugs in porcine muscle via an innovative extraction/clean-up procedure and LC-MS/MS analysis. Besides the high

selectivity, sensitivity and specificity, this high-throughput method proved to be quite general as 34 veterinary drugs (from six distinct classes: tetracyclines, sulfonamides, penicillins, quinolones, macrolides and benzimidazoles) could be successfully detected.

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

Nikolaidou *et al*. (2008) developed and validated a HPLC method for determination of seven tetracycline antibiotic residues in chicken muscle. Overall recoveries were ranged from 92-110.1% and 89-106% for chicken tissues and eggs, respectively. All the relative standard deviations values were lower than 11%. The detection limits (CCα) in chicken tissues ranged from 102.98 to 109.11 µg/kg, while detection capabilities (CCβ) from 113.4 to 118.1 µg/kg. Respective values in eggs were 206.5-214.6 µg/kg for (CCα) and 216.2-228.9 µg/kg. Respective values in eggs were 206.5-214.6 µg/kg for (CCα) and 216.2-228.9 µg/kg for (CCβ).

Donkor *et al*. (2011) determined the prevalence of antibiotic residues in animal source food and estimate the risk to consumers and identify factors

predisposing animal source food to contamination with antibiotic drug residues. Overall, the prevalence of drug residues in animal source food was 21.1%, which translates to an average risk of exposure every fifth time animal source food was consumed. The prevalence rates of drug residues in the various animal source foods were; 30.8% (beef); 29.3% (chevon); 28.6% (pork); 24% (mutton); and 6.8% (egg).

Shahid *et al.* (2007) conducted a study to monitor the status of oxytetracycline (OTC) residue in poultry meat in Rawalpindi and Islamabad area of Pakistan. The preliminary screeninfg of samples for the presence of antibiotic residues was performed by a microbiological assay using *Bacillus subtilis* as test organism. OTC in positive sample is detected and quantified using high performance liquid chromatography (HPLC). A linear calibration curve was obtained with correlation coefficient of 0.9981 while average recoveries were greater 91% with RSD values between 1.64 to 2.07% while the limit of detection (LOD) was 0.01 Mg/ml. Out of 29 meat samples that were analyzed for OTC residues, 13 (44.8%) had detectable residue level for OTC and 6 (20.7%) had higher residue level then the recommended maximum residue level (0.2, 0.6 and 1.2 Mg/gm) for muscles, liver and kidney, respectively.

Lee *et al*. (2007) conducted a study in agricultural and fishery products. A total of 13 antibiotics, including tetracycline, macrolide, penicillin, aminoglycoside, polyester, peptide and chloramphenicol types were analyzed by a microbial assay and high performance liquid chromatography. In the microbial assay, 34 of the 459 screened samples had possible antibiotic residues. The antibiotic concentrations of the 34 samples were analyzed using HPLC with UV and fluorescence detection. The levels of oxytetracycline in pork and eel were 0.01 and 0.05 mg/kg respectively. In eel and oyster, the concentrations of ampicillin were 0.4 and 0.32 mg/kg respectively while in beef, the concentration of tylosin was 0.05 mg/kg.

Biswas *et al.* (2007) developed a simple, rapid and sensitive method for residue monitoring of oxytetracycline, tetracycline and chlortetracycline in buffalo meat samples. The principal steps involved extraction in McIIvaine buffer (pH 3.85) followed by a solid phase clean up step. In HPLC, a reversed phase C8 (RP-C8) column was used and compounds were separated at 35°C using a mobile phase of 0.01 M oxalic acid buffer (pH 1.6)/acetonitrile/methanol (77:18:5, v/v/v) at a flow rate of 0.6 mL/min. A wavelength of PDA detector was set at 355 nm. The detection limit of the method was calculated to be 0.031 ^g/g and the minimum detectable quantity was found to be 0.062 µg/g.

Naeem *et al.* (2006) conducted a study for the estimation of quinolones in 150 poultry samples (120 samples of liver, kidney and muscles and 30 samples of egg) purchased from local markets in Lahore, Pakistan. The quinolones included in the study were ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, ofloxacin, flumequine, oxolinic acid and nalidixic acid. The poultry products included muscle, liver, kidney and egg. Ten gram of each of the samples of liver, muscle, egg and five gram of kidney samples were used for extraction and High Performance Liquid Chromatography (HPLC) system is used for determination of quinolones. The result indicated that 58 to 85% of ciprofloxacin and 55 to 92% samples of enrofloxacin violated the regulation. They concluded that enrofloxacin occur most abundantly and widely in the products and liver and kidney are the most contaminated part of chicken than muscle and egg. They found enrofloxacin concentration in the range of 3.10 to 364 µg kg^{-1} in liver.

Tajick and Shohreh (2006) worked on detection of antibiotic residue in chicken meat using TLC. Nowadays antibiotics are applying for control of infectious diseases. Incorrect use of these drugs deposits some residue in product. This research highlights the importance and existence of antibiotics residue in meat. In this survey 10 grams of chicken meat crashed and squeezed in 10 ml ethanol. After clarifying by centrifuge the solvent evaporated totally. After

loading and running on silica F256 plates, the chromatograms observed on UV light. The results showed more than 50% of meat samples had noticeable antibiotics residue.

Amjad *et al.* (2005) done analysis and comparison of selected residual antibiotics in broiler chicken available in local market. The broiler samples included muscle, kidney and liver. The quinolones included in this study were, oxolinic acid, nalidixic acid, flumequine, enrofloxacin, norfloxacin and ciprofloxacin. The intertissue/organ comparison within each analytical technique and intermethod comparison of results obtained by HPLC, UV spectroscopy and ion association complex techniques were made. TLC was used to separate and identify the quinolone residues. HPLC with ODS column and UV detector and UV/ visible spectroscopy were used for quantification of the residues. Good compatibility of the spectrophotometric results was found with those of high pressure liquid chromatography.

Ng and Linder (2003) compared laser based polarimetric detection with UV detection. A sensitive high performance liquid chromatography method based upon laser based polarimetric detection was developed for the determination of six tetracycline analogues. The six structurally similar tetracycline analogue exhibit significant difference in the specific rotations. Their experiment suggested that specific rotation can be useful in identifying closely related tetracycline analogues. Linear relationships were found to be in the range of 0.342 – 0.0043 mg for the tetracycline analogue. Five of six analogues exhibit excellent linearity (R2 value $\geq$ 0.99). The HPLC-laser based polarimetric detection instrument was able to quantitate the studied tetracycline analogues with high precision, accuracy and sensitivity which make it useful for the development of a standard method for the determination of tetracycline in biological specimen.

Paturkar (2002) did antimicrobial residue surveillance in food animals. For this bioassay and chromatographic methods were used such as TLC/HPTLC and

HPLC to detect and quantify tetracycline and sulfonamide group of antimicrobials. Out of 572 analyzed meat samples 8, 22, 10 and 2 were found to be positive for tetracycline, oxytetracycline, sulphadimidine and sulphadiazine residues, respectively.

Oka *et al.* (2000) designed an improved method for HPTLC analysis of tetracyclines. The plate is predeveloped with a saturated Na_2EDTA aqueous solution and is activated before applying the sample. Using this predeveloping technique, they have reported the successful separation of eight TCs on a silica gel high-performance TLC plate with a solvent system of chloroform-methanol-5% Na_2EDTA (65:20:5, lower phase) and applied this TLC technique to the analysis of the eight residual TCs.

Choma (2000) determined four tetracyclines in milk by a simple thin layer chromatographic method. They perform HPTLC of tetracyclines on silica gel (with concentration zone, impregnated with 5% aqueous Na_2EDTA solution) with the lower layer of chloroform: methanol: 5% aqueous Na_2EDTA 13:4:1 after a predevelopment with n-hexane and acetone to remove lipid fractions. Detection of fluorescent spots was done at 254 and 366 nm. Detection limit is found to be 0.1 µg (two fold development with mobile phase).

Furusawa (1999) described HPLC method for determining residual oxytetracycline in chicken muscle, liver and eggs. Oxytetracycline was extracted by using acetonitrile. It was observed that oxytetracycline in acetonitrile layer was free from interfereing compounds when examined by HPLC using a Lichrospher 100 RP-8 end- capped column with photodiode array detector. Average recoveries from spiked samples with oxytetracycline were >88% and limit of detection was 0.05 ppm.

De Ruyck *et al*. (1999) used HPLC with ion pairing chromatography and diode-array detection at 355 nm to detect tetracycline antibiotics in eggs and broiler meat. The mean recoveries for oxytetracycline for eggs and for tetracycline for breast broiler meat were 76%. The within day precision ranged

from 8.0 to 11.8% for oxytetracycline in eggs and from 6.1 to 15.5% for tetracycline in breast broiler meat, while between day precision was 4.8% and 5%, respectively for oxytetracycline in eggs and tetracycline in breast broiler meat. The limit of detection and the limit of quantification for oxytetracycline in eggs were 2.20 and 13.0 ηg/g, respectively. These limits for tetracycline in breast meat were 10.5 and 20.9, respectively.

Oka *et al.* (1987) develop method of TLC of residual oxytetracycline, tetracycline, chlortetracycline, doxycycline, methacycline, dimethylchlortetracycline and monocycline in honey on silica and RP-8 silica with chloroform- methanol-5% aqueous Na_2EDTA 65:20:5 (lower phase) and methanol - acetonitrile - 0.5M aqueous oxalic acid 1:1:4 (pH 3.0), respectively. Observation done under UV at 360 nm. Detection limit of this method was found to be 0.1 ppm.

2.4 Health risk and maximum residue limits

It is challenging to control the use of antibiotics in agriculture in a uniform way because their use varies significantly from one country to another. For example, it was reported that China is the first country to use antibiotics in food animals (23%), followed by the United States (13%), Brazil (9%), India (3%), and Germany (3%) (Boeckel *et al*., 2015). This statistic seems disproportionate because it assumes that other countries use fewer antibiotics than the five countries. However, international and national regulatory agencies, such as Food and Agriculture Organization/ WHO, Food and Drug Administration, Canadian Food Inspection Agency, the Australian Pesticides and Veterinary Medicines Authority, European Commission, European Food Safety Authority, and the Ministry of Health of each country, continuously attempt to regulate antibiotic use with international standards, considering the specific realities of each country. This harmonization mainly involves the control of parameters, such as (1) acceptable daily intake (ADI), which is a critical standard set from toxicological studies based on the no-observable-effect level and safety factor

(Okocha *et al*., 2018); (2) withdrawal period or waiting time (WT), which refers to the minimum period from the administration of the last dose of medication and the production of meat or other animal-derived products for food (Okocha *et al*., 2018 and Aroeira *et al*., 2021); and (3) MRL, which is the highest level of an antibiotic residue or its metabolites that is legally tolerated in food when antibiotics are correctly applied following Good Agricultural Practice (Yang *et al*., 2020). Although the ADI, WT, and MRL for most antibiotics have been established (for each food) and efforts have been made to regulate the MRL worldwide under the aegis of the World Trade Organization and the Codex Alimentarius, MRLs still vary from one geographical location to another. Meanwhile, although this situation seems to be under control in the European Union countries and other developed countries (Okocha *et al*., 2018), the problem of antibiotic residues remains topical with unprecedented danger in developing countries due to the lack of control mechanisms despite the existing legislation.

Several antibiotic classes are extensively administered to food-producing animals, including tetracyclines, sulfonamides, fluoroquinolones, macrolides, lincosamides, aminoglycosides, beta-lactams, cephalosporins and others (Woodward, 2004 and Jank *et al*., 2017). Almost 90% of all antibiotics used in farm animals and poultry are reported to be administered at sub-therapeutic concentrations. About 70% of this is for the purpose of disease prevention and 30% are for growth promotion (Kebede *et al*., 2014). The risk of residue from the milk is higher in developing countries compared to develop one. This might be related with lack of facilities for detection and regulatory bodies that control the drug residues level in foods in the form of maximum residue limits (MRLs) (Kebede *et al*., 2014). The MRL is defined as the maximum concentration of a residue, resulting from the registered use of an agricultural or veterinary chemical that is recommended to be legally permitted or recognized as acceptable in or on a food, agricultural commodity, or animal feed (Jayalakshmi *et al*., 2017).

A number of national and international organizations are involved in the legislation on residues of veterinary drugs in foods. Countries lend to follow their own guidelines. The Food and Agricultural Organization (FAO) and Word Health Organization (WHO) have set up a Joint FAO/WHO "Codex Alimentarius Commission" to coordinate food standards throughout the world. One of the main tasks of Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) is to establish worldwide Maximum Residue Limits (MRLs). Other international groups active in this area include the European Agency for Evaluation of Medicinal Products (EAEMP), Office International des Epizootics (Oil-) and Consultation Mondiale de I' Industrie de la Sante Animale (COMISA) (Mitchell *et al.,* 1998). Several countries have the specialist groups, i.e., Food and Drug Administration (FDA), USA; Bureau of Veterinary Drugs, Canada and Veterinary Products Committee (Ministry of Agriculture, Fisheries and Foods), UK (Telling, 1990).

The limits of drug residues in foods have been established in the form of tolerances or maximum residue limits (MRLs). The term tolerance is used in United States while MRLs is used in Canada and European Union but these two terms are synonyms (Brynes and Young, 1993). MRL is defined as maximum concentration of residue following administration of a veterinary medicine, which is legally permitted or acceptable in foods and foodstuffs. The MRL is based on the Acceptable Daily Intake (ADI) for that compound. The ADI is rough estimate of the amount of a veterinary drug expressed on a body weight basis that can be ingested daily over a lifetime by a person without any appreciable toxicological risk (Brynes *et al.,* 1996). The Codex maximum residue limits (MRLs) set for OTC/TC/CTC (alone or combination) are 0.1 Mg/g in muscle tissues.

Overview of European Union maximum residue limits (μg/ kg)

Pharmacologically active substance	Animal species	Commission regulation	Target tissues	
			Muscle	Liver
Amoxicillin	All food producing species	508/1999	50	50
Tetracycline	All food-producing species	508/1999	100	300
Oxytetracycline	All food-producing species	508/1999	100	300
Enrofloxacin	Porcine, poultry, rabbits	1181/2002	100	200

MRLs have been determined by various committees and then included in legislation (Food and Drugs Act and Regulations in Canada, List of Codex MRLs for Veterinary Drugs, Official Journal of European Communities, Code of Federal Regulations in the United States) for animal products such as meat, eggs and milk (Code of Federal Regulations, 1994; Codex Alimentarius Commission, 1993).

Withdrawal period is the time between the last recommended treatment and time of slaughter (meat) or collection for use as foods (milk and egg). This time allows the veterinary drug and its residues to decrease to levels below the established MRL. Until the withdrawal period has elapsed the animal or its products are not fit for human consumption. It varies with each drug preparation and target animals. Depending upon the drug, products, dosage, and route of administration, it varies from a day to several days or weeks (Lee *et al.,* 2001). The involvement of many organizations in the legislation of veterinary drugs has made it very difficult to standardize control practices and harmonize tolerance levels internationally in a uniform manner. The differences in tolerance levels are mainly due to differences in the use of compounds, food habits, choice of safety factors and food consumption

values (Brynes and Young, 1993). Therefore, it has been proposed that the ADI is better choice for determination of food safety rather than MRL (Brynes *et al.*, 1996).

Nisha (2008) reported deposition of antibiotic residues in meat, milk and eggs and concluded that if use of antibiotics is necessary as in prevention and treatment of animal diseases, a withholding period must be observed until the residues are negligible or no longer detected.

Antibiotic residues are a severe public health issue (Beyene, 2016), with their presence in foods capable of causing mild to adverse complications that are difficult to manage. Some antibiotic residues, such as sulfamethazine, oxytetracycline and furazolidone, have carcinogenic effects (Bacanli *et al*., 2019; Darwish *et al*., 2013; Treiber and Beranek-Knauer, 2021). Aside from their carcinogenicity, other effects, including bone marrow toxicity (mainly due to chloramphenicol) and nephropathy (mainly due to gentamicin), were also reported (Bacanlı *et al*., 2019). Beyond all these adverse effects, antibiotic resistance, an indirect consequence of antibiotic residues in food, is the most catastrophic issue, with the WHO estimating that if nothing is done to address this problem, drug-resistant diseases may cause 10 million deaths each year by 2050, consequently damaging the economy as catastrophic as the 2008-2009 global financial crisis (Mbarga *et al*., 2021).

Hautekeete (1996) reported that penicillin, oxacillin, cloxacillin, flucloxacillin, and amoxicillin-clavulanate could cause hepatitis (mainly cholestatic). He also reported that tetracyclines could cause a syndrome mimicking acute fatty liver of pregnancy, indicating the potential of erythromycin and several other macrolides to cause hepatitis (usually cholestatic) (Hautekeete, 1996). Furthermore, Van Gerven *et al*. (2016) and Hautekeete (1996) reported that nitrofurantoin could cause chronic hepatitis mimicking chronic autoimmune hepatitis acute cholestatic and hepatocellular reactions. Finally, it has been reported that ceftriaxone can cause drug-induced gallstones and quinolone

cholestasis, and sulfamethoxazole/trimethoprim can cause severe hepatotoxicity, especially in patients with acquired immunodeficiency syndrome (Hautekeete, 1996).

It is evident from the aforesaid reported that information regarding the present topic of research is meager and scanty in Bangladesh. A few published report findings failed to reach a conducive idea about the persistence of antibiotic residues in chicken feed and meat. It justified the research gap of this problem oriented research topic. Extensive research should be conducted in all parts of Bangladesh as because, this is an issue of food safety.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area and duration

The research work was conducted in the Food Toxicology Research Laboratory, (IFST) at Bangladesh Council of Scientific and Industrial Research (BCSIR) and a part of the sample processing was done at the Laboratory of Department of Agricultural Chemistry, at Sher-e-Bangla Agricultural University. The study was done on broiler and layer and poultry feed on antibiotic residue at Sirajgonj and Gazipur district, Bangladesh, during the period of March 2020 to June, 2021.

3.2 Surveillance study

The antibiotics which are more frequently used, or at least which are detected most often in the carcasses or meat, information was gathered by conducting a surveillance study pertaining to use of antibiotic in various poultry farms located in Sirajgonj and Gazipur district, Bangladesh. The information was also gathered to shortlist most commonly and frequently used two antibiotics in chickens for further investigations and hence tetracycline and oxytetracycline were short listed for determination of their residual concentration in samples of muscle and liver of chickens (broiler and layer) and also in poultry feed.

3.3 Study population

A total of 40 samples of broiler and layer meat and liver and feed were collected from different poultry (broiler and layer) farms of Sirajgonj and Gazipur district subjected to determine antibiotic residue and health risk characterization during the study period.

3.4 Sample Collection

A total number of 40 samples (meat and liver of broiler and layer chicken and also poultry feed were collected (Table 1 and 2) from totally different poultry farm of Sirajgonj and Gazipur district, Bangladesh. Meat and liver samples of local breed chicken were also collected for comparison on antibiotic residue.

Table 1. A total of 30 muscle and liver samples of broiler and layer chicken were collected from Sirajgonj and Gazipur district

SL. No	Sample id	Name of poultry farms of sample collection	Village	Upazila	District	No. of sample
Layer muscle						
1	SKLCRM	Ratan poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSLCAM	Amjad poultry farm	Ekdala	Sirajganj sadar	Sirajganj	1
3	GCLCPSM	Sohag poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCLCPRM	Rashid poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCLCPAM	Asma poultry farm	Pajulia	Gazipur sadar	Gazipur	1
Layer liver						
1	SKLCRL	Ratan poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSLCAL	Amjad poultry farm	Ekdala	Sirajganj sadar	Sirajganj	1
3	GCLCPSL	Sohag poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCLCPRL	Rashid poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCLCPAL	Asma poultry farm	Pajulia	Gazipur sadar	Gazipur	1
Broiler muscle						
1	SKBCGSM	Samad poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSBCEMUM	Mukul poultry farm	Ekdala	Sirajganj sadar	Sirajganj	1
3	GCBCPAM	Akbar poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCBCPROM	Robin poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCBCPMAM	Mamun poultry farm	Pajulia	Gazipur sadar	Gazipur	1

Broiler liver						
1	SKBCGSL	Samad poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSBCEMUL	Mukul poultry farm	Ekdala	Sirajganj sadar	Sirajganj	1
3	GCBCPAL	Akbar poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCBCPROL	Robin poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCBCPMAL	Mamun poultry farm	Pajulia	Gazipur sadar	Gazipur	1
Control						
	Muscle					
1	PGM-01	Local breed-01 (Paragon muscle)	--	Gazipur sadar	Gazipur	1
2	PGM-02	Local breed-02 (Paragon muscle)	--	Gazipur sadar	Gazipur	1
3	AGM-01	Local breed (AG Agro muscle)	--	Gazipur sadar	Gazipur	1
4	AGM-02	Local breed(AG Agro muscle)	--	Gazipur sadar	Gazipur	1
5	LOSSM-01	Local breed-01	--	Sirajganj sadar	Sirajganj	1
6	LOSSM-02	Local breed-02	--	Sirajganj sadar	Sirajganj	1
7	LOGM-03	Local breed-03	--	Gazipur sadar	Gazipur	1
	Liver					
8	LOSSLVR-01	Local breed-01	--	Sirajganj sadar	Sirajganj	1
9	LOSKLVR-02	Local breed-02	--	Sirajganj sadar	Sirajganj	1
10	LOGLVR-03	Local breed-03	--	Gazipur sadar	Gazipur	1
Total						30

Table 2. A total of 10 feed samples of broiler and layer chicken were collected from Sirajgonj and Gazipur district

SL. No	Sample id	Name of poultry farms of sample collection	Village	Upazila	District	No. of sample
Layer feed						
1	SKLCRaF	Ratan poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSLCAmF	Amjad poultry	Ekdala	Sirajganj	Sirajganj	1

		farm		sadar		
3	GCLCSoF	Sohag poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCZCRaF	Rashid poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCLCAsF	Asma poultry farm	Pajulia	Gazipur sadar	Gazipur	1
Broiler feed						
1	SKBCSaF	Samad poultry farm	Gandail	Kazipur	Sirajganj	1
2	SSBCMuF	Mukul poultry farm	Ekdala	Sirajganj sadar	Sirajganj	1
3	GCBCAkF	Akbar poultry farm	Pajulia	Gazipur sadar	Gazipur	1
4	GCBCRoF	Robin poultry farm	Pajulia	Gazipur sadar	Gazipur	1
5	GCBCMaF	Mamun poultry farm	Pajulia	Gazipur sadar	Gazipur	1
Total						10

3.5 Transport of meat and feed sample

Meat samples were collected in clean sample collection container. After notation of samples characteristics they were transported to the laboratory in thermo-cooled container jacket with ice and were stored in refrigerator at 0°C till processing. Feed samples were collected in dried and cleaned polythene bag and each sample was separated to one another with valid identification number.

3.6 Sample preservation

Samples were stored at a cool and dry place untill working (Lab work) at BCSIR Laboratory.

3.7 Materials used for Tetracycline analysis:

3.7.1 Tetracycline standard

1) Oxytetracycline hydrochloride (Sigma Aldrich)
2) Tetracycline hydrochloride (Sigma Aldrich)
3) Chlortetracycline hydrochloride (Sigma Aldrich)

3.7.2 Chemicals

3.7.2.1 Solvent and Reagents

1) Methanol- HPLC Grade (Merck)
2) **Magnesium** Acetate (Extra Pure, BDH)
3) Citric Acid- Monohydrate (Merck)
4) Sodium Hydrogen Phosphate-anhydrous (Merck)
5) EDTA- disodium dehydrate (Scharlu)
6) Acetic Acid (Riedel de Harein)
7) Imidazole (Merck)
8) n-Hexane(Merck)
9) Water (filter de-ionised water)

3.7.2.2 Solutions

1) Mellvaine Buffer
2) Mellvaine Solution: (Mellvaine Buffer/ 0.1 M EDTA)
3) Extraction Solution
4) Imidazole Buffer (1M)
5) Mobile Phase

3.7.3 Equipments

1) Analytical Balance (Shimadzu Corp. AUY- 120)
2) Bucher Funnel- 5.5 cm diameter
3) Refrigerated centrifuge (Hettich, Universal 420)
4) Centrifuge tubes- polypropylene, 50 mL, Disposable Extra GENE
5) Centrifuge tubes, polypropylene, 15 mL Disposable Extra GENE
6) Filter paper glass microfiber, grade GFB, 5.5 cm (Whatman)
7) Ground joint flask- 250 mL
8) Chopping board and Knife
9) Mechanical Shaker- flatbed (Denley Ins. RM 521)
10) Sample concentrator (Techne, DB-3)

11) Digital *pH* meter (Jenway)
12) Sample Cartridge- 13 mm diameter × 0.2 micron
13) SPE Cartridge -3mL, 500 mg C18 packing, Bond- Elut (Varian)
14) SPE Vacuum Manifold (Supelco Visiprep 5-7030)
15) SPE reservoirs- 30 mL (Varian)
16) Vortex Mixer (Remi Equip)
17) Syringes, disposable- 3mL (Opsonin)
18) Volumetric Flask- 25 mL and 10mL

3.7.4 Analytical apparatus

1. **HPLC System:**
   a) Brand: JASCO, Japan
   b) Model: 2000 plus Series
   c) Software: JASCO chrompass Chromatography Data System, Version 1.7.403.1
2. **Column:**
   a) C18, 5µ 250 mm×4.6 mm

3.7.5 Methods

3.7.5.1 Sample Preparation and Extraction:

1) At first, the livers and gizzards were chopped homogeneously using a sharp knife and board. From that chopped sample, 5 gm of it was taken in a 50 mL centrifuge tube after weighing.
2) 20 ml Extraction Solution (6ml Mcllvaine Solution + 14 ml HPLC grade Methanol) was then added.
3) The tube was capped and then shaken vigorously until sample was uniformly mixed.
4) It was then shaken for 10-15 minutes on a flat-bed shaker at high speed.
5) Centrifugation for 15 minutes at 3°C, 8000 rpm was then carried out
6) The supernatant was poured into a second centrifuge tube, whilst being

careful not to transfer any tissue.

7) The SPE Clean-up procedure was then performed.
8) 5 ml glass vial was placed in the vacuum manifold to serve as a collection vessel.
9) Tetracyclines were eluted from the SPE cartridge with 5 mL elution solution.
10) The vial was dried at 40-50°C under a stream of dry nitrogen in tire Sample Concentration (Total dryness was not allowed)
11) 1 mL methanol + water (1:1) was added to the vial and was transferred to vortex.
12) It was then filtered through the syringe filter into an HPLC auto sampler vial.
13) Lastly, HPLC analysis was then carried out.

3.7.5.2: Preparation of Tetracycline Extract Buffer:

1) 11.35 g of anhydrous disodium hydrogen phosphate in 400mL of distilled water was taken.
2) 5.25 g of Citric acid in 250 mL of distilled water was then taken.
3) In (2), (1) was added gradually so that the final p^H becomes 6.
4) 18.76 g of disodium EDTA to 500 mL distilled water was added. This solution was then added to the solvent with p^H 6 so that it becomes 5.5.

3.7.5.3: Preparation of Tetracycline Mobile Phase:

1) **1M Imidazole buffer (pH 12):** Dissolve 68.08 g imidazole, 10.72 g magnesium acetate and 0.37 g disodium salt of EDTA in 800mL of HPLC Grade Water. Adjust to pH 7.2 using acetic acid and make up to 1 L (litre) with HPLC Grade Water.
2) **Mobile phase solution:** Mix 1M Imidazole buffer and HPLC Grade Methanol in the ratio 70:30 by volume. It is very important to filter the mobile phase solution through a 0.45µm Millipore Filter.

3.7.5.4: HPLC Parameters for analysis of Tetracycline residues:

1) Mobile Phase: Buffer Methanol= 70:30
2) Column: C18, 5p 250 mmx4.6 mm
3) Injection Volume: 25pL
4) Flow Rate: 1 mL/min
5) Column Temperature: 35°C
6) Detector: Fluorescence Detector; JASCO 2000 plus Series
7) Excitation Wavelength- 380 nm
8) Emission Wavelength-520 nm
9) Run Time-15 minutes
10) Retention time:
    - Tetracycline (TC): 5.60 ±5%
    - Oxytetracycline (OTQ: 4.90 ±5%

3.8 Materials used for oxytetracycline analysis

3.8.1 Oxytetracycline standard

1. Oxytetracycline hydrochloride (Sigma Aldrich)

3.8.2 Chemicals

3.8.2.1. Solvent and Reagents

1) Methanol — HPLC grade (Merck)
2) Magnesium Acetate (Extra Pure, BDH)
3) Citric Acid — Monohydrate (Merck)
4) Sodium Hydrogen phosphate - anhydrous (Merck)
5) EDTA - disodium dehydrate (Scharlu)
6) Acetic acid (Riedel de Haein)
7) Imidazole (Merck)
8) n-Hexane (Merck)
9) HPLC grade water

3.8.2.2. Solutions

1) Mcllvaine Buffer
2) Mcllvaine Solution: (Mcllvaine Buffer/0.1 M EDTA)
3) Extraction Solution
4) Imidazole Buffer (1M)
5) Mobile Phase

3.8.3 Equipments

1) Analytical balance (Shimadzu Corp. AUY -120)
2) Bucher Funnel - 5.5 cm diameter
3) Refrigerated centrifuge (Hettich, Universal 420R)
4) Centrifuge tubes- polypropylene, 50 mL, Disposable (BD Falcon)
5) Centrifuge tubes- polypropylene, 5 mL (Tarson)
6) Filter paper-glass microfiber, grade GFB, 5.5 cm (Whatman)
7) Sidearm Erlenmeyer flask - 250 mL
8) Ultra Turrax,IKAT18B
9) Mechanical shaker - flatbed (Denley Ins., RM 521)
10) Sample concentrator (Teche, DN-3)
11) Digital pH meter (Jenway)
12) Sample filter cartridge — 13 mm diameter x 0.2 micron
13) SPE Cartridge - 3 mL, 500 mg C18 packing, Bond -Elut (Vanan)
14) SPE vacuum manifold (Supelco Visiprep 5-7030)
15) SPE reservoirs - 75 mL (Varian)
16) SPE adapters (Varian)
17) Vortex mixer (Remi Equip.)
18) Syringes, disposable - 3 mL (Opsonin)
19) Volumetric flask - 25 mL and 10 mL

3.8.4 Analytical apparatus

1. **HPLC system: Agilent Liquid chromatography consisting of**
    a) Agilent: Solvent delivery system series 1100 (Isocratic pump)
    b) Agilent series 1100 Column oven
    c) Agilent 1200 series Fluorescence detector for HPLC
    d) Manual injector capable of injection volumes up to 50 microliters
    e) Software : Chem Station Rev A. 10.02
2. **Column:**
    a) Phenomenex — Gemini 5u C18 110A (250 x4.60 mm)

3.8.5 Methods

3.8.5.1 Sample preparation and extraction

1) At first after adequate thawing few grams of muscle sample were collected from the fish and minced using chopping board and knife.
2) Then weighed 5.0 g of partially thawed intact samples was taken separately into 50 mL polypropylene centrifuge tubes.
3) Another weighed 5.0 g of partially thawed appropriate blank tissue (previously analyzed and found to contain no tetracyclines) was separately taken into 50 mL polypropylene tubes. Use one tube as control and fortify the other tubes with 125 pL of mixed standard for 0.5 ppm recovery.
4) Then 20 mL extraction solution was added to each sample and homogenized by using Ultra Turrax until samples were uniformly blended (15- 30 seconds). After rinsing probe with 4 mL of extraction solution, rinses were added to centrifuge tube.
5) Homogenizer was cleaned carefully with methanol followed by de-ionized water to ensure no carry over to other samples.
6) Tubes were capped and shaken 10 minutes on a flatbed shaker at speed.
7) Contents of tubes were centrifuged at a minimum 8000 rpm for 20

minutes at approximately 15°C. Supernatants were poured into a second centrifuge tube carefully not to transfer any tissue.

8) Five (5) mL n-Hexane was added to solution and briefly shaked. Upper layer was removed.
9) A single Whatman #1 filter paper was placed into a 5.5 cm Bucher filtering funnel and attached to a 250 mL sidearm flask with vacuum condition. Paper was moistened with Extraction solution to assure that the filter paper is well-seated and then the combined sample extracts were filtered. Centrifuge tubes were rinsed with 4 mL Extraction solution and filtered into a flask.
10) An SPE cartridge was attached to an SPE vacuum manifold. The cartridge was conditioned with 10 mL methanol followed by 15-20 mL distilled water at approximately 1.5-2.5 mL/minute with vacuum as necessary. The elute were discarded.
11) A 75 mL reservoir was connected to the cartridge. The filtered sample extracts were added to the SPE reservoir. The flask was rinsed with approximately 4 mL buffer solution and was added to the rinses to the reservoir. Extract was drained through the column by gravity. (If gravity is not sufficient for some slow samples gently apply vacuum and adjust stopcocks to achieve a flow rate of 1.5 - 2.5 mL/minute. After sample has been applied column). The sidearm flask was rinsed with 20 mL distilled water and added to reservoir. After draining under — 10 mm Hg vacuum cartridges were allowed to go dry after the water rinse is completed, and continue to draw air through the cartridge for at least 2 minutes. Then Elute was discarded.
12) A 15 mL graduated centrifuge tube was placed in the vacuum apparatus to serve as a collection vessel and elute oxytetracycline from the cartridge with 6 mL elution solution. Vacuum condition was applied to initiate flow continue elution. Once flow stops, vacuum applied to remove residual solvent from the cartridge. Tubes were removed from

vacuum manifold and vortex was done.

13) The tube containing elute were placed in the sample concentrator at the temperature at 40-50°C to reduce volume of the elute to 0.5-0.25 mL under a stream of dry nitrogen. Final volume was adjusted tol mL with methanol + water (1:1) and briefly vortexing.
14) Then approximately 1.0 mL extract were drawn into a 3 mL syringe and was filtered through a syringe into an HPLC vial (1.5 mL). The remaining extract was store at -20° C.

3.8.5.2 HPLC parameters for analysis of oxytetracycline residues

The concentrate extract were subjected to analysis by Agilent 1100 series HPLC system.

1) Mobile phase: Buffer: Methanol = 70:30
2) Column: Phenomenex - Gemini 5 u Cl8 110A (250 x4.60 mm)
3) Injection volume: 20 pL
4) Flow rate: 1 mL/min
5) Column temperature: 30°C
6) Detector: Fluorescence detector (Agilent 1200 series)
7) Excitation wavelength: 380 nm
8) Emission wavelength: 520 nm
9) Run time: 12 minutes
10) Software : Chem Station Rev A. 10.02

3.9 Preparation of calibration curve

3.9.1 Tetracycline

Calibration curve was prepared from injecting corresponding concentrations of Tetracycline standard solution of 25, 50, 100, 150 and 200 µg/kg . The linear fit curve obtained using

$$y = bx + a = 0.11456x + 0.34187$$

Where y = peak area and x = concentration of Tetracycline (µg/kg) and the

correlation coefficient = 0.9970. The detection limit for Tetracycline was 4.69 µg/kg . The mean retention times (RT) of the tetracycline was found 5.60±5%.

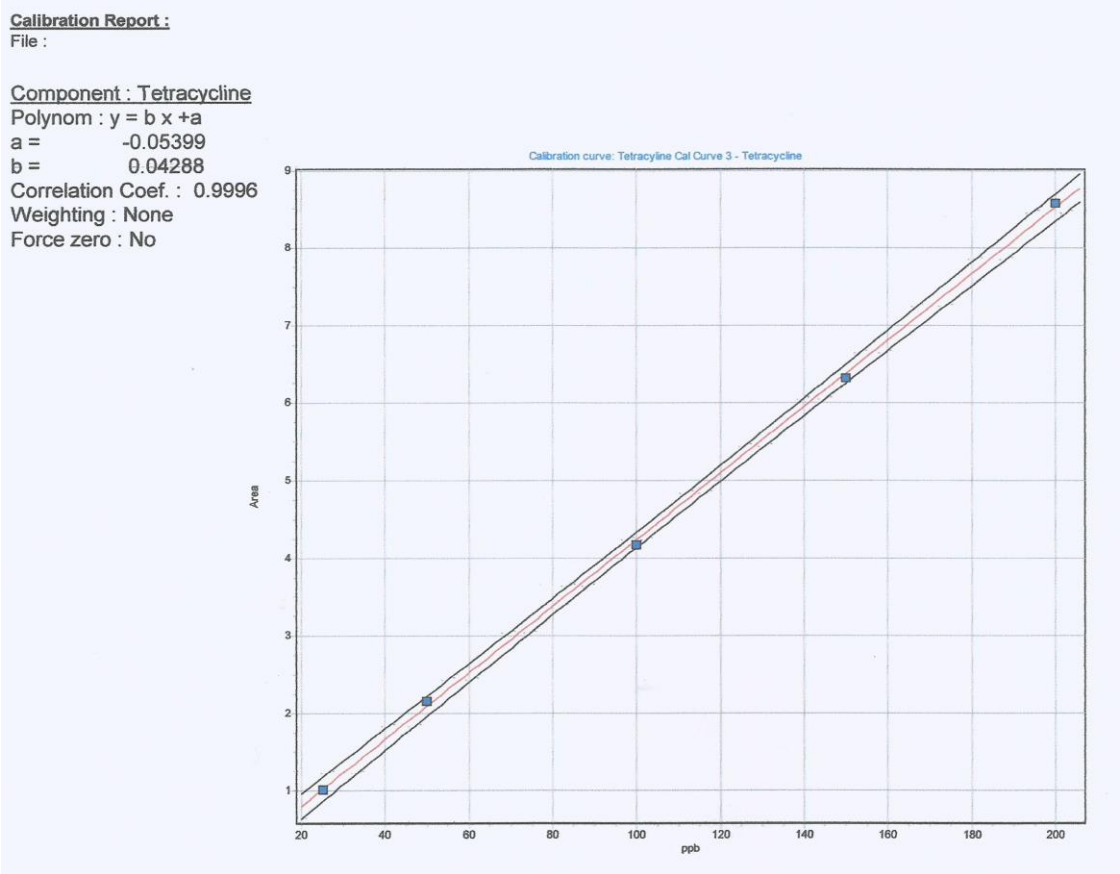


Figure 1. Five point calibration curve for Tetracycline with retention time (RT) 5.60±5%.

3.9.2 Oxytetracycline

Calibration curve was prepared from injecting corresponding concentrations of Tetracycline standard solution of 25, 50, 100, 150 and 200 µg/kg . The linear fit curve obtained using

$$y = bx + a = 0.11456x + 0.34187$$

Where y = peak area and x = concentration of Tetracycline (µg/kg) and the correlation coefficient = 0.9970. The detection limit for Tetracycline was 4.69 µg/kg . The mean retention times (RT) of the tetracycline was found 4.90±5%.

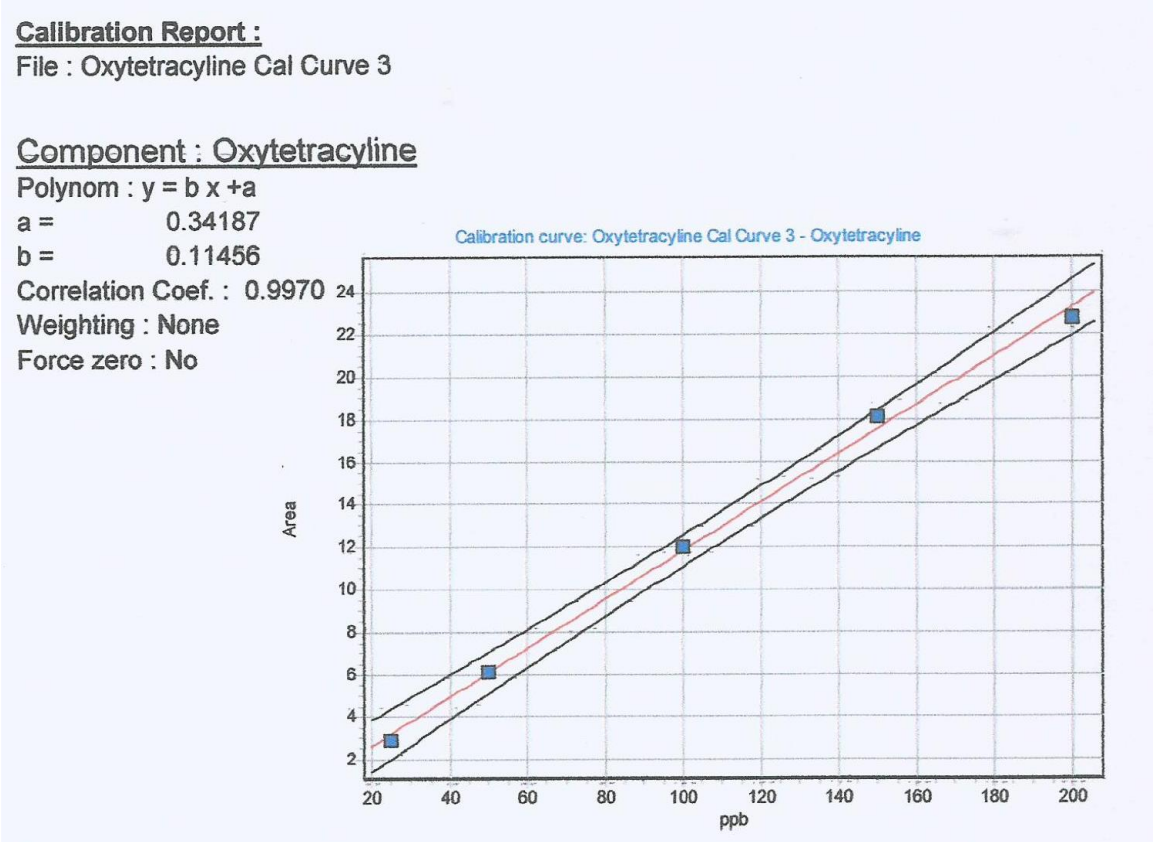


Figure 2. Six point calibration curve for Oxytetracycline with retention time (RT) 4.90±5%.

3.10 Limitation of the study

First of all, there was extreme lack of resources. Also there was lack of co-operation. 40 samples were extracted from mid September to October and were labeled and refrigerated. But due to the inconvenience of the Lab correspondents, the samples got ruined. Due to lack of time, I had to carry out my experimental samples with very short times.

3.11 Statistical analysis

For initial processing of raw data obtained from this stud was analysis by using the computer software like Microsoft Excel, SPSS and so on.

Pictorial presentation of my work

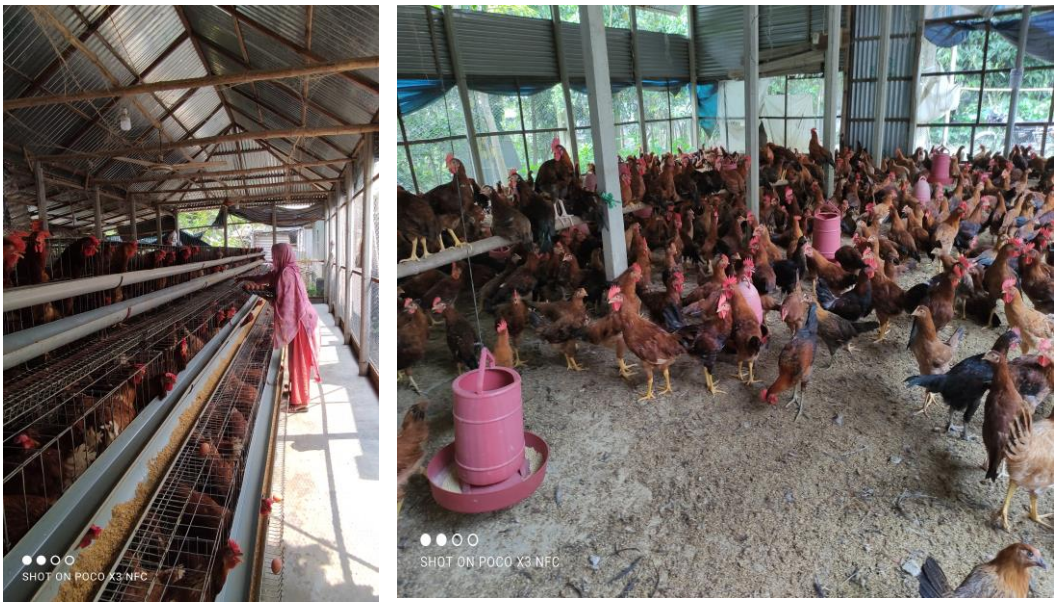


Plate 1. Visited layer poultry farm

Plate 2. Visited broiler poultry farm

Plate 3. Chopping and weighing of poultry samples

Plate 4. Leveling of chopped poultry (layer and broiler) samples

Plate 5. Centrifugation of samples

Plate 6. Supernatant collection

Plate 7. Drain of filtrate sample extract through SPE by using vacuum manifold

Plate 8. Placing of vial in sample concentrator and drying by N_2 gas

Plate 9. Collection of solution in syringe and filtration through syringe filter

Plate 10. Inject of sample solution in to HPLC

CHAPTER IV

RESULTS AND DISCUSSION

Adulteration of poultry meat and products is a serious public health concern which have detrimental effects for human life and health. Poultry meat and poultry feed can be contaminated by variety of contaminates (Sarker *et al*., 2020; Altherwi *et al*., 2018). For the longest time, there has been numerous studies and research that have been conducted and are still now conducting to examine the presence of the different veterinary drugs, mostly antibiotic residues in animal products due to the adverse effects they inflict on the health and environment of human consumers (Elnasri *et al*., 2014).

The purpose of the study was to detect the presence two antibiotic residues, particularly Tetracycline and Oxytetracycline in broiler and layer meat and liver and also in poultry feed. As meat and liver are namely popular among the poultry giblets in Bangladesh. To perform this study, 5 layer chicken muscle, 5 broiler chicken muscle, 5 layer chicken liver, 5 broiler chicken broiler liver, in total 20 chicken samples were randomly collected from different farm of Sirajgonj and Gazipur district, Bangladesh. Similarly, 10 feed samples from the respected poultry farm were collected. Again, 5 chicken meat samples and 5 chicken liver samples of local breed considered as control, in total 10 chicken samples were also collected from Sirajgonj and Gazipur district, Bangladesh.

Then the collected chicken samples and feed samples were analyzed at Food Toxicology Laboratory of Bangladesh Council of Scientific and Industrial Research, Dhaka. Tetracycline and Oxytetracycline residues of collected samples were determined by analysis of chicken samples and feed samples using High Performance Liquid Chromatography (HPLC) system.

After analysis evident amounts of Tetracycline and Oxytetracycline residues were found in a number of collected chicken samples and feed samples used for the current study. Results are presented in tabular forms which are obtained from the chromatogram of the analyzed samples. Overall results of antibiotic

residues analysis of the given samples are organized according to the type of antibiotic and are given in Table 3 to 5. Detected antibiotic residues are shown in figure 3 to 8. The mean of the detected Oxytetracycline residues were calculated as Mean±SEM (Standard Error of Mean). Chromatograms of analyzed chicken samples containing visible or non-detectable residues of TC and OTC are shown in Appendices, which were made by JASCO chrompass Chromatography Data System, Version 1.7.403.1 software.

4.1 Antibiotic residues obtained from chicken feed samples

4.1.1 Tetracycline residues in layer and broiler feed samples

In this study, 10 chicken feed samples were collected from different selected farms of Sirajgonj and Gazipur district, Bangladesh. Tetracycline (TC) residues were tested in the collected samples in the laboratory. Among the 5 layer feed samples, 3(60%) samples were positive regarding the presence of Tetracycline (TC) which was higher than LOD whereas 2(40%) feed samples showed no TC (Table 5 and Figure 7). Again, among the 5 broiler feed samples, 2(40%) had Tetracycline (TC) residue which were higher than LOD whereas 3(60%) samples had no detected TC (Table 5 and Figure 7). In this experiment, no sample exceeded the maximum residue limit (MRL) of TC (200 mg/kg) set by European Commission (2010). Poultry feed can be contaminated by variety of contaminates (Sarker *et al*., 2020; Altherwi *et al*., 2018). The imprudent use of antibiotics both for prophylactic and therapeutic purposes has considered as global concern due to its residual effect, and subsequent adverse health hazards of consumers (Ferdous *et al*., 2019; Chanda *et al*., 2014). Alam *et al*. (2021) reported from a study that 18.89% feed samples were positive on antibiotic residues out of 94 broiler feed samples.

4.1.2 Oxytetracycline residues in layer and broiler feed samples

In this study, 10 chicken feed samples were collected from different selected farms of Sirajgonj and Gazipur district, Bangladesh. Oxytetracycline (OTC) residues were tested in the collected samples in the Laboratory. Among the 5

layer feed samples, 3(60%) samples were positive regarding the presence of Oxytetracycline (OTC) which was higher than LOD whereas 2(40%) feed samples showed no OTC (Table 5 and Figure 8). Again, among the 5 broiler feed samples, 2(40%) had Oxytetracycline (OTC) residue which were higher than LOD whereas 3(60%) samples had no detected OTC (Table 5 and Figure 8). In this experiment, no sample exceeded the maximum residue limit (MRL) of OTC (200 mg/kg) set by European Commission (2010). Similar result was also observed by Alam *et al*. (2021) from a study carried out with 94 broiler feed samples and reported that antibiotic residues were detected in 18.89% of the feed samples, whereas, among the antibiotic positive samples, Oxytetracycline (OTC) was found predominant and detected in 12.22% cases. Alhendi *et al*. (2000) also found similar result with the present study and observed the presence of Oxytetracycline residue in broiler feed samples.

Table 3. Level of Tetracycline and Oxytetracycline residue in layer and broiler feed samples

Antibiotic name	Chicken organ	Sample id	Area of collection	Test result (µg/kg)	Minimum detection limit (MDL)	Comments
Tetracycline	Layer feed	SKLCRM	Sirajganj	81.52	23.45 µg/kg	Obtained TC amounts were less than recommended dose by BD authority, Department of fisheries (DOF) (200 mg/kg)
		SSLCAM	Sirajganj	120.17		
		GCLCPSM	Gazipur	122.51		
		GCLCPRM	Gazipur	Not detected		
		GCLCPAM	Gazipur	Not detected		
	Broiler feed	SKLCRL	Sirajganj	99.21		
		SSLCAL	Sirajganj	Not detected		
		GCLCPSL	Gazipur	Not detected		
		GCLCPRL	Gazipur	Not detected		
		GCLCPAL	Gazipur	130.23		
Oxyetracycline	Layer feed	SKBCGSM	Sirajganj	13.12		
		SSBCEMUM	Sirajganj	16.38		
		GCBCPAM	Gazipur	12.92		
		GCBCPROM	Gazipur	Not detected		
		GCBCPMAM	Gazipur	Not detected		
	Broiler feed	SKBCGSL	Sirajganj	20.81		
		SSBCEMUL	Sirajganj	Not detected		
		GCBCPAL	Gazipur	Not detected		
		GCBCPROL	Gazipur	Not detected		
		GCBCPMAL	Gazipur	15.22		

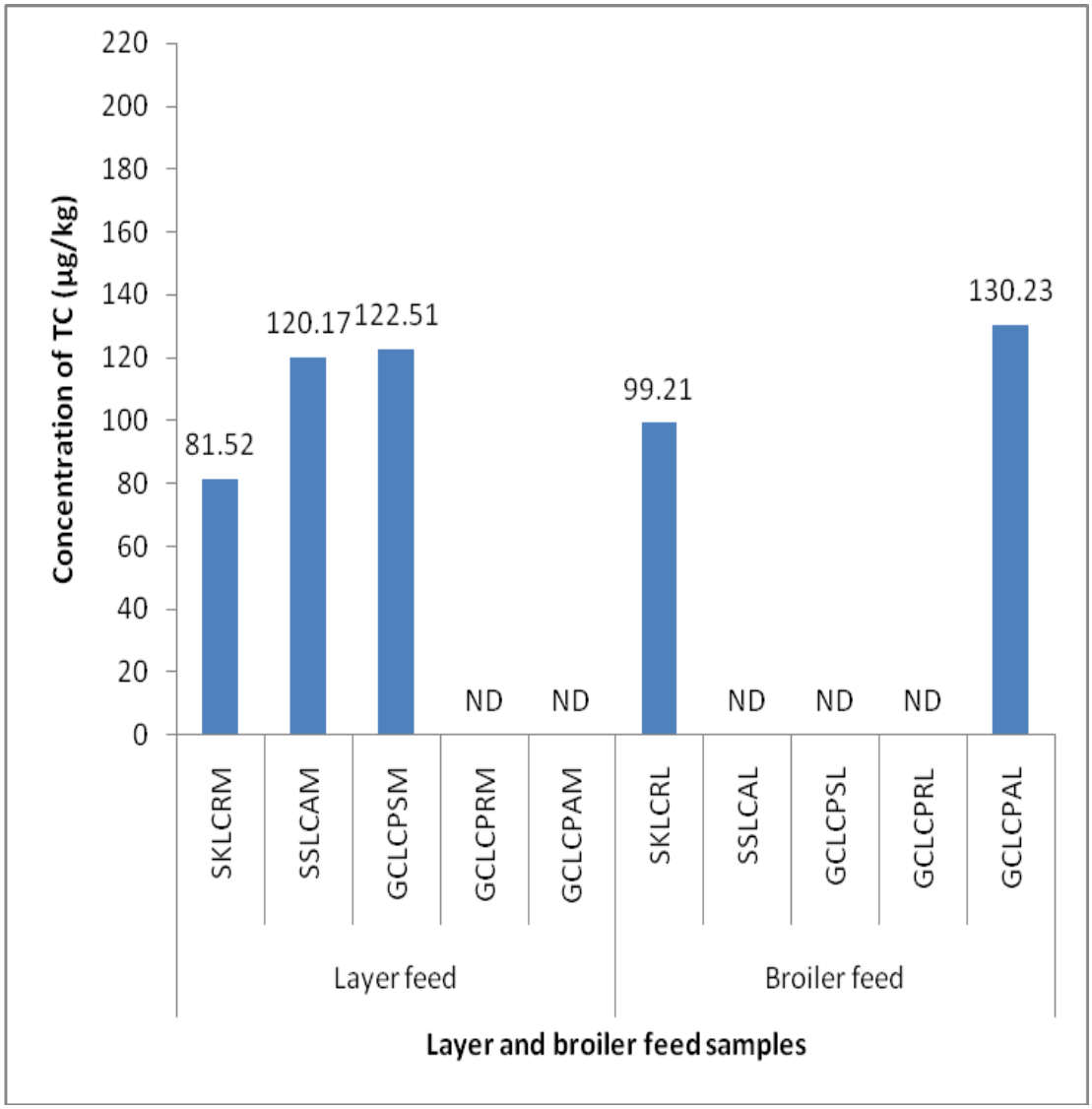


Figure 3. Detected Tetracycline (TC) residues in layer and broiler feed samples

*ND = Not detected

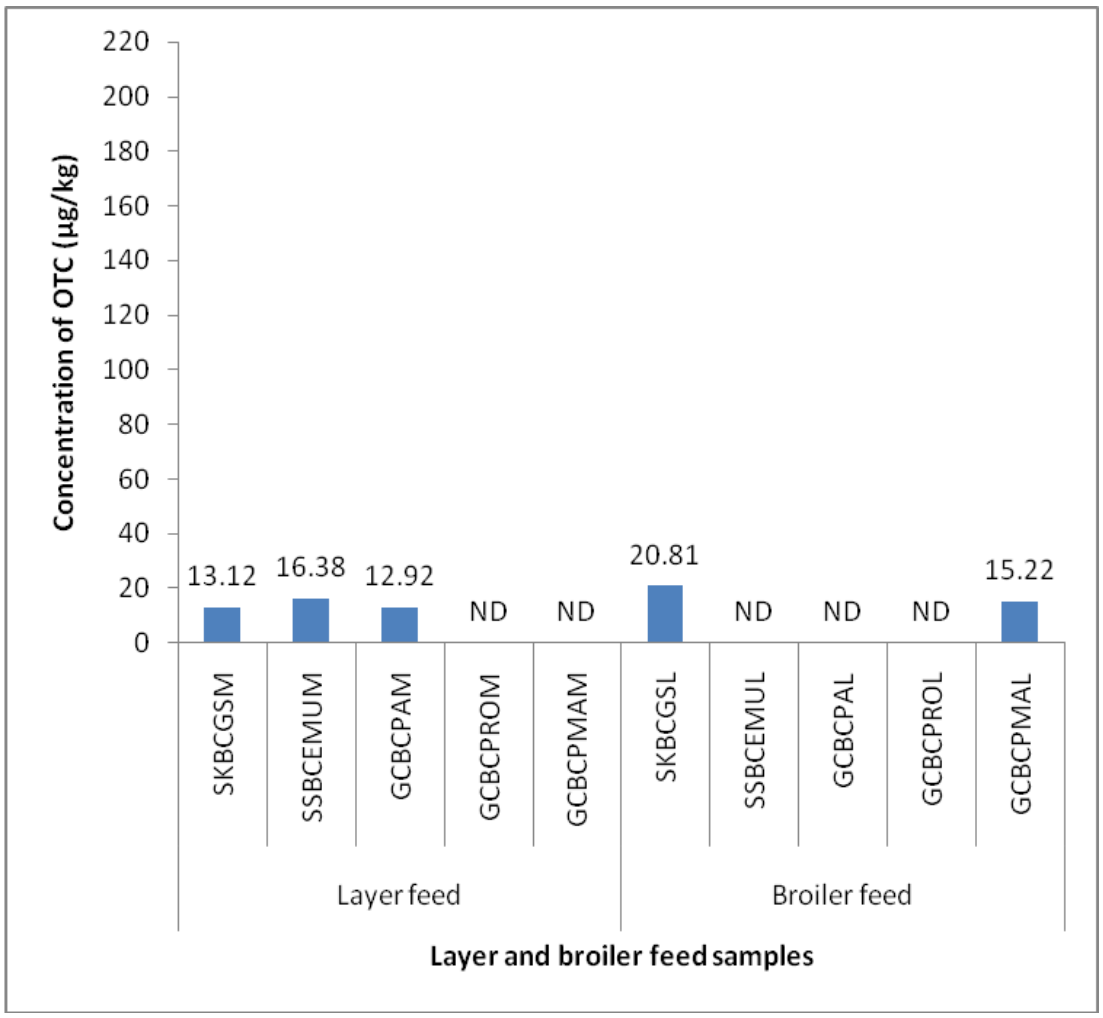


Figure 4. Detected Oxytetracycline (OTC) residues in layer and broiler feed samples

*ND = Not detected

4.2 Antibiotic residues obtained from chicken muscles and livers

4.2.1 Tetracycline residues in layer and broiler

In this study as mentioned before, 30 chicken samples were collected from different selected farms of Sirajgonj and Gazipur district, Bangladesh. Tetracycline (TC) residues were tested in the collected samples. Among the 5 layer muscle samples, 2(40%) had Tetracycline (TC) present which were higher than LOD whereas 3(60%) samples couldn't detect TC (Table 3 and

Figure 3). Again, among the 5 layer liver samples, 3(60%) samples had Tetracycline (TC) residue which were higher than LOD whereas 2(40%) samples couldn't detect TC (Table 3 and Figure 3). Similarly, among the 5 broiler muscle samples, Tetracycline (TC) residues were detected in 2(40%) samples which were higher than LOD whereas no TC was detected in 3(60%) samples (Table 3 and Figure 4). Among the 5 broiler liver samples, Tetracycline (TC) residues were detected in 2(40%) samples which were higher than LOD whereas no TC was detected in 3(60%) samples (Table 3 and Figure 4). Considering control muscle and liver samples (five samples of each from local breed), no TC residue was detected (Table 3).

Table 4. Level of Tetracycline residue in the chicken sample

Antibiotic name	Chicken organ	Sample id	Area of collection	Test result (µg/kg)	Minimum detection limit (MDL)*	Comments
Tetracycline	Layer muscle	SKLCRM	Sirajganj	Not detected	23.45 µg/kg	Obtained TC amounts are less than MRL level set as Codex CAC/MRL 2-2015 (100 µg/kg in muscle, 300 µg/kg in liver)
		SSLCAM	Sirajganj	41.68		
		GCLCPSM	Gazipur	43.12		
		GCLCPRM	Gazipur			
		GCLCPAM	Gazipur	Not detected		
	Layer liver	SKLCRL	Sirajganj	28.49		
		SSLCAL	Sirajganj	64.51		
		GCLCPSL	Gazipur	69.74		
		GCLCPRL	Gazipur	Not detected		
		GCLCPAL	Gazipur	Not detected		
	Broiler muscle	SKBCGSM	Sirajganj	30.60		
		SSBCEMUM	Sirajganj	Not detected		
		GCBCPAM	Gazipur	Not detected		
		GCBCPROM	Gazipur	Not detected		
		GCBCPMAM	Gazipur	47.55		
	Broiler liver	SKBCGSL	Sirajganj	39.22		
		SSBCEMUL	Sirajganj	Not detected		
		GCBCPAL	Gazipur	Not detected		
		GCBCPROL	Gazipur	Not detected		
		GCBCPMAL	Gazipur	78.16		
	Control muscle	PGM-01	Gazipur	Not detected	23.45 µg/kg	
		PGM-02	Gazipur	Not detected		
		AGM-01	Gazipur	Not detected		
		AGM-02	Gazipur	Not detected		

		LOSSM-01	Sirajganj	Not detected		
		LOSSM-02	Sirajganj	Not detected		
		LOGM-03	Gazipur	Not detected		
	Control liver	LOSSLVR-01	Sirajganj	Not detected	23.45 µg/kg	
		LOSKLVR-02	Sirajganj	Not detected		
		LOGLVR-03	Gazipur	Unknown		

* MDL = Minimum detection limit

In this experiment, no sample exceeded the maximum residue limit (MRL) of Tetracycline (100 µg/kg in muscle and 300 µg/kg in liver) set by European Commission (2010). The obtained result agrees well with the findings of (Ekaterina, *et al*., 2017) where they analyzed the presence of Tetracycline (TC) in broiler chicken. Tetracycline is one of the most common and universally acknowledged antibiotics in poultry forming, most farms use it high doses, in a previous study it is shown that the mean levels of tetracycline antibiotic residue was as high as 2125 µg/kg out of 140 samples (Dahshan *et al.*, 2014). Al-Ghamdi *et al.* (2000) found similar result with the present study and reported 73.9% broiler farms were positive for tetracycline among 33 broiler farms.

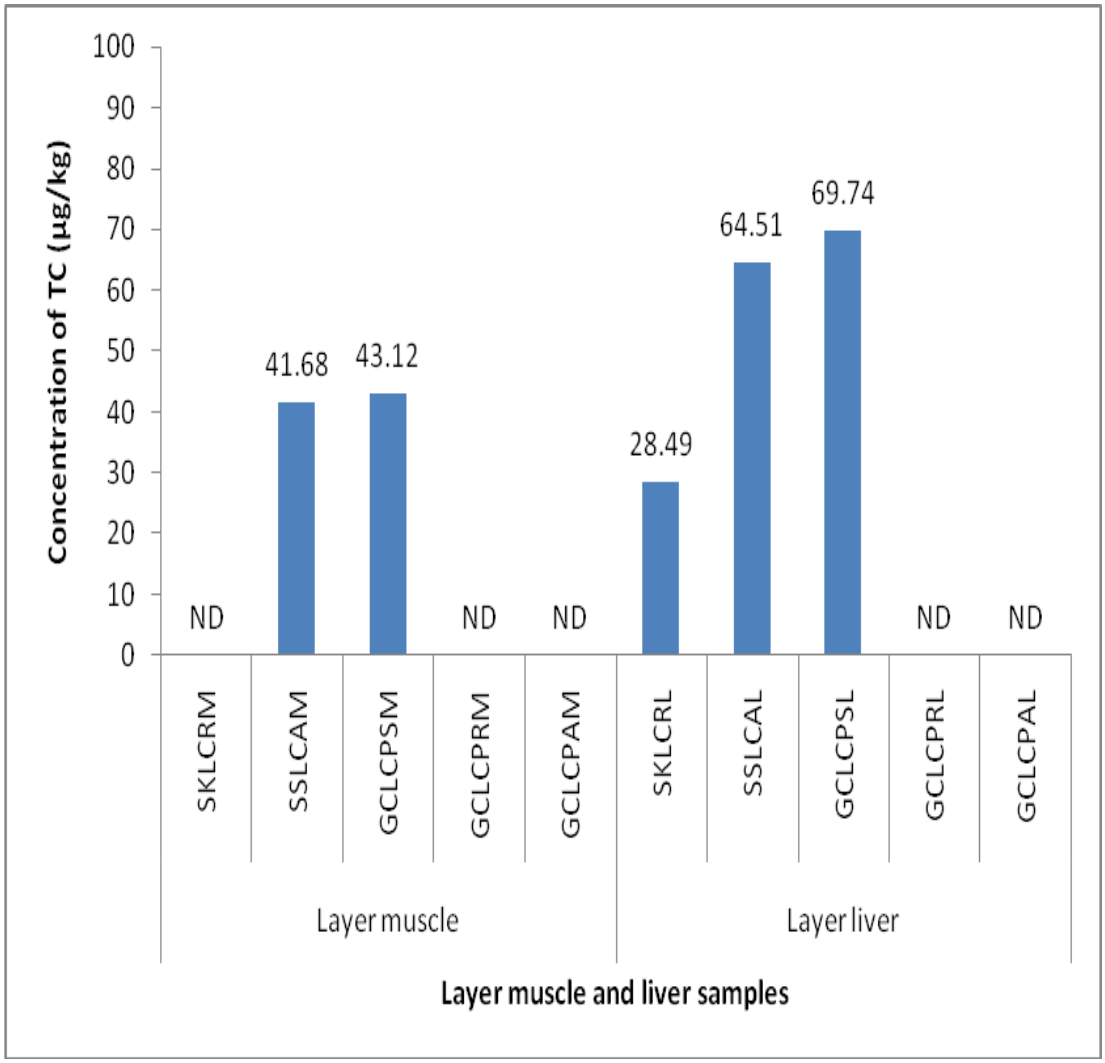


Figure 5. Detected Tetracycline (TC) residues in layer muscle and liver samples

*ND = Not detected

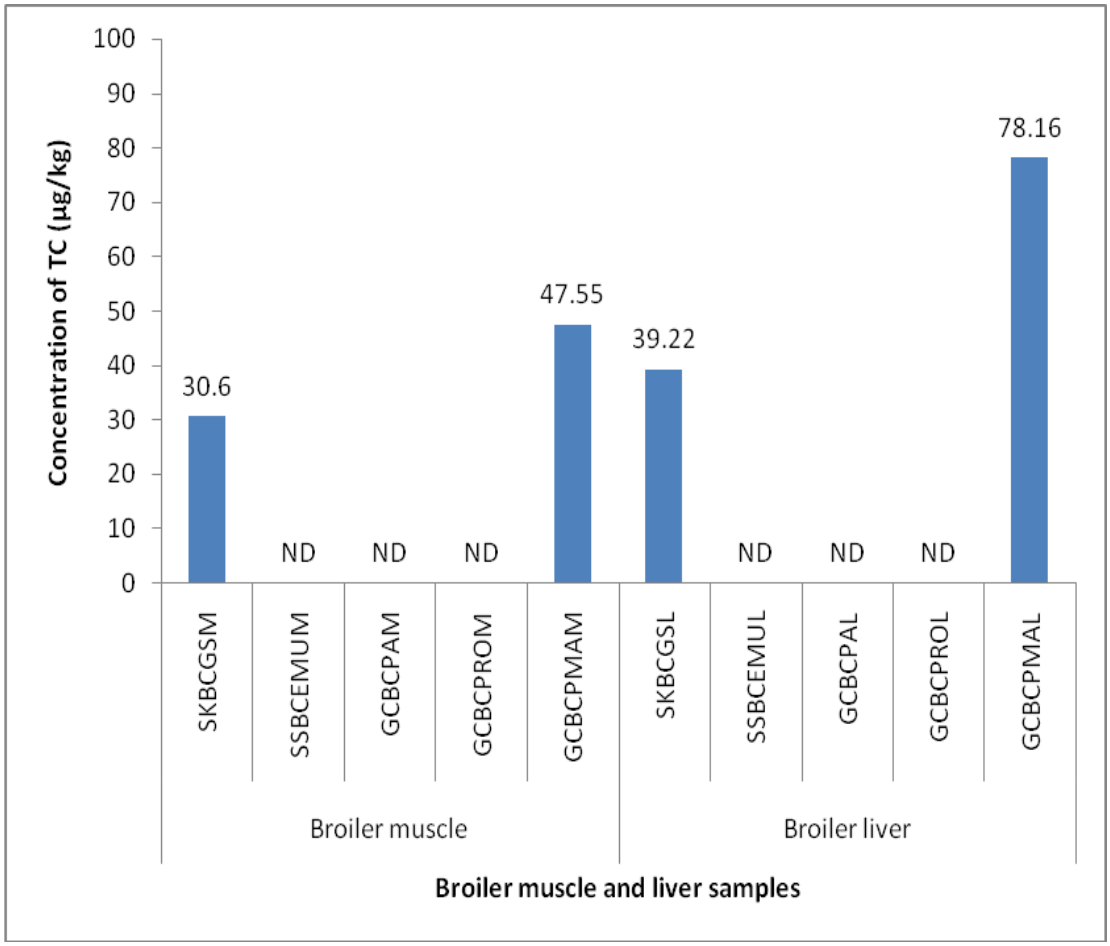


Figure 6. Detected Tetracycline (TC) residues in broiler muscle and liver samples

*ND = Not detected

4.2.2 Oxytetracycline residues in layer and broiler

The collected 30 chicken samples from different selected farms of Sirajgonj and Gazipur district, Bangladesh, Oxytetracycline (TC) residues were tested in the Laboratory. Among the collected 5 samples of layer muscle, 2(40%) had Oxytetracycline (OTC) residue which were less than LOD whereas no OTC was detected in 3(60%) samples (Table 4 and Figure 5). Among the 5 layer liver samples, 3(60%) samples had OTC residue which were less than LOD whereas 2(40%) samples couldn't detect OTC (Table 4 and Figure 5). Similarly, among the 5 samples of broiler muscle, Oxytetracycline (OTC) residues were detected in 2(40%) samples which were less than LOD whereas

no OTC was detected in 3(60%) samples (Table 4 and Figure 6). Among the 5 broiler liver samples, Oxytetracycline (OTC) residues were detected in 2(40%) samples which were less than LOD whereas no OTC was detected in 3(60%) samples (Table 3 and Figure 6).

Table 5. Level of Oxytetracycline residue in the chicken sample

Antibiotic name	Chicken organ	Sample id	Area of collection	Test result (µg/kg)	Minimum detection limit (MDL)	Comments
Oxytetracycline	Layer muscle	SKLCRM	Sirajganj	Not detected	23.67 µg/kg	Obtained TC amounts are less than MRL level set as Codex CAC/MRL 2-2015 (100 µg/kg in muscle, 300 µg/kg in liver)
		SSLCAM	Sirajganj	8.20 (BDL)		
		GCLCPSM	Gazipur	6.74 (BDL)		
		GCLCPRM	Gazipur	Not detected		
		GCLCPAM	Gazipur	Unknown		
	Layer liver	SKLCRL	Sirajganj	8.75 (BDL)		
		SSLCAL	Sirajganj	17.08 (BDL)		
		GCLCPSL	Gazipur	11.86 (BDL)		
		GCLCPRL	Gazipur	Not detected		
		GCLCPAL	Gazipur	Not detected		
	Broiler muscle	SKBCGSM	Sirajganj	8.56 (BDL)		
		SSBCEMUM	Sirajganj	Not detected		
		GCBCPAM	Gazipur	Not detected		
		GCBCPROM	Gazipur	Not detected		
		GCBCPMAM	Gazipur	9.93 (BDL)		
	Broiler liver	SKBCGSL	Sirajganj	13.20 (BDL)		
		SSBCEMUL	Sirajganj	Not detected		
		GCBCPAL	Gazipur	Not detected		
		GCBCPROL	Gazipur	Not detected		
		GCBCPMAL	Gazipur	19.62 (BDL)		
	Control muscle	PGM-01	Gazipur	Not detected		
		PGM-02	Gazipur	Not detected		
		AGM-01	Gazipur	Not detected		
		AGM-02	Gazipur	Not detected		
		LOSSM-01	Sirajganj	Not detected		
		LOSSM-02	Sirajganj	Not detected		
		LOGM-03	Gazipur	Not detected		
	Control liver	LOSSLVR-01	Sirajganj	Not detected		
		LOSKLVR-02	Sirajganj	Not detected		
		LOGLVR-03	Gazipur	Not detected		

* BDL = Below detection limit

Considering control muscle and liver samples (five samples of each from local breed), no OTC residue was detected (Table 3). In this experiment, no sample exceeded the maximum residue limit (MRL) of OTC (100 µg/kg in muscle and 300 µg/kg in liver) set by European Commission (2010). The obtained result agrees well with the findings of (Ekaterina, *el al*., 2017) where they analyzed the presence of Tetracycline (TC) in broiler chicken. Similar result was also observed by Sarker *et al*. (2017) who carried out a study with 160 broiler meat samples of breast and liver and reported 23% OTC in breast and 46% OTC in liver samples.

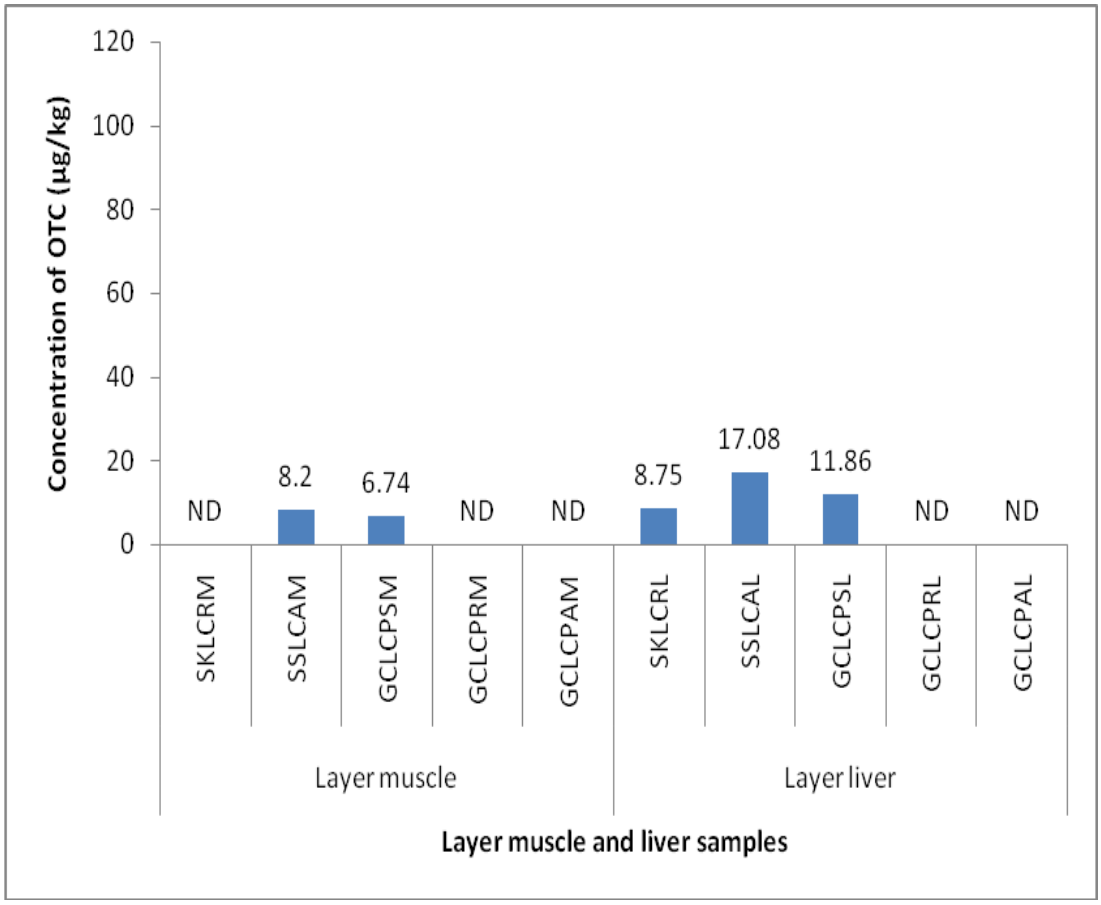


Figure 7. Detected Oxytetracycline (OTC) residues in layer muscle and liver samples

*ND = Not detected

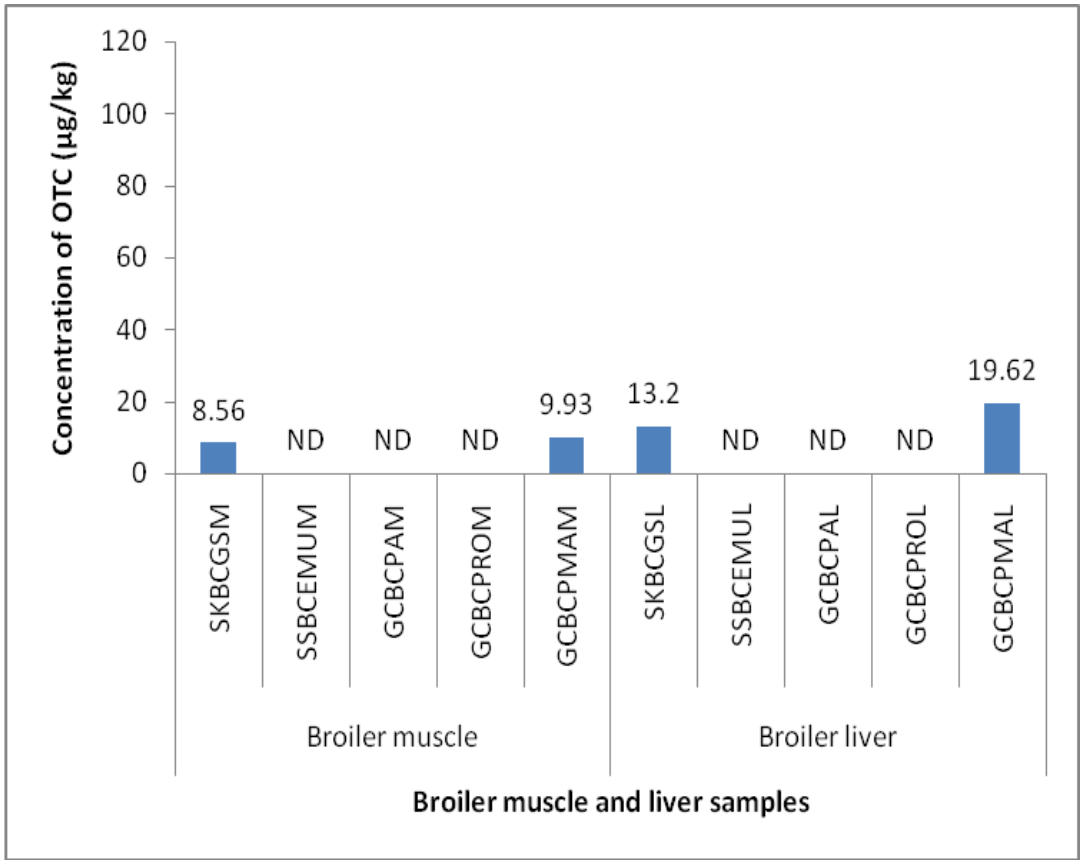


Figure 8. Detected Oxytetracycline (OTC) residues in broiler muscle and liver samples

*ND = Not detected

4.3 Risk characterization

Risk assessment regarding EDI (Estimated Daily Intake), HQ (Hazard Quotient) and Hazard index (HI) were measured using following formula according to the FAO/WHO (2010):

$$\text{EDI (layer/broiler muscle/liver)} = \frac{\text{Daily intake} \times \text{mean antibiotic}}{\text{Average body weight}}$$

$$\text{HQ (layer/broiler muscle/liver)} = \frac{\text{EDI}}{\text{MRL}}$$

Hazard index (HI) = HQ (layer muscle) + HQ (layer liver) + HQ (broiler muscle) + HQ (broiler liver)

Table 6. Risk assessment for broiler or layer muscle or liver

Samples	Daily intake (g) (DLS, 2021-22)	Average body weight (kg)	EDI (µg/kg)	HQ	HI
Broiler muscle	120	57	0.08	0.008	0.002<1
Broiler liver	120		0.06	0.0002	
Layer muscle	122		0.09	0.0009	
Layer liver	122		0.043	0.00014	

From the health risk assessment, it was observed that no sample was under health hazardous condition regarding antibiotic residue in broiler or layer muscle or liver.

4.4 Antibiotics residues and its impact on the public health

Food safety is a term broadly applied to food quality that may adversely affect human health. There is an increased demand for animal products in Bangladesh and also in the world which is related to rising incomes and growing population. Therefore, in order to stay competitive, producers mainly rely on antibiotics and other antimicrobial agents to promote growth, prevent the spread of diseases and treat sick animals (Biswas *et al*., 2019). Antibiotics are the chemical agents which were formulated for saving the life of people and livestock from infections of bacteria (Hyung *et al*., 2017). The imprudent use of antibiotics both for prophylactic and therapeutic purposes has considered as

global concern due to its residual effect, and subsequent adverse health hazards of consumers (Ferdous *et al*., 2019; Chanda *et al*., 2014).

Indiscriminate in the form of overuse and misuse of antibiotics or other antimicrobial agents are the main contributing factor for the development of such high rates of resistance (WHO, 2014). The public health researchers all over the world have termed these resistant microorganisms as 'nightmare bacteria' that often called 'pose a catastrophic threat' to human beings.

The issue of antibiotic resistance becomes more pronounced and concerned due to the fact that most of the commonly used antibiotics in animals are the same that are used for the treatment of human beings. This has raised concerns about reducing the effectiveness of such antibiotics when used for treating human infections (Biswas *et al*., 2019). The use of antibiotics and other antimicrobial agents in chickens, in particular, is expected to increase by triple in developing countries such as India, Bangladesh, Pakistan. Increasing and indiscriminate in the form of overuse and misuse of antibiotics or other antimicrobial agents is considered as main factor for increase in antibiotic resistance, in both humans and animals. Resistant bacteria or microorganisms could be transmitted between humans and animals through various routes such as contact, food products, and the environment (Hyung *et al*., 2017). It has been also reported that antibiotic residues, as well as resistant microorganisms, have been found in live animals in India as well as in related animal food products and in many cases, within the same community, the same strains of resistant bacteria are also detected in human and environmental sources (Biswas *et al*., 2019).

Antibiotics can generally be used for therapeutic purposes by administering high doses for a relatively shorter period to individual or small groups of animals or for prophylactic purposes, where animals are exposed to moderate doses for a relatively longer duration (Marshall and Levy, 2011). In case of livestock production and management, use of antibiotics or antimicrobial agents is inevitable as they are not only crucial for treatment of diseases,

prevention of diseases, modification of physiological function, promoting growth and production but also for ensuring food safety point of view (Falowo and Festus, 2019).

Residues are the active ingredients of the metabolites that remain in meat or any other foodstuff for the animal through which the medicinal products in question have been administered (EC, 2002). The access of antibiotics into meat products including milk and fish usually come from its use for different purposes while controlling/ preventing diseases. Sometimes prolonged use in low doses result into the transfer of antibiotics into the products in a very larger form. It has been reported that about 90 percent of antibiotics used in various food animals including poultry are at subtherapeutic concentration (Jayalakshmi *et al*., 2017) and out of that 70% is for the purpose of diseases prevention and 30% is for growth promotion. Antibiotics generally get eliminated from the body rapidly and disappear from blood and tissue within few days (Tadesse and Tadesse, 2017). However, indirect contamination of antibiotics through feeds along with contamination of feed with faecal recycling is also a very common incidence and usually through this route it enters into the body of the untreated animals also (Darwish *et al*., 2013).

Such presence of antibiotic residues constantly get the chance and opportunity to get entrance into the human body system and consequently there will be systemic response within the physiological limit of the human body and develop antibiotic resistance and remain recessive along with the immune system. When that very individual get treated for obvious reasons with antibiotics, fails to get the benefits of it and succumbed to diseases owing to the reason of antibiotics resistance phenomenon. World Health Organization has given a caution that human society is likely to face a catastrophe by 2050 where the factor of antibiotic resistance would be a giant killer of human lives, no way inferior to any deadly diseases including cancer (Kuhn *et al*., 2000; Biswas *et al*., 2019).

The residues from antibiotics in meat and meat products can potentially be transmitted to humans via consumption of contaminated edible tissues and may lead to several pathological implications. Continuous consumption of meat or meat products contaminated with toxic residues induce changes in the biotransformation of endogenous and exogenous compounds resulting in a variety of health problems, particularly endocrine dysfunction, carcinomas and neurological disorders (Muthukumar and Mandal, 2017).

Residual levels of tetracycline have been reported to lead to poor development of foetuses, staining of teeth in young children, gastrointestinal disorders, and pro-inflammatory, cytotoxic, and immuno-pathological effects (Mund et al., 2017). Chronic exposure to oxytetracycline may lead to leucocytosis, lung congestion, toxic granulation of granulocytes and thrombocytopenic purpura (Lawal *et al*., 2015; Palmieri *et al*., 2014).

Antimicrobial resistance (AMR) means naturally susceptible bacteria acquire ways to withstand the effects of drugs and not being affected by it. The U.S. Centers for Disease Control and Prevention has described AMR as “one of the world’s most pressing health problems”. Long term overexposure to antimicrobials or its repeated use in humans and food animals is the single most causative factor responsible for increased antimicrobial resistance. Whereas other factors that influence the development of antibiotic resistance include concentration of drug, duration of exposure, organism type, antimicrobial type and immune status of host (WHO, 1997). Resistance to antibiotics is one of the most serious global medical problems as we enter the 21st Century. Medical authorities are already confronted with infections for which no antibiotic is effective because the causative bacteria have acquired resistance to all available antibiotic agents. One of the issues receiving close attention at the moment is the link between use of antibiotics in animals and the development of resistance in human pathogens. There is evidence that resistance in some human enteric pathogens has arisen because of transfer of

resistant bacteria or resistance genes from animals to people via the food chain (Barton, 2000). Over-use and misuse of antibiotics therapeutically has driven the resistance problem in human medicine whereas it would seem that prophylactic use to some extent and growth promotant use in particular have contributed most to the emergence of resistant bacteria in animals (van den Bogaard and Stobberingh, 1999).

Residue level of antibiotics in food is a global concern in today's day and age. In this study, the occurrence of Tetracycline and Oxytetracycline in edible chicken parts were found out. Though the detected levels of both these antibiotics were far lower than the maximum residue limit (100 µg/kg in muscle and 300 µg/kg in liver) and these levels are safe for human. But their co-occurrence could be harmful for public health in case of higher concentration with long term exposure. Therefore, supervision of antibiotics use in poultry farming at farmers level is needed with residue and food safety education of producers and consumers.

CHAPTER V

SUMMARY AND CONCLUSION AND RECOMMENDATION

SUMMARY

The present study was conducted to determine the Tetracycline and oxytetracycline residues in chicken layer and broiler muscle and liver samples and also in feed samples. To perform this experiment, 5 samples of layer muscle, 5 samples of layer liver, 5 samples of broiler muscle, 5 samples of broiler liver, 7 samples of local breed chicken muscle (control) and three samples of local breed liver (control) that is total 30 chicken samples and 10 feed samples (5 samples of layer feed and 5 samples of broiler feed) were collected from different poultry farm of Sirajgonj and Gazipur district, Bangladesh during the study period of March 2020 to June, 2021. The samples were collected in separate marked polythene bags and kept an ice box with sufficient amount of ice. Then the samples were transferred to the SAU Medicine and Public Heath Laboratory via cool-chain maintaining in coolbox and stored at 0°C temperature untill working (Lab work). Feed samples were stored at a cool and dry place untill working (Lab work). For analysis, samples were transported to Food Toxicology Laboratory of Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka with icing condition. Collected chicken and feed samples were analyzed using High Performance Liquid Chromatography (HPLC) method to detect residues of Tetracycline and Oxytetracycline.

In this study, 2 Layer muscle samples, 3 Layer liver samples, 2 Broiler muscle samples and 2 Broiler liver samples were Tetracycline and Oxytetracycline positive whereas no control muscle and liver were Tetracycline and Oxytetracycline positive. Among the collected feed samples, 3 layer feed samples and 2 broiler feed samples were Tetracycline and Oxytetracycline positive but the rest of the samples showed no residue detection.

CONCLUSION

From the results and findings of the present investigation it can be concluded that near about 50% of the meat and feed samples collected from different poultry farm of Sirajgonj and Gazipur district, Bangladesh are contaminated with Tetracycline and Oxytetracycline residues. In this experiment, no chicken sample exceeded the maximum residue limit (MRL) of Tetracycline and Oxytetracycline (100 µg/kg in muscle and 300 µg/kg in liver) set by European Commission (2010). Again, no feed sample also exceeded the maximum residue limit (MRL) of TC and OTC (200 mg/kg) recommended by the European Commission (2010).

RECOMMENDATIONS

1. Antibiotics should only be used for treatment or growth purposes in poultry with prescribed dose.
2. Appropriate MRLs need to be set by the regulatory body m the country and enforced.
3. Withdrawal periods should be strictly followed and enforced to make the muscles and liver safer for human consumption.
4. Poultry farmers need to be made aware to best poultry practices to preventand evade the use of antibiotics.
5. Alternatives to antibiotics in poultry feed need to be developed and used where ever possible.
6. Organic poultry forming may be encouraged by providing appropriate incentives tn the farmers in form of subsidy.
7. Use of proper processing techniques to inactivate the antibiotic residue e.g. Refrigeration causes inactivation of penicillin.
8. Establish a routine screening program for antibiotic residues by the appropriate authorities

REFERENCES

Adams, H.R. (2001). Veterinary pharmacology and therapeutics. 8 ed. Iowa state press. USA. Pp: 310-311.

Alaboudi, A., Oboegbulem, S.I., Nwanta, J.A., Onunkwo, J.I. (2013). Chlortetracycline and sulfanilamide residues in table eggs: Prevalence, distribution between yolk and white and effect of refrigeration and heat treatment. *Food Control*. **33**: 281-86.

Alam, M.M., Mallick, D.L., Ahsan, M.M., Akhter, A.H.M.T., Eftesum, Rahman, F., Kabir, S.M.L. and Rahman, A.A. (2021). Heavy metal contamination and antibiotic residues in poultry feed and meat in Bangladesh. Asian Australas. *J. Food Saf. Secur*. **5**(2): 71-78.

Al-Ghamdi, M.S., Al-Mustafa, Z.H., El-Morsy, F., Al-Faky, A., Haider, I. and Essa, H. (2000). Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. *Public Health.* **114**: 300-304.

Alhendi, A.B., Homeida, A.A.M. and Gaili, E.S. (2000). Drug residues in broiler chickens fed with antibiotics in ration. *Vet. Arhiv.* **70**: 199-205.

Altherwi, E.M., Soliman, Y.M. and Khalifa, F.K. (2018). Detection of heavy metals and antibiotic compounds in some local and export meats. *Int. J. Adv. Res. Biol. Sci*. **5**: 114-121.

Amjad, H., Iqbal, J. and Naeem, M. (2005). Analysis of Some Residual Antibiotics in Muscle, Kidney and Liver Samples of Broiler Chicken by Various Methods. *Pakistan Acad. Sci.* **42**(4):223-231.

Annan-Prah, A., Agbemafle, E., Asare, P.T. and Akorli, S.Y. (2012). Antibiotic use, Abuse and their Public Health Implication: The Contributory role of management Flaws in the Poultry Industry in two Agro-Ecological Zones in Ghana. *J. Vet. Adv.* **2**(4): 199-208.

Aroeira, C.N., Feddern, V., Gressler, V., ContrerasCastillo, C.J. and Hopkins, D.L. (2021). Growth promoters in cattle and pigs: A review of

legislation and implications for human health. *Food Rev*. Int., Avaialbe from: https://doi.org/ 10.1080/87559129.2021.1961268.

Arsene, M.M.J., Davares, A.K.L., Viktorovna, P.I., Andreevna, S.L., Sarra, S., Khelifi, I., Sergueievna, D.M. (2022). The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. *Vet. World*. **15**(3): 662-671.

Bacanli, M. and Başaran, N. (2019). Importance of antibiotic residues in animal food. *Food Chem. Toxicol*., **125**: 462-466.

Bahmani, K., Shahbazi, Y. and Nikousefat, Z. (2020). Monitoring and risk assessment of tetracycline residues in foods of animal origin. *Food Sci Biotechnol*. **29**(3): 441–448.

Barton, M.D. (2000). Antibiotic use in animal feed and its impact on human health. *Nut. Res. Rev*. **13**: 1-22.

Beyene, T. (2016) Veterinary drug residues in food-animal products: Its risk factors and potential effects on public health. *J. Vet. Sci. Technol*., **7**(1): 1-7.

Bhuiyan, M.M.I., Islam, M.S., Hasan, M.R. and Islam, K.R. (2021). Thin layer chromatographic detection of enrofloxacin antibiotic residues in poultry tissue. Asian Australas. *J. Food Saf. Secur*., **5**: 11-18.

Biswas, A.K., Rao, G.S., Kondaiah, N., Anjaneyulu, A.S.R., Mendiratta, S.K., Prasad, R. and Malik, J.K. (2007). Simple Multi-residue Method for Determination of Oxytetracycline, Tetracycline and Chlortetracycline in Export Buffalo Meat by HPLC-Photodiode Array Detector. *J. Food Drug Anal*. **15**(3):278-284.

Biswas, S., Banerjee, R., Das, A.K. (2019). Antibiotic residues in meat products and public health importance in the perspective of drug resistance. *Indian J. Anim. Hlth*. **58**(2): Special Issue: 87-104.

Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A. and Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. U.S.A.* **112**(18): 5649-5654.

Boix, C., Ibanez, M., Sancho, J.V., Leon, N., Yusa, V. and Hernandez, F. (2014). Qualitative screening of 116 veterinary drugs in feed by liquid chromatography-high resolution mass spectrometry: Potential application to quantitative analysis. *Food Chem*. **160**: 313-20.

Bristy, N.I., Das, S., Noman, Z.A., Ferdous, J., Sachi, S., Kabir, S.M.L. and Sikder, M.H. (2019). Colistin residue in broiler: detection in different growth stages. Asian Australas. *J. Food Saf. Secur*., **3**: 43-47.

Brynes, S.D. and Yong, M.S. (1993). Harmonization of tolerances under the free trade agreement between the United States and Canada. *Proceedings of the Euro-Residue II Conference,* Veldhoven, The Netherlands, 3-5 May.

Brynes, S.D., Sundlof, S.F., Vilim, A., Lambert, G., Yong, M.S. and Fitzpatrick, S.Z. (1996). International harmonization of maximum residue levels for veterinary drugs via dietary intake estimates. *Proceedings of the Euro- Residue III conference*, Veldhoven, The Netherlands, 6-8 May.

Butaye, P., Devriese, L.A. and Haesebrouck, F. (2001). Differences in antibiotic resistance patterns of Enterococcus faecalis and Enterococcus faecium strains isolated from farm and pet animals. *Antimicorbial Agents and Chemotherapy*, **45**:1374-1378.

Cerniglia, C.E. and Kotarski, S. (1999). Evaluation of Veterinary Drug Residues in Food for Their Potential to Affect Human Intestinal Microflora. *Regulatory Toxicol. Pharmacol.* **29**: 238-261.

Chafer-Pericas C., Maquieira, A. and Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *Trends Analytical Chem.* **29**(9): 1038-1049.

Chanda, R., Fincham, R. and Venter P. (2014). Review of the Regulation of Veterinary Drugs and Residues in South Africa. *Critical Rev. Food Sci. Nutr*. **54**: 488–494.

Choma, I.M. (2000). Determination of four tetracyclines in milk by a simple Thin- Layer Chromatographic method. *Planar chromatography-Mod. TLC*, 13:261, 159-169.

Code of Federal Regulations (1994). U.S. department of Health and Human Serv ices, Food and Drug Administration. (1 April) 21 CFR1, part 556.

Codex Alimentarius Commission (1993). List of Codex MRLs for vet drugs. *Res. Vet. drugs in foods*, 3(1), FAO/WIK Rome.

Cunha, B.A. (2001). Antibiotic side effects. *Med. Clin. North. Am.*, **85**(1):149-185.

Dahshan, El., Lamiaa, I.K. and Laila S.D. (2014). Talent Management and Its Effect on Organization Performance among Nurses at Shebin El-Kom Hospitals. *Int. J. Nursing*. **5**(2): 108-123

Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S. and Ishizuka, M. (2013) Antibiotic residues in food: The African scenario. *Jpn. J. Vet. Res*., **61**(Suppl): S13-S22.

De Ruyck, H., De-Ridder, Renterghem, R.V. and Wambeke, F.V. (1999). Validation of HPLC method of analysis of tetracycline residues in eggs and broiler meat and its application to feeding trial. *Food Additives Contaminants***. 16**(2): 4756.

Dibner, J.J. and Richards, J.D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci.*, **84**: 634-643.

Dipeolu, M. A. and Alonge D. O., (2002). Residues of streptomycin antibiotic in meat sold for human consumption in some states of south-western Nigeria. *Arch. Zootec.* **51**: 477-480.

Donkor, E. S., Newman, M. J., Tay, S. C. K., Dayie, N. T. K. D., Bannerman, E. and Olu-Taiwo, M. (2011). Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana**.** *Food Control***.** **22**: 869-73.

Donoghue, D. J. (2003). Antibiotic Residues In Poultry Tissues and Eggs: Human Health Concerns?. *Poultry Sci.*, **82**:618-621.

Doyle, M. (2006). Veterinary Drug Residues in Processed Meats-Potential Health Risk, FRI Briefings; Food Research Institute, University of Visconsin: Madison, WI, USA. p. 148–196.

EC, European Commission. (2002). Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *J. Eur. Communi*. **221**: 8-36

Ekaterina, V., Pokrant, A.A., Maddaleno, B.C., Arya, B.B., San, M., Cornejo, J. (2017). In-House validation of HPLC-MS/MS Methods for detection and quantification of Tetracycline in Edible tissues and feathers of broiler chickens. *Chem. Soc*. pp. 1-10.

Ellin-Doyle, M. (2001). Alternative to antibiotic use for growth promotion in animal Husbandary. FRI briefing.

Elnasri, H.A., Salman, A.M., and El-Rade, S.A. (2014). Screening of antibiotic residues in poultry liver, kidney and muscle in Kharoum State, Sudan, *J. Appl. India*. Sci. **2**(3): 116-122.

Falowo, A.B. and Festus, A.O. (2019). Veterinary Drug Residues in Meat and Meat Products: Occurrence, Detection and Implications. Publisher: IntechOpen, DOI: 10.5772/ intechopen.83616.

FAO/WHO. (2010). Residue evaluation of certain veterinary drugs. In J. F. W. E. C. o. F. Additives (Ed.), (15 ed.). Rome: Food And Agriculture Organization Of The United Nations.

Ferdous, J., S Sachi, Z.A. Noman, S.M.A.K. Hussani, Y.A. and Sikder, M.H. (2019). Assessing farmers' perspective on antibiotic usage and management practices in small-scale layer farms of Mymensingh district, Bangladesh. *Vet. World*, **12**: 1441-1447.

Furusawa N. (1999). Spiramycin, oxytetracycline and sulphamonomethoxine contents of eggs and egg-forming tissues of laying hens**.** *Zentralbl Veterinarmed A***. 46**(10): 599-603.

Goetting, V., Lee, K. A. and Tell, L. A. (2011). Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. Journal of Veterinary Pharmacology Therapeutics 34(6): 521-56.

Graham, J.P., Boland, J.J. and Silbergeld, E. (2007). Growth promoting antibiotics in food animal production: An economic analysis. *Public Health Rep*., **122**(1): 79-87.

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

Hamid, M.A., Rahman, M.A., Ahmed, S. and Hossain, K.M. (2017). Status of Poultry Industry in Bangladesh and the Role of Private Sector for its Development. *Asian J. Poult. Sci*., **11**: 1-13.

Hautekeete, M.L. (1996) Hepatotoxicity of antibiotics. *Acta Gastroenterol. Belg*., **58**(3): 290-296.

Hu, B., Jia, X., Hu, J., Xu, D., Xia, F. and Li, Y. (2017). Assessment of heavy metal pollution and health risks in the soil-plant-human system in the Yangtze River delta, China. I. *J. Environ. Res. Public Health*. **14**: 1042.

Hussein, M.A. and Khalil, S. (2013). Screening of some antibiotics and anabolic steroids residues in broiler breast marketed in El-Sharkia governorate. *Life Sci. J*., **10**: 2111-2118.

Hyung, S.W., Lee, C.H. and Kim, B. (2017). Development of certified reference materials for accurate determination of fluoroquinolone antibiotics in chicken meat. *Food Chem*., **229**: 472-478.

Islam, M.S., Islam, M.Z. and Islam, M.S. (2019). Discriminate and indiscriminate use of amoxicillin antibiotic and detection of its residue in poultry edible tissue by thin layer chromatography (TLC) method. Asian Australas. *J. Food Saf. Secur*., **3**: 96-102.

Jammoul, A. and Darra, N.E.l. (2019). Evaluation of antibiotics residues in chicken meat samples in Lebanon. *J. Antibiotics*. **8**: 69.

Jank, L., Martins, M.T., Arsand, J.B., Motta, T.M.C., Feij, T.C., Castilhos, T.DS. (2017). Liquid chromatography-tandem mass spectrometry multiclass method for 46 antibiotics residues in milk and meat: Development and validation. *Food Analytical Methods*. pp. 1-13.

Jayalakshmi, K., Paramasivam, M., Sasikala, M., Tamilam, TV. and Sumithra, A. (2017). Review on antibiotic residues in animal products and its impact on environments and human health. *J. Entomol. Zool. Studies*. **5**(3): 1446-1451.

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

Kantiani, L., Farré, M., Grases I., Freixiedas, J.M. and Barceló, D. (2010). Development and validation of a pressurised liquid extraction liquid chromatography-electrospray-tandem mass spectrometry method for β-

lactams and sulfonamides in animal feed. *J. Chromatogr*. **1217**: 4247–4254.

Katz, S.E. and Brady, M.S. (2000). Antibiotic residues in food and their significance. *Food Biotechnol*., **14**(3): 147-171.

Kebede, G., Zenebe, T., Disassa, H., Tolosa, T. (2014). Review on detection of antimicrobial residues in raw bulk milk in dairy farms. African Journal of Basic & Applied Sciences. **6**(4):87-97.

Kempe, M. and Verachtert, B. (2000). Cartridges with Molecularly Imprinted Recognition Elements for Antibiotic Residues Monitoring in Milk Cream. Pure and Applied Biochemistry, Lunds Universitét Centre for Chemistry and Chemical Engineering Getingevagen, Lund Sweden. p1-10.

Khan, M.I., Ferdous , J., Ferdous, M.R.A., islam, M.S., Rafiq, K. and Rima, U.K. (2018). Study on indiscriminate use of antibiotics in poultry feed and residues in broilers of Mymensingh city in Bangladesh. Progressive Agriculture. **29**(4): 345-352.

Kirbis, A. (2006). Microbilogical 5-plate screening method for detection of tetracyclines, aminoglycosides, cephalosporines and macrolides in milk. *Slo. Vet. Res.*, **43**(4): 161-168.

Kuhn, I., Iversen, A., Burman, L. G., Olson-Li1jecluist, B., Franklin, A., Finn, M., F. Aarestrup, F. M., Scyfarih, A. M., Blanch, A. R., Taylor, H., Caplin, J., Moreno, M. A., Dominguez, L. and Molby, R. (2000). Epidemiology and ecology of enterococci, with special reference to antibiotic resistant strain, in animals, humans and the environment. Example of an ongoing project within the European research programme. *Int. J. Antimicrob. Agents*. **14**: 337–342.

Lawal, J.R., Jajere, S.M., Geidam, Y.A., Bello, AM. and Wakil, Y. (2015). Antibiotic residues in edible poultry tissues and products in Nigeria: A potential public health hazard. *Int. J. Anim. Vet. Adv*. **7**(3): 55-61.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M. and Goossens, H. (2013). Antibiotic resistance-the need for global solutions. *Lancet Infect. Dis*. **13**: 1057–1098.

Lee, K. G., Lee, J. B., Chung, H. H. and Chung, Y. H. (2007). Development of an analytical protocol for detecting antibiotic residues in various foods. *Food Chem*. **105**: 1726-31.

Lee, M. H., H. L. Lee and P. D. Ryu (2001). Public Health risks: Chemical and Antibiotic residues. *Asian-Australian J. Anim. Scie.* **14**(3): 402-413.

Lolo, M., Pedreira, S., Miranda, J. M., Vazquez, B. I., Franco, C. M., Cepeda, A. and Fente, C. (2006). Effect of cooking on Enrofloxacin residues in chicken tissue. *F. Add. Cont. Part A*., **23**(10):988-993.

Lopes, R. P., Eustaquia de Freitas Passos, E., de Alkimim Filho, J. F., Vargas, E. A., Augusti, D. V. and Augusti, R. (2012). Development and validation of a method for the determination of sulfonamides in animal feed by modified QuEChERS and LCeMS/MS analysis. *Food Control***.** **28**: 192-98.

Maraschiello, C., Cusido, E., Abellan, M. and Vilageliu, J. (2001). Validation of an analytical procedure for the determination of the fluoroquinolone Enrofloxacin in chicken tissues. *J. Chromatogr. B: Biomed. Sci. Appl.,* **754** (2):311-318.

Marazuela, M.D. and Bogialli, S. A. (2009). Review of novel strategies of sample preparation for the determination of antibacterial residues in foodstuffs using liquid chromatography-based analytical methods. Anal. *Chim. Acta*. **645**: 5–17.

Marshall, B.M. and Levy, S.B. (2011). Food animals and antimicrobials: Impacts on human health. *Clin Microbiol Rev*. **24**(4): 718-733.

Mbarga, M.J.A., Podoprigora, I.V., Davares, A.K.L., Razan, M., Das, M.S. and Senyagin, A.N. (2021) Antibacterial activity of grapefruit peel extracts and green-synthesized silver nanoparticles. *Vet. World*. **14**(5): 1330-1341.

McEwen, S.A., Black, W.D., Meek, A.H. (1992). Antibiotic residues (bacterial inhibitory substances) in the milk of cows treated under label and extra-label conditions. *Canadian Vet. J*. **33**:527-534.

McEwen, S. A. and P. J. Fedorka-Cray (2002). Antimicrobial use and resistance in animals. *Clin Infect Dis.,* **34**(3):S93-S106.

Milhaud, G. and Pinault, L. (2001) Evaluation des Médicaments Vétérinaires: Autorisation de Mise sur le Marché (AMM), limites Maximales de Résidus (LMR). Editions Le point Veterinaire. p. 25-40.

Mitchell, J. M., Griffiths, M. W. McEwen, S. A. McNab, W. B. and Yee, A. J. (1998). Antimicrobial Drug Residues in Milk and Meat: Causes, Concerns, Prevalence, Regulations, Tests, and Test Performance. *J Food Prot.* **61**: 742-756.

Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam, Tanzania and public health implications. *American J. Res. Commun*. **2**(4): 51-63.

Mund, M.D., Khan, U.H., Tahir, U., Bahar-EMustafa, Fayyaz, A. (2017). Antimicrobial drug residues in poultry products and implications on public health: A review. *Int. J. Food Properties*. **20**(7):1433–1446.

Muthukumar, M. and Mandal, P.K. (2017). Concerns and consequences of industrial livestock and meat production. *J. Meat Sci*. **12**(2): 1-9.

Naeem, M., Khan K. and Rafiq, S. (2006). Determination of residues of quinolones in poultry products by High Performance Liquid Chromatography. *J. Appl Sci.,* **6**(2):373-379.

Ng, K. and Linder, S. W. (2003). HPLC separation of tetracycline analogues: comparison study of laser based polarimetric detection with UV detection. *J. Chromatographic Sci***. 41**: 460-66.

Niewold, T.A. (2007). The non antibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poult. Sci.,* **86**: 605-609.

Nikolaidou, K. I., Samanidou, F. and Papadoyannis. (2008). Development and validation of an HPLC method for the determination of seven tetracycline antibiotics residues in chicken muscle and egg yolk according to 2002/657/EC. *J. Liquid Chromatography Technol*. **31**: 2141-58.

Nisha, A.R. (2008). Antibiotic Residues - A Global Health Hazard. *Veterinary World.* **1**(12): 375-377.

Nonga, H.E.; Mariki, M.; Karimuribo, E.D.; Mdegela, R.H. (2009). Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro Municipality, Tanzania. *Pakistan J. Nutr*. **8**: 203–207.

Oka, H., Ikai, Y., Kawamura, N., Uno, K., Yamada, M. and Suzuki, M. (1987). Improvement of chemical analysis of antibiotics XII. Simultaneous analysis of seven tetracyclines in honey. *J. Chromatogr.,* **400**: 253261.

Oka, H., Ito, Y. and Matsumoto, H. (2000). Chromatographic analysis of tetracycline antibiotics in foods. *J. Chromatography A,* **882**: 109-133.

Okerman, L., Van, J.H. and Debeuckelaere, W. (1998). Evaluation of the European four-plate test as a tool for screening antibiotic residues in meat samples from retail outlets. *J. Assoc. off. Anal. Chem.,* **81**:51-56.

Okocha, R.C., Olatoye, I.O. and Adedeji, O.B. (2018) Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev*., **39**(1): 21.

Omeiza, G. K., Kabir, J., Mamman, M., Ibrahim, H. and Fagbamila, I. O. (2012). Response of Nigerian farmers to questionnaire on application in commercial farmers. ***V****eterinariaItaliana***. 48**: 87-93.

Palmieri, M.D., Cerbo, A.D. and Laurino, C. (2014). Antibiotic Treatments in zootechnology and effects induced on the food chain of domestic species and comparatively, the human species. *Nutricion Hospitalaria*. **29**: 1427- 1433.

Paturkar, A. M. (2002). Antimicrobial residue surveillance in food animals.' Ph.D. dissertation. Maharashtra Animal and Fishery Sciences University, Nagpur, India.

Paul, T.K., Hasan, M.M. Haque, M.A., Talukder, S., Sarker, Y.A., Sikder, M.H., Mahna Khan, and Kumar, A. (2020). Dietary supplementation of Neem (Azadirachta indica) leaf extracts improved growth performance and reduced production cost in broilers. *Vet. World*. **13**: 1050-1055.

Rahman, M.S., Jang, D.H., and Yu, C.J. (2017). Poultry industry of Bangladesh: entering a new phase. *Korean J. Agric. Sci*. **44**(2): 272-282.

Raihan, S. and Mahmud, N. (2008). Trade and Poverty Linkages: A Case Study of the Poultry Industry in Bangladesh, Cuts Citee Working Paper No.6.

Ramatla, T., Ngoma, L., Adetunji, M., Mwanza, M. (2017). Evaluation of Antibiotic Residues in Raw Meat Using Different Analytical Methods. *Antibiotics*. **6**(4):1–17.

Rashid, M.A., Sarker, M.S.K. Khatun, H., Sarker, N.R. Ali, M.Y. and Islam, M.N. (2018). Detection of heavy metals in poultry feed, meat and eggs. Asian Australas. *J. Food Saf. Secur*., **2**: 1-5.

Salehzadeh, F., A. Salehzadeh, N. Rokni, R. Madani and F. Golchinefar (2007). Enrofloxacin residue in chicken tissues from Tehran slaughter houses in Iran. *Pak. J. Nutr,* **6**(4):409-413.

Sarker, Y.A., Hasan, M.M., Alam, M.N. and Sikder, M.H. (2017). Measurement of antibiotic residues in chicken feed and meat in Bangladesh. *J. Adv. Vet. Anim. Res*., **5**(1): 131-136.

Sarker, M.S., Ahaduzzaman, M., Ghosh, S.K., Sayeed, M.A., Bary, M.A. (2016). Crosssectional survey on prescribing patterns for food animal medications in Bangladesh. *J. Dairy Vet. Animal Res*. **3**: 3–5.

Sarker, Y.A., Hasan, M.M., Paul, T.K., Rashid, S.Z., Alam, M.N., Sikder, M.H. (2018). Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. *J. Adv. Vet. Animal Res*. **5**(2):140-145.

Sarker, Y.A., Rashid, S.Z., Sachi, S., Ferdous, J., Das, B.L. Tarannum, S.S. and Sikder, M.H. (2020). Exposure pathways and ecological risk assessment of common veterinary antibiotics in the environment through poultry litter in Bangladesh. *J. Environ. Sci. Health B*. **55**: 1061-1068.

Shahid, M. A., Siddique, M., Rehman, S. U., Hameed, S. and Hussain, A. (2007). Evaluation of a Microbiological Growth Inhibition Assay as a Screening Test for the Presence of Antibiotic Residues in Poultry Meat. *American J. food technol.* **2**(5): 457-461.

Shareef, A. M., Jamel, Z. T. and Yonis, K. M. (2009). Detection of antibiotic residues in stored poultry products. *Iraqi Journal of Veterinary Sciences,* **23**(1): 45- 48.

Skoog, D. A., Holler, F. J. and Nieman, T. A. (1998). Principles of Instrumental Analysis. 5th ed. *Saunders, Philadelphia.* USA.

Swatantra, S., Tandia, N., Nitesh, K., Paliwal, R. (2014). Antibiotic Residues: A global challenge. *Pharma Sci. Monitor*. **5**(3):184-197.

Tadesse T and Tadesse T, 2017. Public health impacts of antibiotic residues in foods of animal origin: A review. *Public Policy Adm Res*. **7**: 6-11.

Tajick, M. A. and Shohreh, B. (2006). Detection of Antibiotics Residue in Chicken Meat Using TLC. *International J Poultry Sci.,* **5** (7):611-612.

Talukder ,S., Hasan, M.M., Noman, Z.A., Sarker, Y.A., Paul ,T.K. and Sikder, M.H. 2017. Effect of dietary supplementation of ginger extract on growth, carcass characteristics and haematological parameters in broilers. *Asian J. Med. Biol. Res*., **3**: 211-215.

Telling, G. M. (1990). Control of Veterinary drug residues in Food. *Proceedings of the Euro-Residue Conference,* Noordwij-kerhout, The Netherlands, 21-23 May.

Thiele-Bruhn, S. (2003). Pharmaceutical antibiotic compounds in soils - A review. *J. Plant Nutr. Soil*. **166**, 145–167.

Treiber, F.M. and Beranek-Knauer, H. (2021) Antimicrobial residues in food from animal origin a review of the literature focusing on products collected in stores and markets worldwide. *Antibiotics (Basel)*. **10**(5): 534.

Van-den-Bogaard, A.E. and Stobberingh, E.E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. *Int. J. Antimicrob Agents*. **14**: 327-335.

Van-Gerven, N.M., de Boer, Y.S., Mulder, C.J., van Nieuwkerk, C.M. and Bouma, G. (2016) Auto immune hepatitis. *World J. Gastroenterol*., **22**(19): 4651.

Vollard, E.J. and Clasener, H.A.L. (1994). Colonization resistance. *Antimicrobial Agents Chemotherapy*, **38**: 409-414.

Vragovic, N., Bazulic, D. and Zdolec, N. (2012). Dietary exposure assessment of β-lactum antibiotic residues in milk on Croatian market. Croatian Journal of Food Science and Technology **4**(1): 81-4.

Wageh, S., Darwish, Eldaly, Elsaid, A., El-Abbasy, Tharwat, M., Ikenaka, Yoshinori, Nakayama, Shouta, Ishizuka and Mayumi. (2013). Antibiotic residues in food: the African scenario. Japanese Journal of Veterinary Research**. 61**: 13-22.

WHO (World Health Organization), 1997. The Medical Impact of the Use of Antimicrobials in Food Animals. Available in https://apps.who.int/iris/ bitstream/handle/10665/64439/WHOEMC_ZOO_97.4. pdf? sequence=1 [9 Sep, 2019].

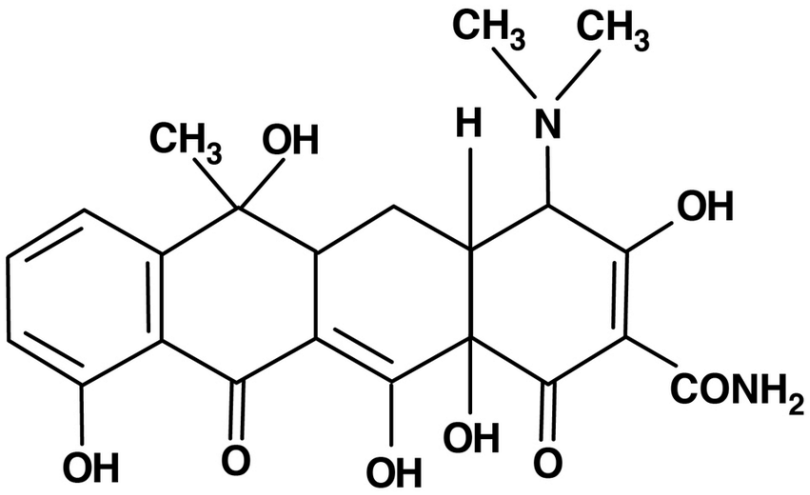
WHO. (2014). Residue evaluation of certain veterinary drugs. In J. F. W. E. C. o. F. Additives (Ed.), (15 ed.). Rome: Food And Agriculture Organization Of The United Nations.

Woodward, K. (2004). The evolution of safety assessments for veterinary medicinal products the European Union. *Vet. Human Toxicol*. **46**: 199-205.

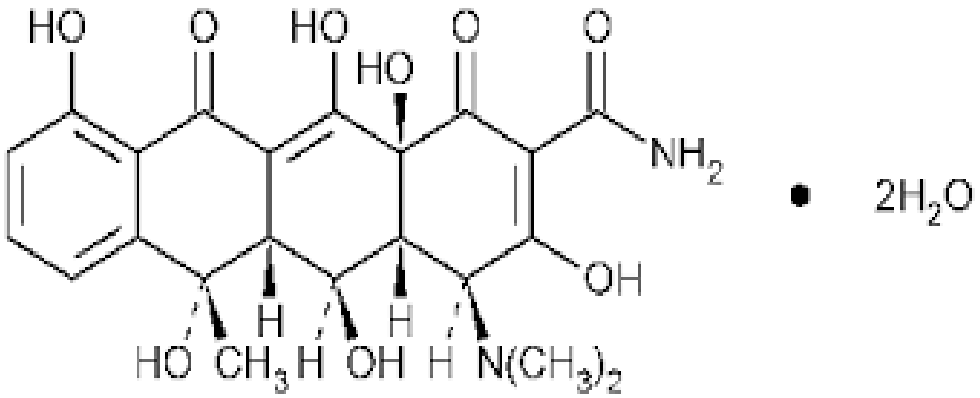
Yang, Y., Qiu, W., Li, Y. and Liu, L. (2020) Antibiotic residues in poultry food in Fujian Province of China. Food Addit. *Contam. Part B Surveill*., **13**(3): 177-184.

APPENDICES

Appendix I: Chemical structure of Tetracycline

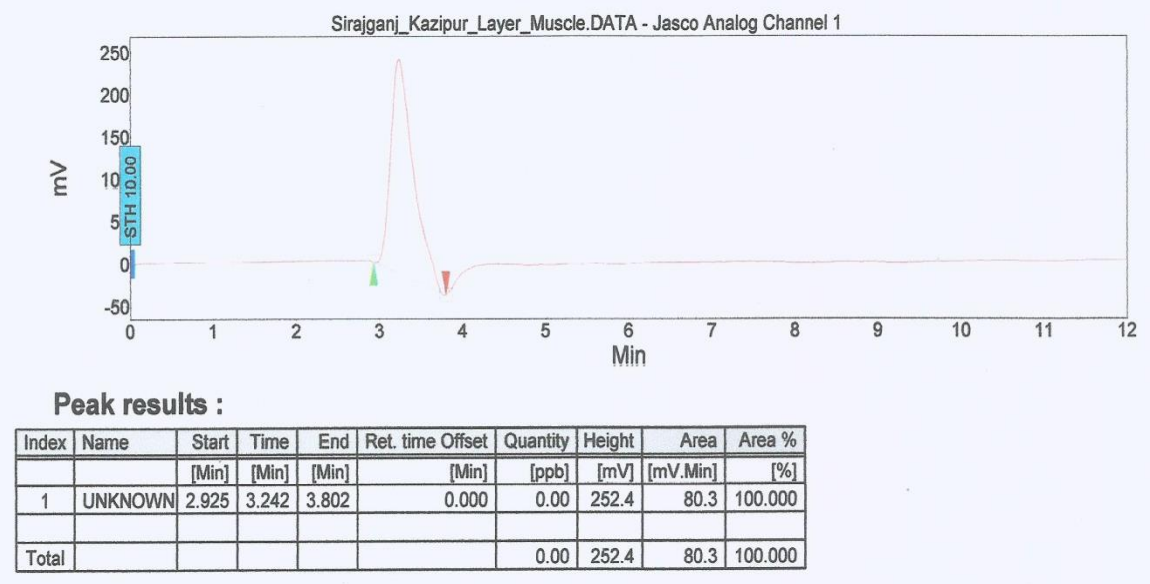


Appendix II: Chemical structure of Oxytetracycline



Appendix III. Chromatogram of Tetracycline and Oxytetracycline in layer and broiler muscle and liver samples

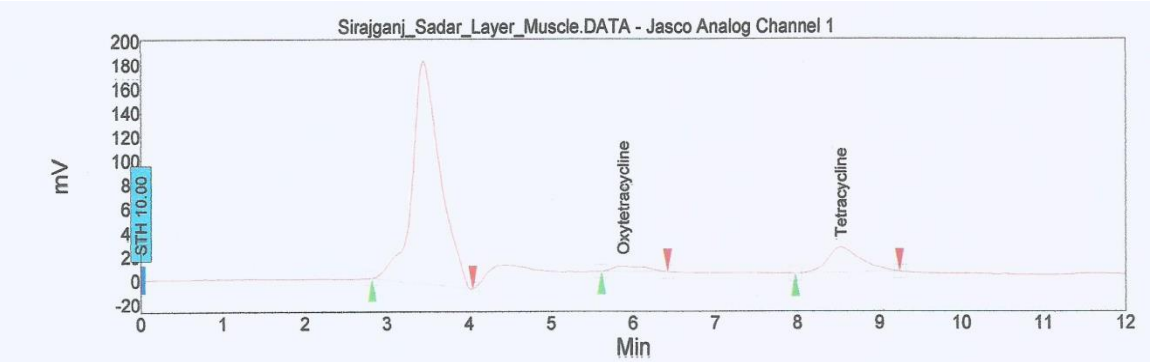
A. Layer muscle



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.925	3.242	3.802	0.000	0.00	252.4	80.3	100.000
Total						0.00	252.4	80.3	100.000

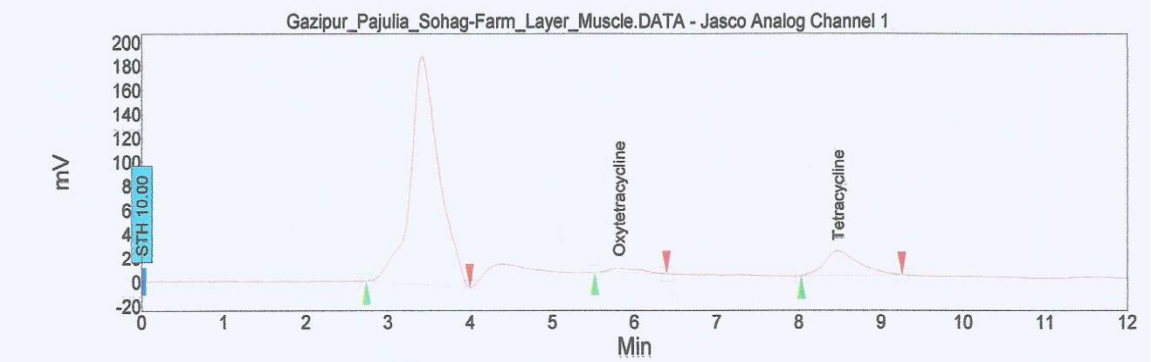
Figure 9. Chromatogram of TC and OTC in layer muscle (sample id SKLCRM**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.817	3.450	4.046	0.000	0.00	185.0	73.4	86.182
2	Oxytetracycline	5.608	5.875	6.420	-0.225	8.20	4.1	1.9	2.268
3	Tetracycline	7.970	8.525	9.247	0.025	41.68	21.1	9.8	11.550
Total						49.89	210.2	85.2	100.000

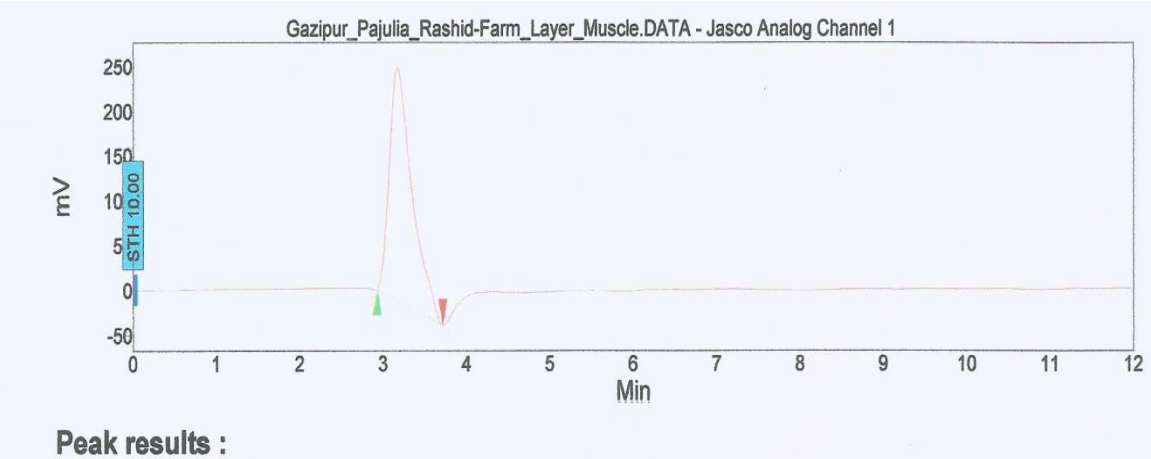
Figure 10. Chromatogram of TC and OTC in layer muscle (sample id SSLCAM**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.734	3.417	3.996	0.000	0.00	189.9	76.7	86.934
2	Oxytetracycline	5.512	5.808	6.392	-0.292	6.74	3.6	1.6	1.790
3	Tetracycline	8.023	8.475	9.259	-0.025	43.12	20.8	9.9	11.276
Total						49.86	214.3	88.2	100.000

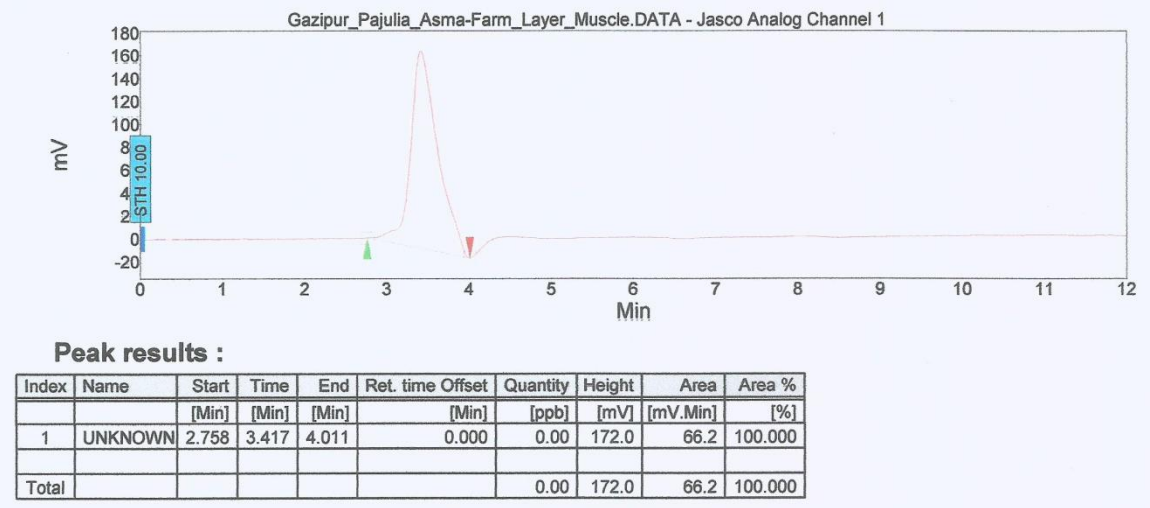
Figure 11. Chromatogram of TC and OTC in layer muscle (sample id GCLCPSM**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.925	3.175	3.719	0.000	0.00	259.6	81.9	100.000
Total						0.00	259.6	81.9	100.000

Figure 12. Chromatogram of TC and OTC in layer muscle (sample id GCLCPRM**)**

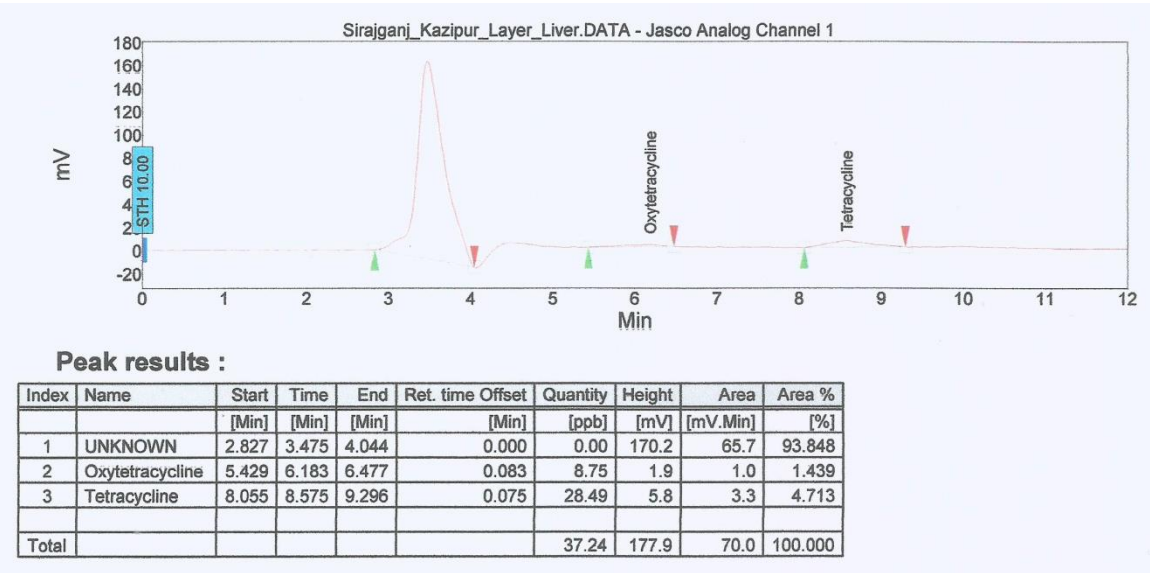


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.758	3.417	4.011	0.000	0.00	172.0	66.2	100.000
Total						0.00	172.0	66.2	100.000

Figure 13. Chromatogram of TC and OTC in layer muscle (sample id GCLCPAM**)**

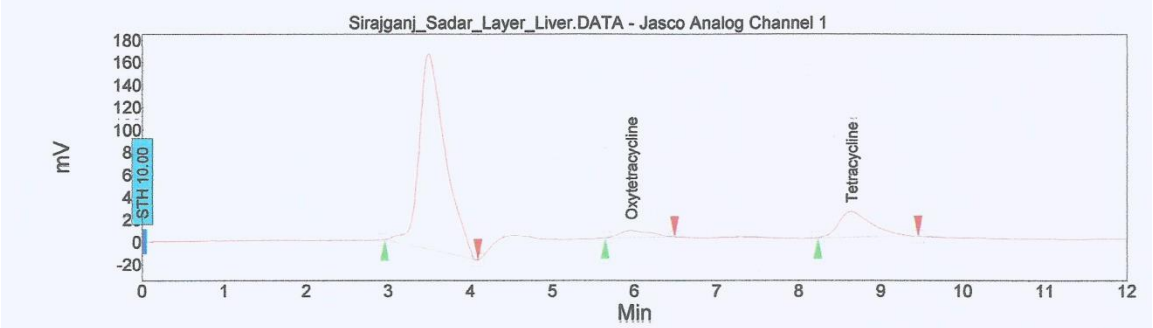
B. Layer liver



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.827	3.475	4.044	0.000	0.00	170.2	65.7	93.848
2	Oxytetracycline	5.429	6.183	6.477	0.083	8.75	1.9	1.0	1.439
3	Tetracycline	8.055	8.575	9.296	0.075	28.49	5.8	3.3	4.713
Total						37.24	177.9	70.0	100.000

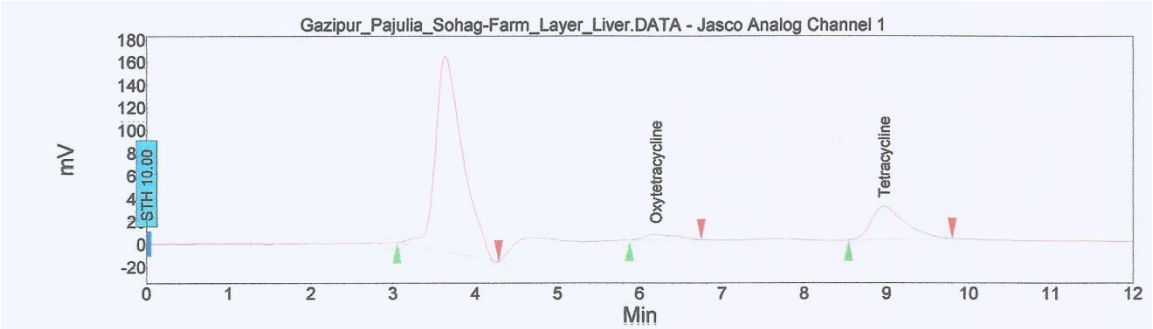
Figure 14. Chromatogram of TC and OTC in layer liver (sample id SKLCRL**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.951	3.492	4.094	0.000	0.00	173.4	64.3	82.547
2	Oxytetracycline	5.638	5.958	6.490	-0.142	17.08	6.4	2.8	3.591
3	Tetracycline	8.229	8.642	9.457	0.142	64.51	22.9	10.8	13.861
Total						81.59	202.7	77.8	100.000

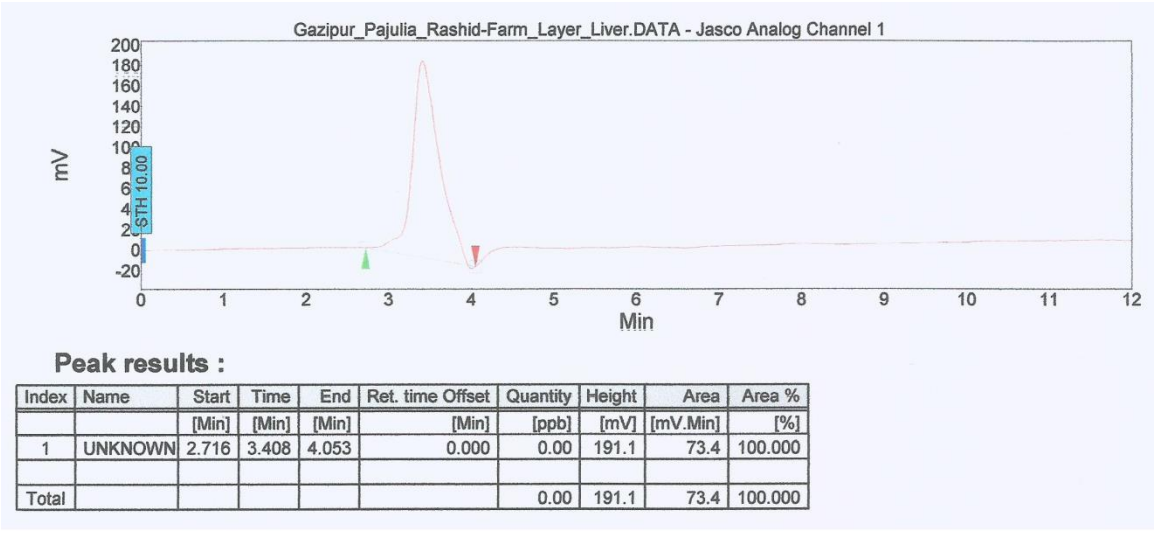
Figure 15. Chromatogram of TC and OTC in layer liver (sample id SSLCAL)



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.044	3.642	4.285	0.000	0.00	171.4	65.7	80.136
2	Oxytetracycline	5.867	6.192	6.751	0.092	11.86	5.1	2.4	2.875
3	Tetracycline	8.533	8.967	9.802	0.467	69.74	29.1	13.9	16.989
Total						81.60	205.6	82.0	100.000

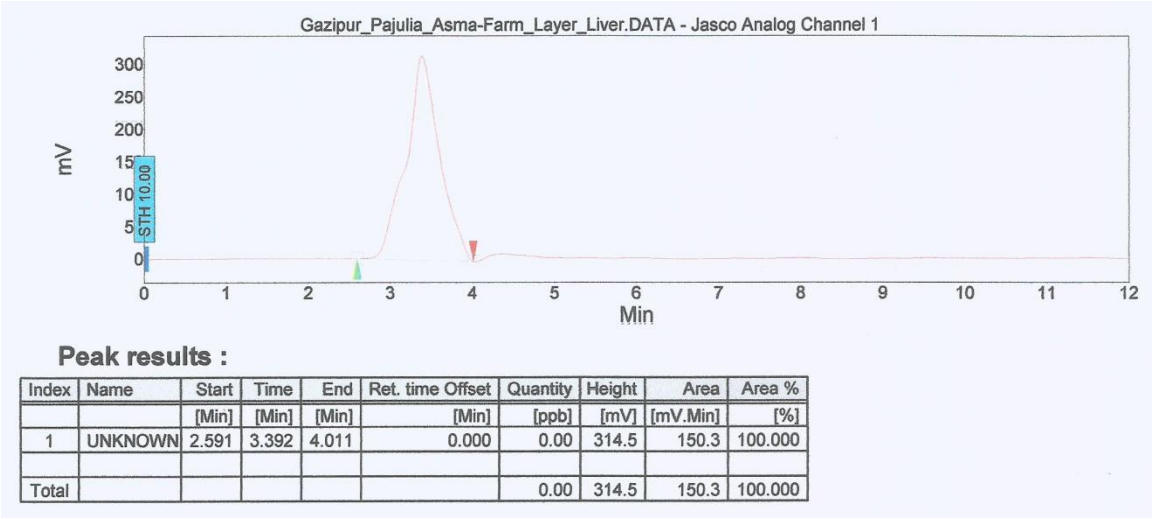
Figure 16. Chromatogram of TC and OTC in layer liver (sample id GCLCPSL)



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.716	3.408	4.053	0.000	0.00	191.1	73.4	100.000
Total						0.00	191.1	73.4	100.000

Figure 17. Chromatogram of TC and OTC in layer liver (sample id GCLCPRL)

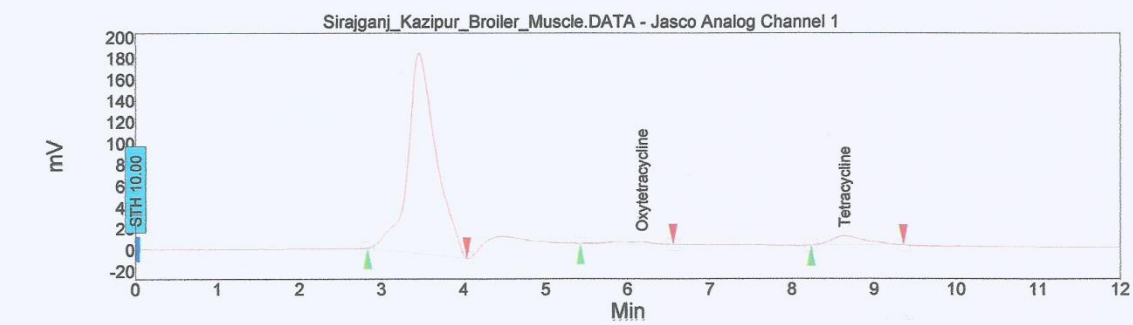


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.591	3.392	4.011	0.000	0.00	314.5	150.3	100.000
Total						0.00	314.5	150.3	100.000

Figure 18. Chromatogram of TC and OTC in layer liver (sample id GCLCPAL)

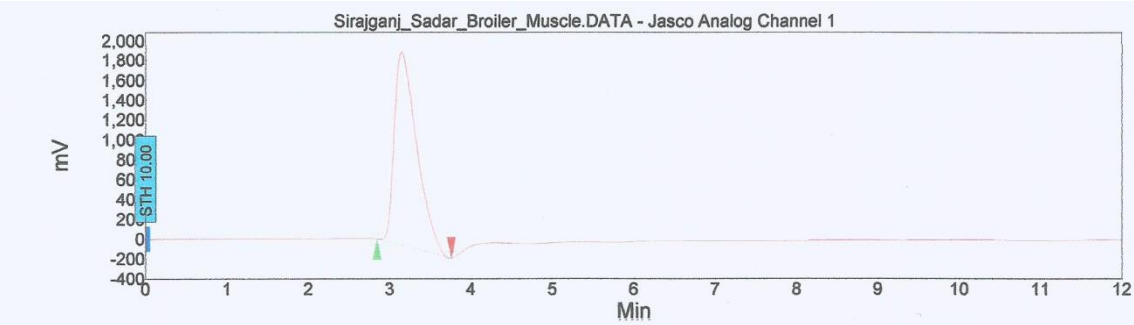
C. Broiler muscle



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
3	UNKNOWN	2.828	3.458	4.039	0.000	0.00	188.1	75.4	93.326
1	Oxytetracycline	5.419	6.167	6.553	0.067	8.56	2.0	1.2	1.456
2	Tetracycline	8.231	8.633	9.359	0.133	30.60	8.7	4.2	5.218
Total						39.15	198.8	80.8	100.000

Figure 19. Chromatogram of TC and OTC in broiler muscle (sample id SKBCGSM**)**

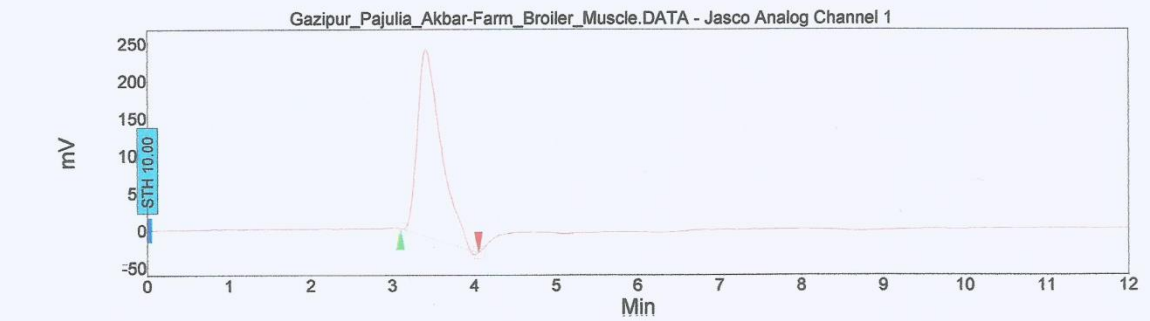


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.841	3.150	3.760	0.000	0.00	1943.1	624.2	100.000
Total						0.00	1943.1	624.2	100.000

Total						39.15	198.8	80.8	100.000

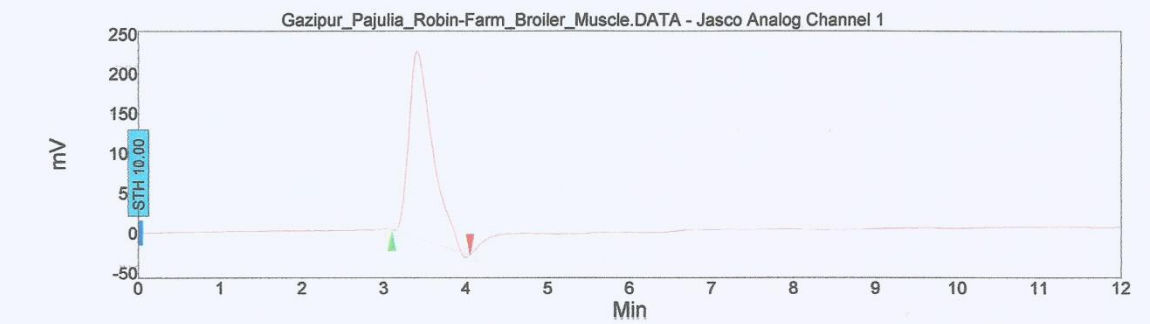
Figure 20. Chromatogram of TC and OTC in broiler muscle (sample id SSBCEMUM**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.092	3.408	4.053	0.000	0.00	249.9	83.5	100.000
Total						0.00	249.9	83.5	100.000

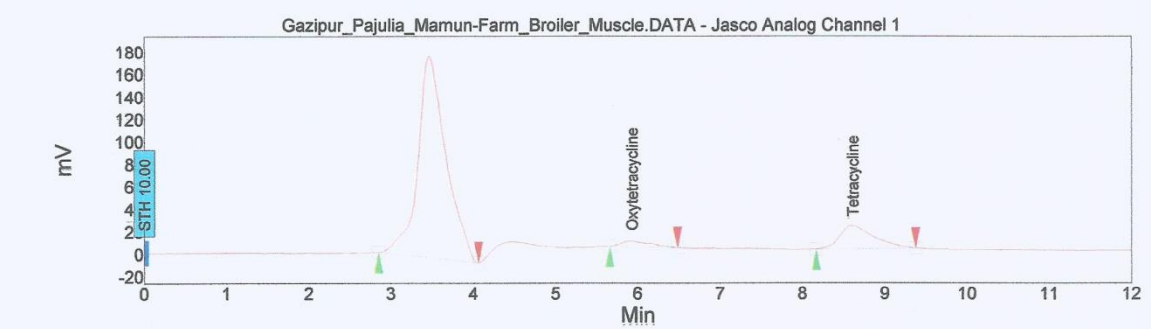
Figure 21. Chromatogram of TC and OTC in broiler muscle (sample id GCBCPAM**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.092	3.408	4.053	0.000	0.00	234.2	78.3	100.000
Total						0.00	234.2	78.3	100.000

Figure 22. Chromatogram of TC and OTC in broiler muscle (sample id GCBCPROM**)**

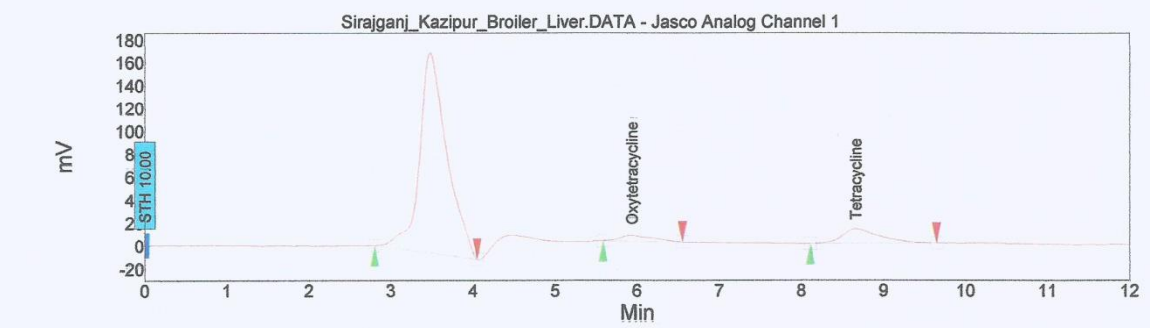


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.848	3.467	4.064	0.000	0.00	178.8	71.1	85.520
2	Oxytetracycline	5.657	5.925	6.484	-0.175	9.93	4.8	2.1	2.488
3	Tetracycline	8.163	8.608	9.379	0.108	47.55	20.5	10.0	11.992
Total						57.48	204.2	83.1	100.000

Figure 23. Chromatogram of TC and OTC in broiler muscle (sample id GCBCPMAM**)**

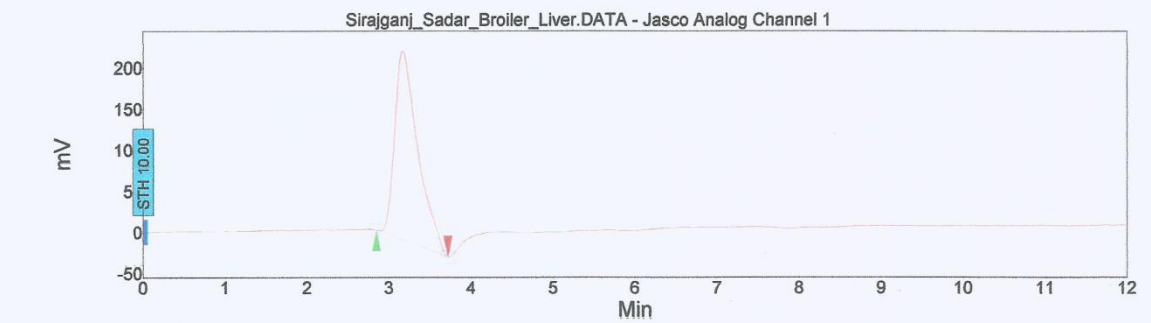
D. Broiler liver



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.799	3.483	4.053	0.000	0.00	174.3	68.0	88.289
3	Oxytetracycline	5.580	5.933	6.552	-0.167	13.20	4.7	2.2	2.887
2	Tetracycline	8.106	8.658	9.652	0.158	39.22	12.8	6.8	8.825
Total						52.42	191.8	77.0	100.000

Figure 24. Chromatogram of TC and OTC in broiler liver (sample id SKBCGSL**)**

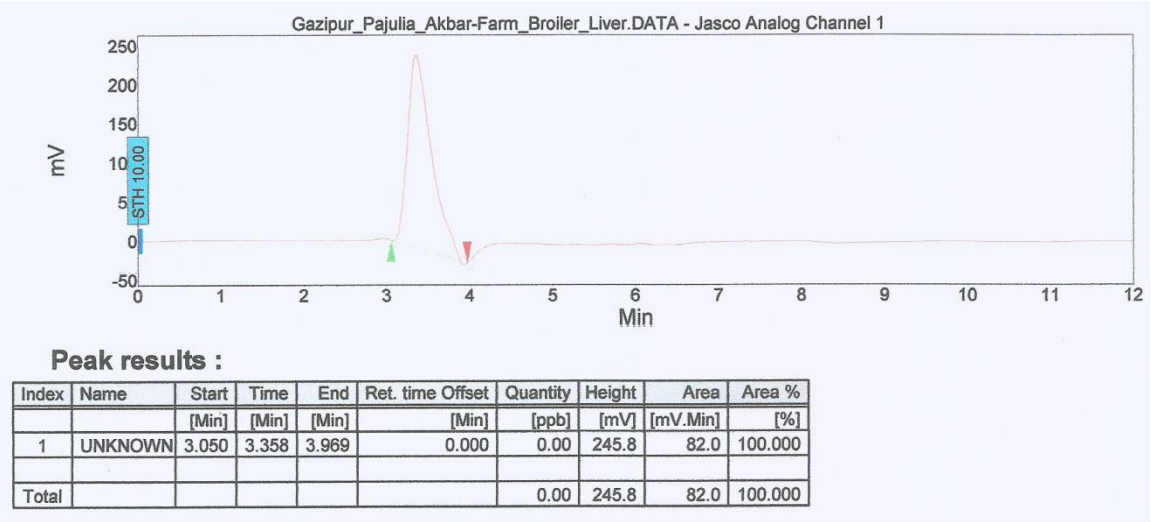


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.841	3.175	3.719	0.000	0.00	229.8	75.2	100.000
Total						0.00	229.8	75.2	100.000

Total						39.15	198.8	80.8	100.000

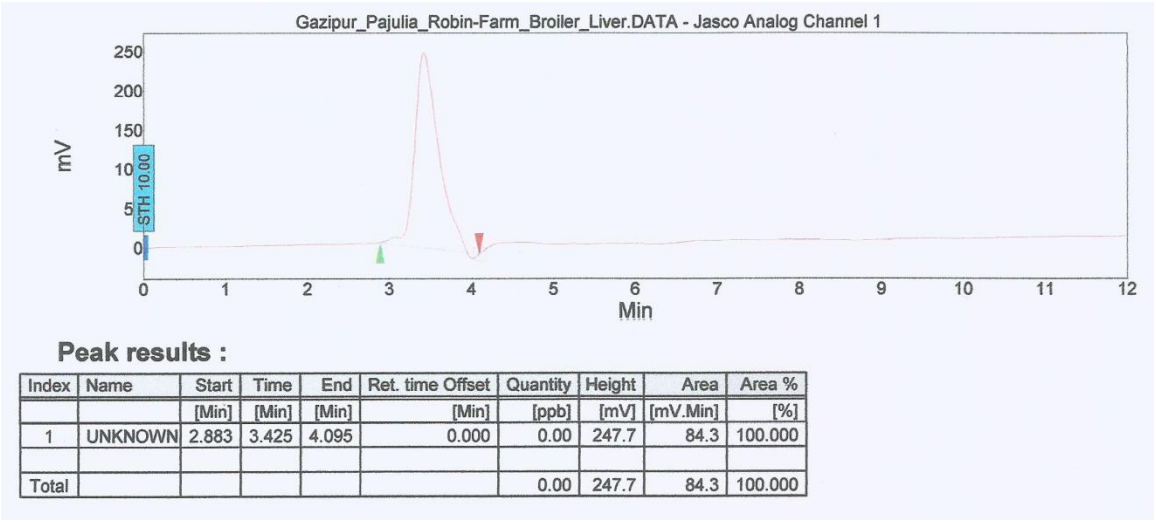
Figure 25. Chromatogram of TC and OTC in broiler liver (sample id SSBCEMUL**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.050	3.358	3.969	0.000	0.00	245.8	82.0	100.000
Total						0.00	245.8	82.0	100.000

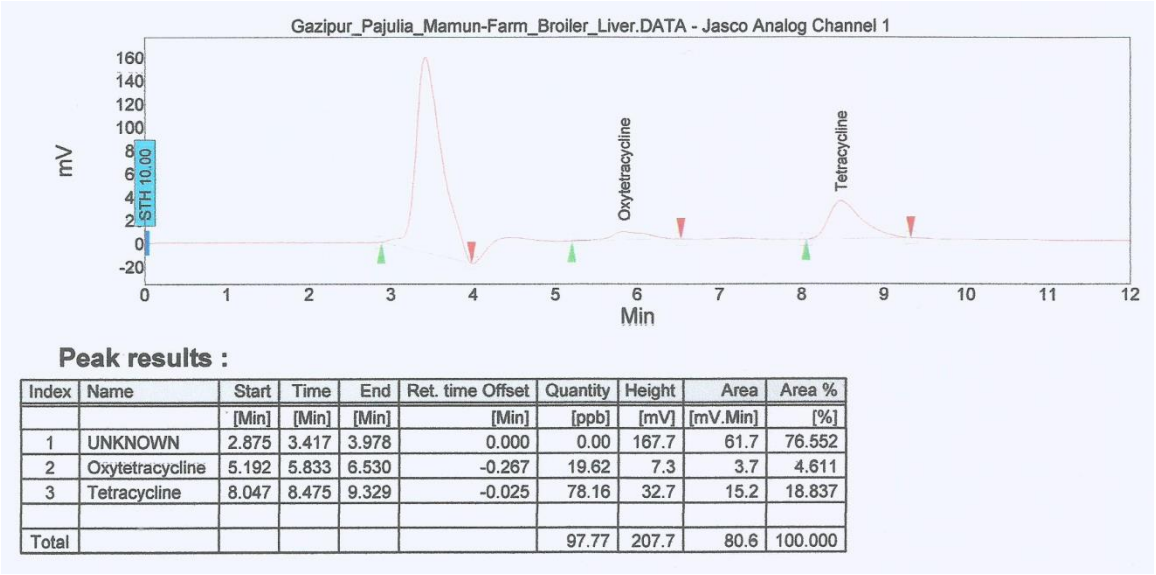
Figure 26. Chromatogram of TC and OTC in broiler liver (sample id GCBCPAL**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.883	3.425	4.095	0.000	0.00	247.7	84.3	100.000
Total						0.00	247.7	84.3	100.000

Figure 27. Chromatogram of TC and OTC in broiler liver (sample id GCBCPROL**)**

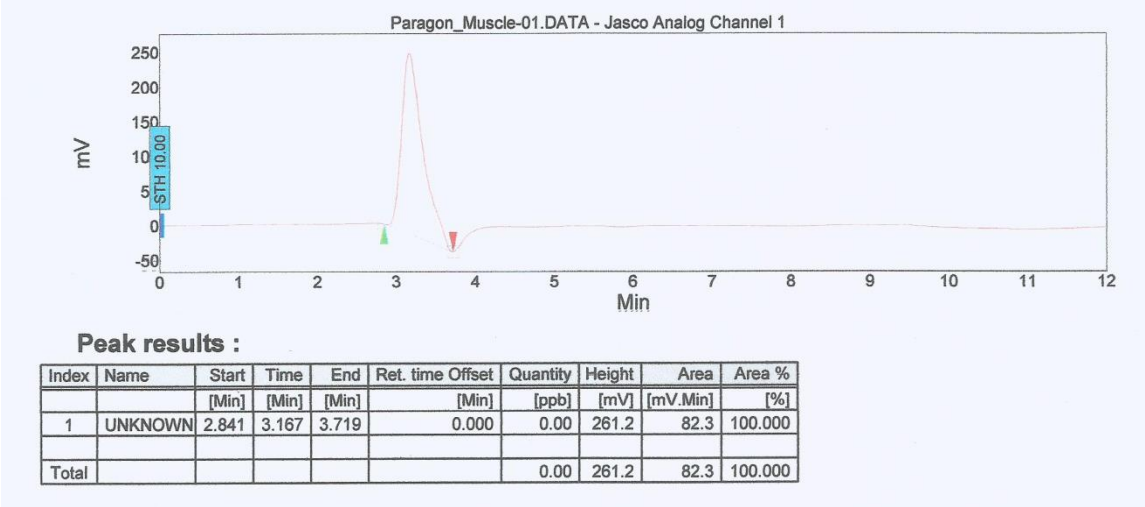


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.875	3.417	3.978	0.000	0.00	167.7	61.7	76.552
2	Oxytetracycline	5.192	5.833	6.530	-0.267	19.62	7.3	3.7	4.611
3	Tetracycline	8.047	8.475	9.329	-0.025	78.16	32.7	15.2	18.837
Total						97.77	207.7	80.6	100.000

Figure 28. Chromatogram of TC and OTC in broiler liver (sample id GCBCPMAL**)**

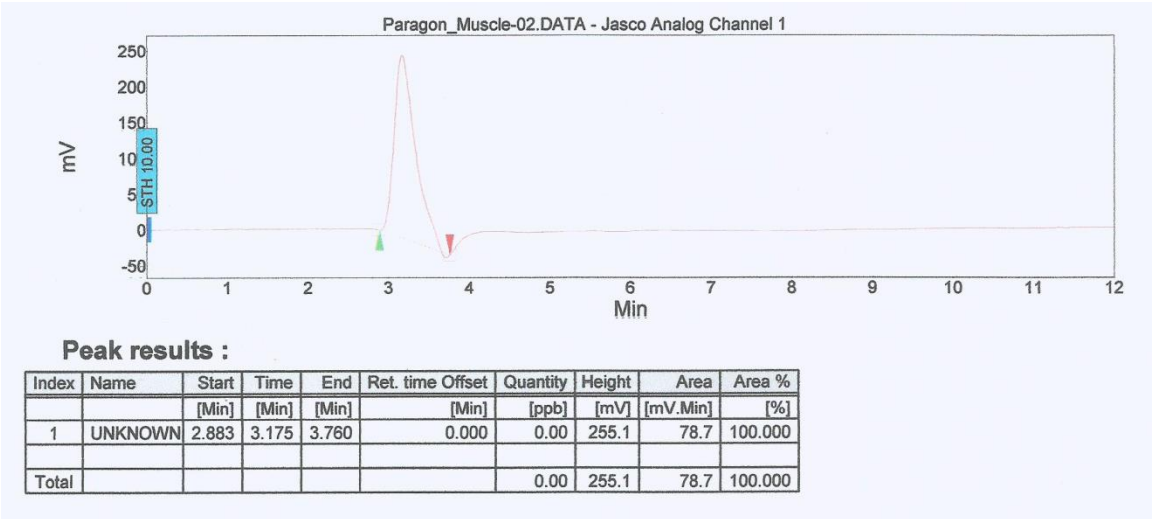
E. Control muscle



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.841	3.167	3.719	0.000	0.00	261.2	82.3	100.000
Total						0.00	261.2	82.3	100.000

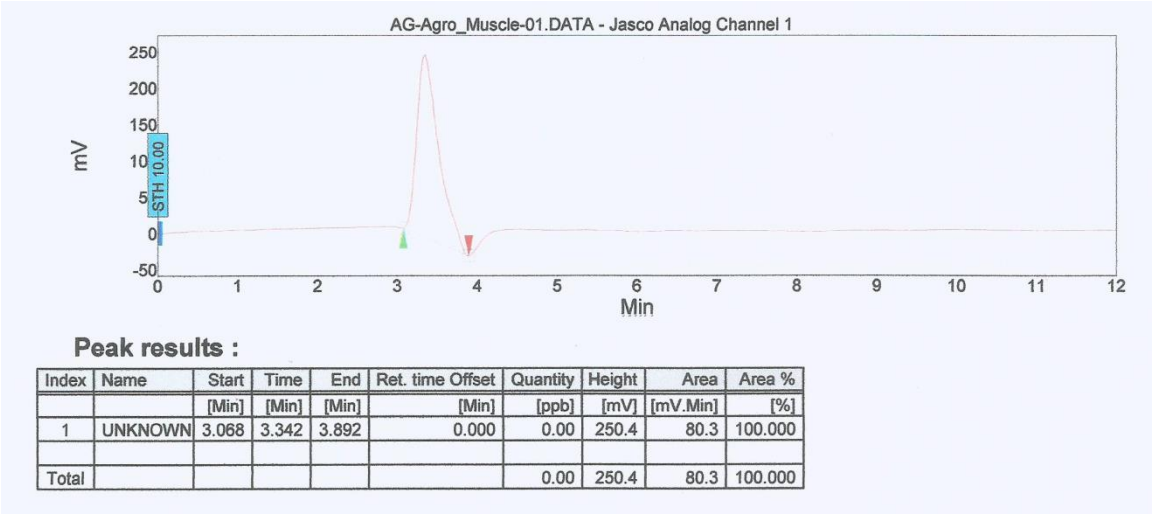
Figure 29. Chromatogram of TC and OTC in control; Paragon muscle-01 (local breed) (sample id PGM-01**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.883	3.175	3.760	0.000	0.00	255.1	78.7	100.000
Total						0.00	255.1	78.7	100.000

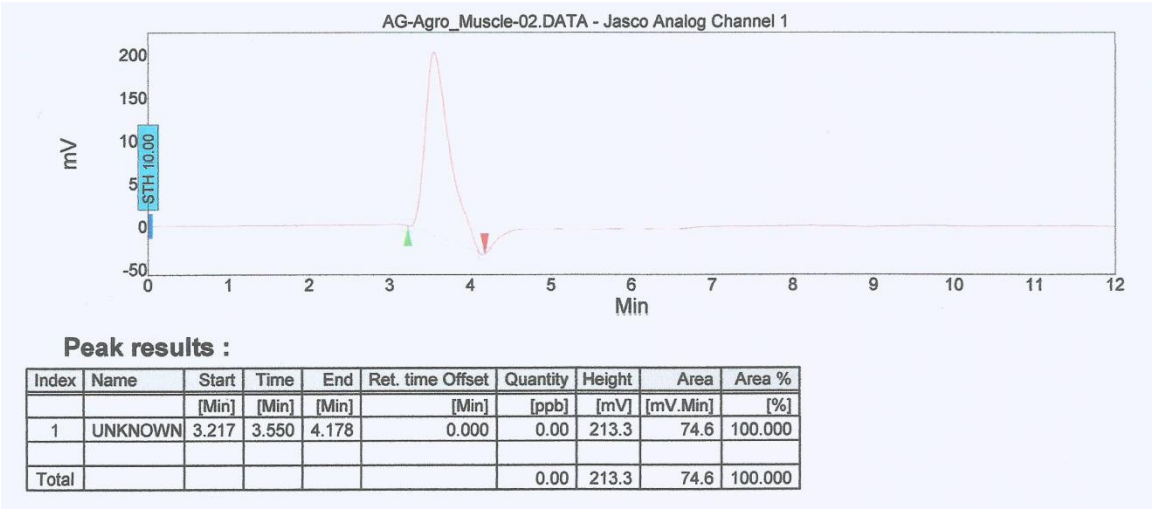
Figure 30. Chromatogram of TC and OTC in control; Paragon muscle-02 (local breed) (sample id PGM-02**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.068	3.342	3.892	0.000	0.00	250.4	80.3	100.000
Total						0.00	250.4	80.3	100.000

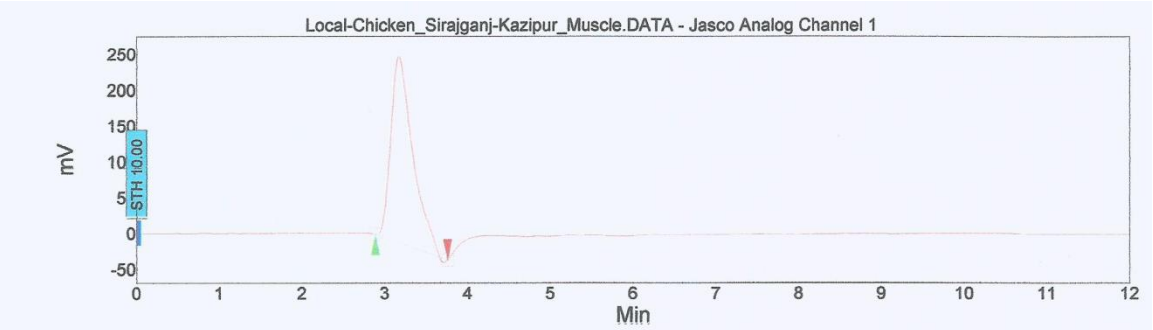
Figure 31. Chromatogram of TC and OTC in control; AG-Agro muscle-01 from local breed (sample id AGM-01**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.217	3.550	4.178	0.000	0.00	213.3	74.6	100.000
Total						0.00	213.3	74.6	100.000

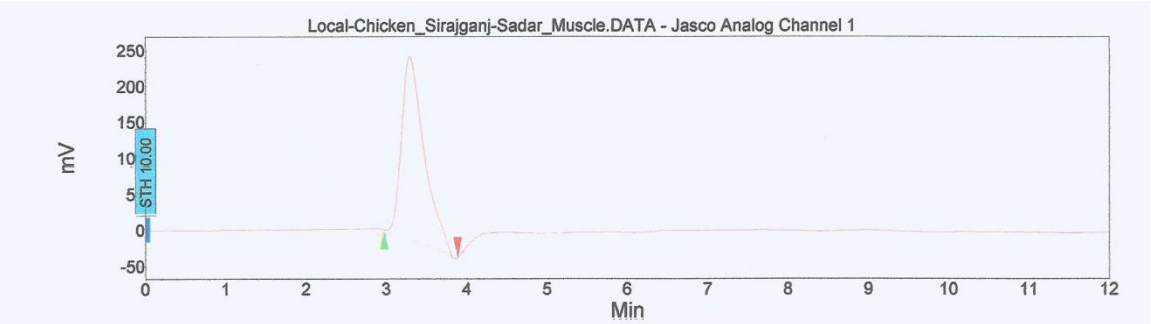
Figure 32. Chromatogram of TC and OTC in control; AG-Agro muscle-02 from local breed (sample id AGM-02**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.883	3.175	3.760	0.000	0.00	259.7	80.2	100.000
Total						0.00	259.7	80.2	100.000

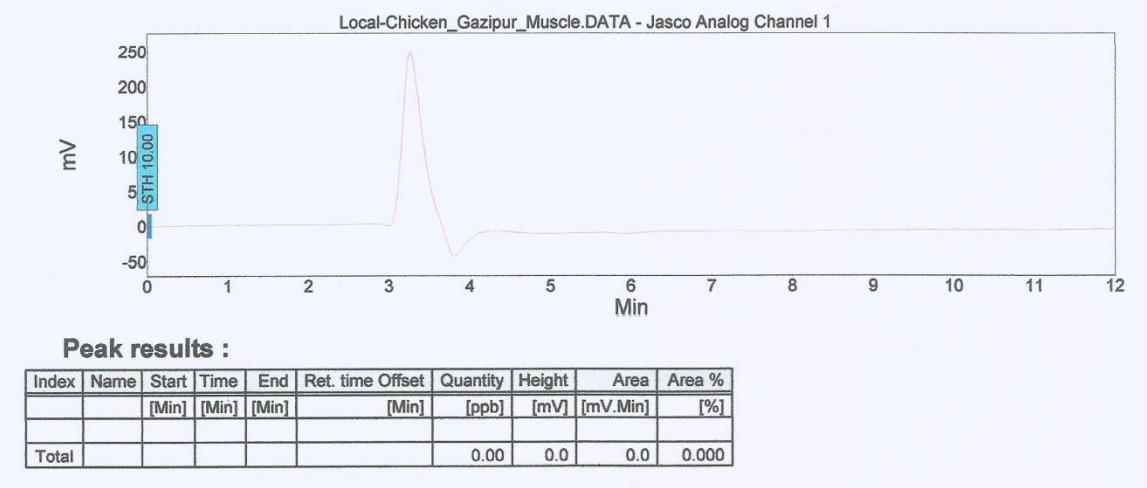
Figure 33. Chromatogram of TC and OTC in control; local chicken, Sirajganj-Kazipur muscle-01 from local breed (sample id LOSSM-01**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.967	3.292	3.886	0.000	0.00	253.9	83.0	100.000
Total						0.00	253.9	83.0	100.000

Figure 34. Chromatogram of TC and OTC in control; local chicken, Sirajganj-Sadar muscle-02 from local breed (sample id LOSSM-02**)**

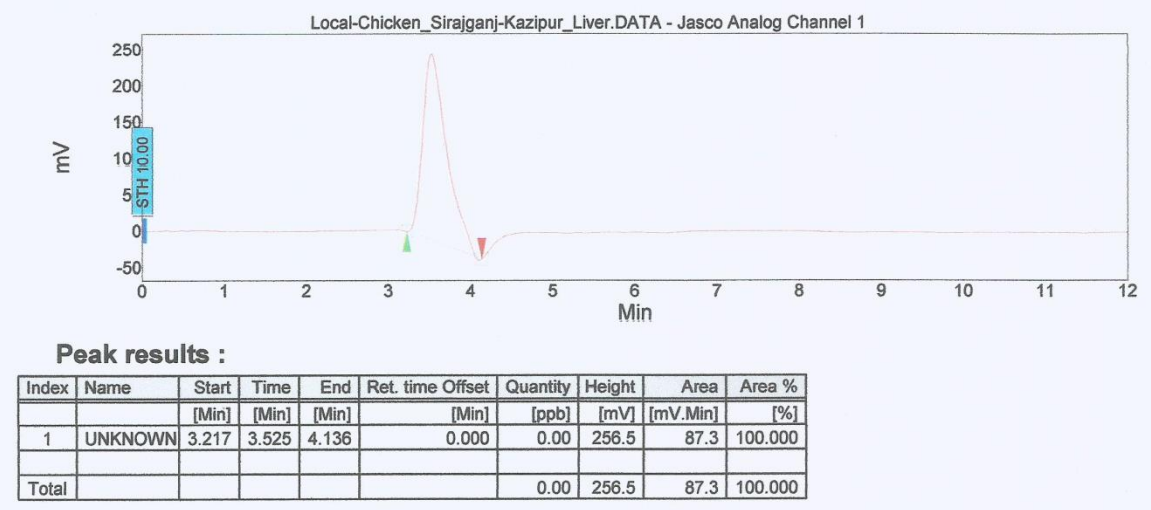


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
Total						0.00	0.0	0.0	0.000

Figure 35. Chromatogram of TC and OTC in control; local chicken, Gazipur-muscle-03 from local breed (sample id LOGM-03**)**

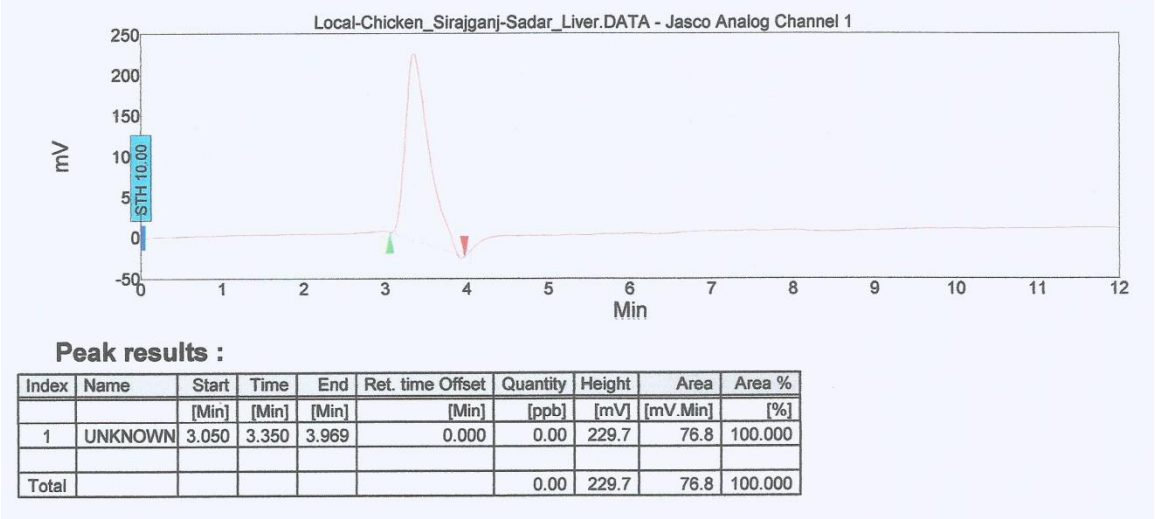
F. Control liver



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.217	3.525	4.136	0.000	0.00	256.5	87.3	100.000
Total						0.00	256.5	87.3	100.000

Figure 36. Chromatogram of TC and OTC in control; local chicken, Sirajganj-Kazipur liver-01 from local breed (sample id LOSSLVR-01**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.050	3.350	3.969	0.000	0.00	229.7	76.8	100.000
Total						0.00	229.7	76.8	100.000

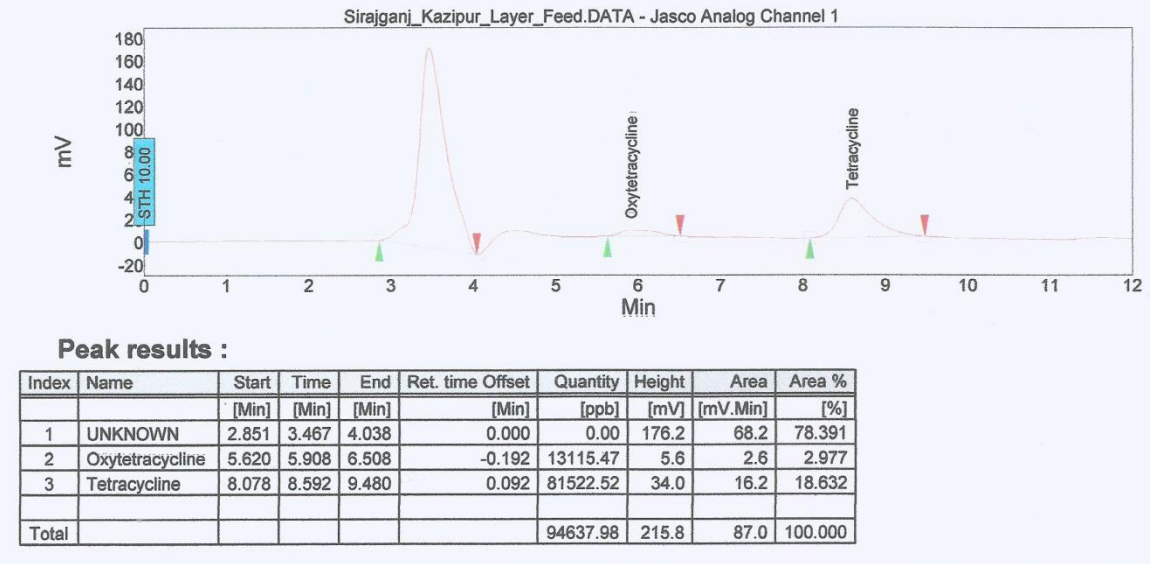
Figure 37. Chromatogram of TC and OTC in control; local chicken, Sirajganj-Sadar liver-02 from local breed (sample id LOSKLVR-02**)**

Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.008	3.292	3.886	0.000	0.00	253.8	82.1	100.000
Total						0.00	253.8	82.1	100.000

Figure 38. Chromatogram of TC and OTC in control; local chicken, Gazipur - liver-03 from local breed (sample id LOGLVR-03**)**

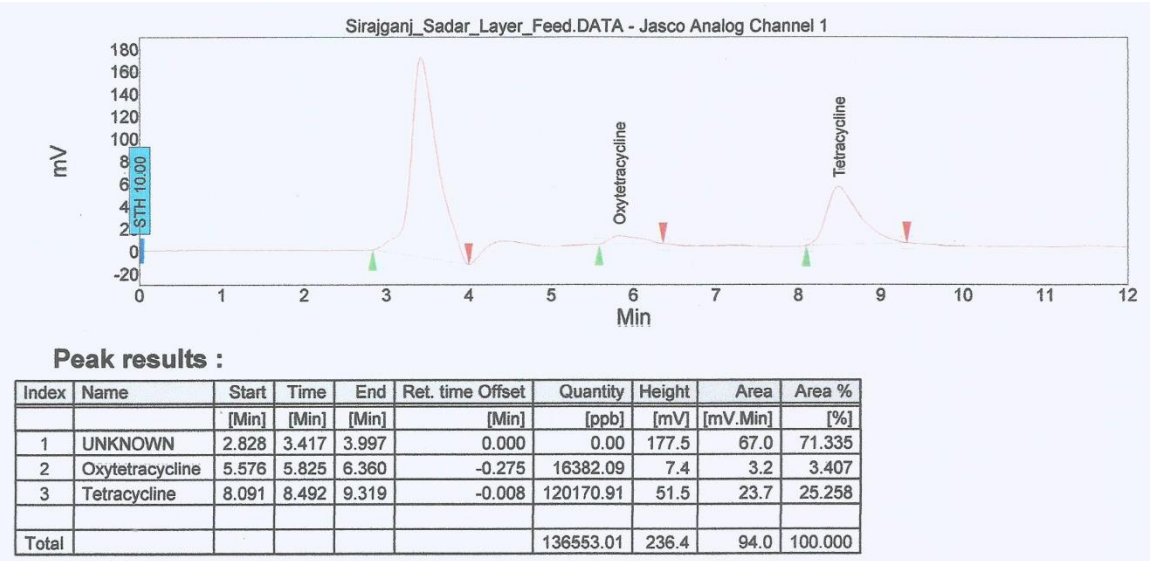
G. Layer feed



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.851	3.467	4.038	0.000	0.00	176.2	68.2	78.391
2	Oxytetracycline	5.620	5.908	6.508	-0.192	13115.47	5.6	2.6	2.977
3	Tetracycline	8.078	8.592	9.480	0.092	81522.52	34.0	16.2	18.632
Total						94637.98	215.8	87.0	100.000

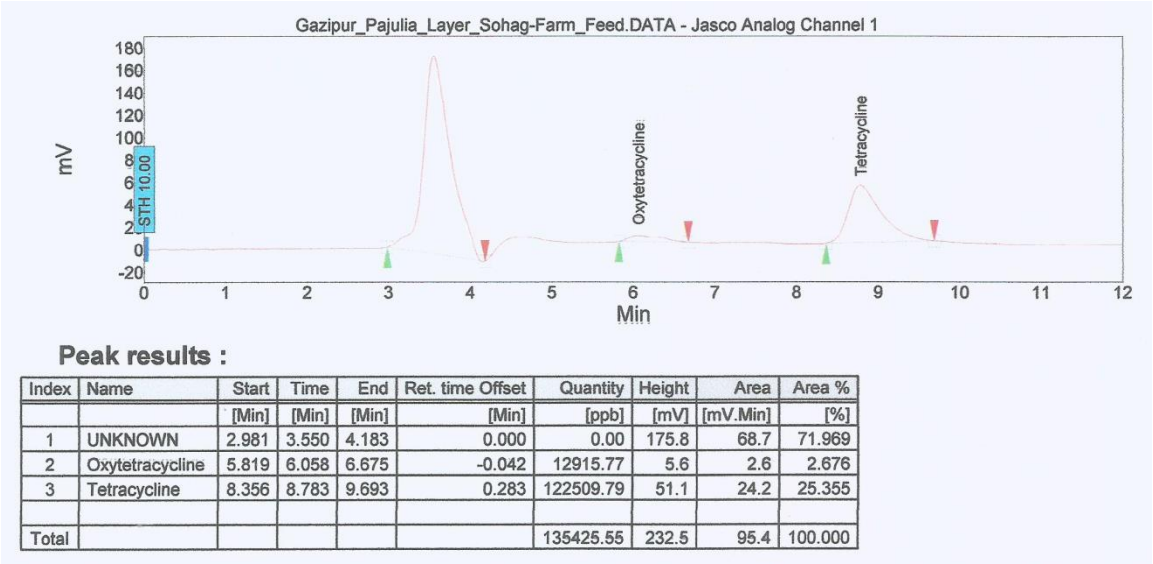
Figure 39. Chromatogram of TC and OTC in layer feed (sample id SKLCRaF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.828	3.417	3.997	0.000	0.00	177.5	67.0	71.335
2	Oxytetracycline	5.576	5.825	6.360	-0.275	16382.09	7.4	3.2	3.407
3	Tetracycline	8.091	8.492	9.319	-0.008	120170.91	51.5	23.7	25.258
Total						136553.01	236.4	94.0	100.000

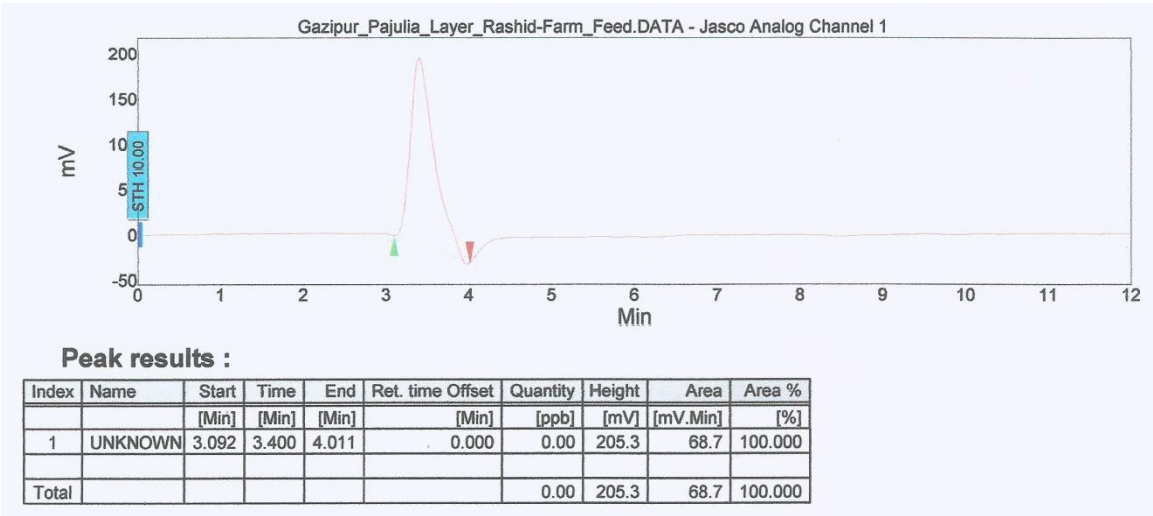
Figure 40. Chromatogram of TC and OTC in layer feed (sample id SSLCAmF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.981	3.550	4.183	0.000	0.00	175.8	68.7	71.969
2	Oxytetracycline	5.819	6.058	6.675	-0.042	12915.77	5.6	2.6	2.676
3	Tetracycline	8.356	8.783	9.693	0.283	122509.79	51.1	24.2	25.355
Total						135425.55	232.5	95.4	100.000

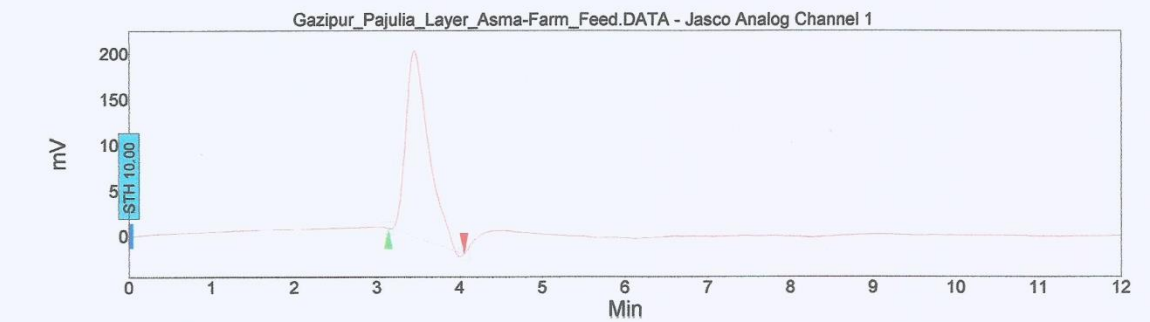
Figure 41. Chromatogram of TC and OTC in layer feed (sample id GCLCSoF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.092	3.400	4.011	0.000	0.00	205.3	68.7	100.000
Total						0.00	205.3	68.7	100.000

Figure 42. Chromatogram of TC and OTC in layer feed (sample id GCZCRaF**)**

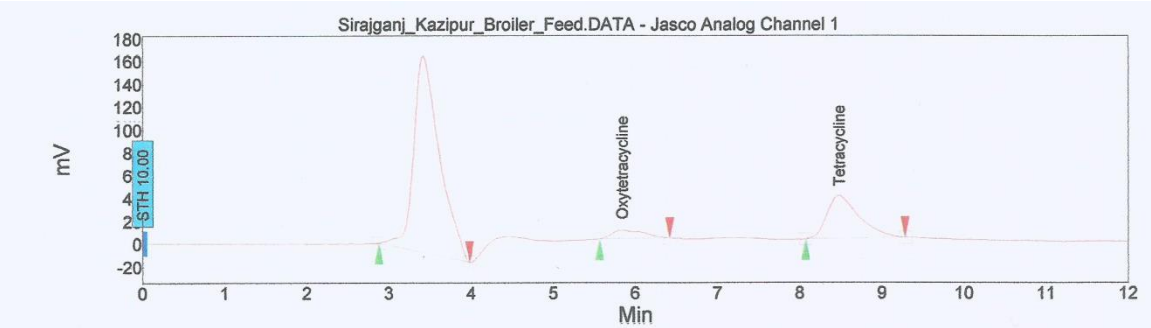


Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	3.134	3.458	4.053	0.000	0.00	203.7	65.0	100.000
Total						0.00	203.7	65.0	100.000

Figure 43. Chromatogram of TC and OTC in layer feed (sample id GCLCAsF**)**

H. Broiler feed



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.874	3.417	3.982	0.000	0.00	171.8	62.8	75.302
2	Oxytetracycline	5.561	5.833	6.420	-0.267	20811.84	7.7	3.5	4.212
3	Tetracycline	8.068	8.475	9.287	-0.025	99206.69	37.6	17.1	20.486
Total						120018.53	217.1	83.4	100.000

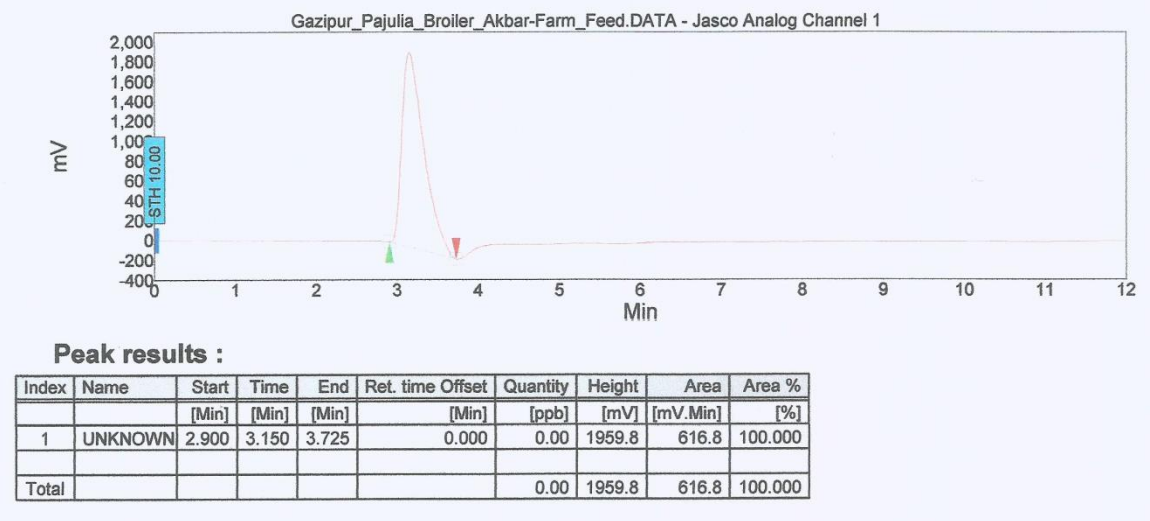
Figure 44. Chromatogram of TC and OTC in broiler feed (sample id SKBCSaF**)**

Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.925	3.242	3.844	0.000	0.00	215.9	67.1	100.000
Total						0.00	215.9	67.1	100.000

Total						39.15	198.8	80.8	100.000

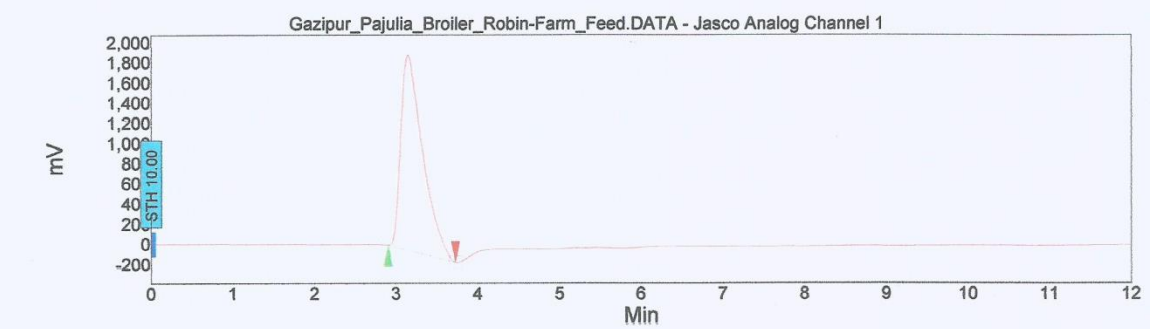
Figure 45. Chromatogram of TC and OTC in broiler feed (sample id SSBCMuF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.900	3.150	3.725	0.000	0.00	1959.8	616.8	100.000
Total						0.00	1959.8	616.8	100.000

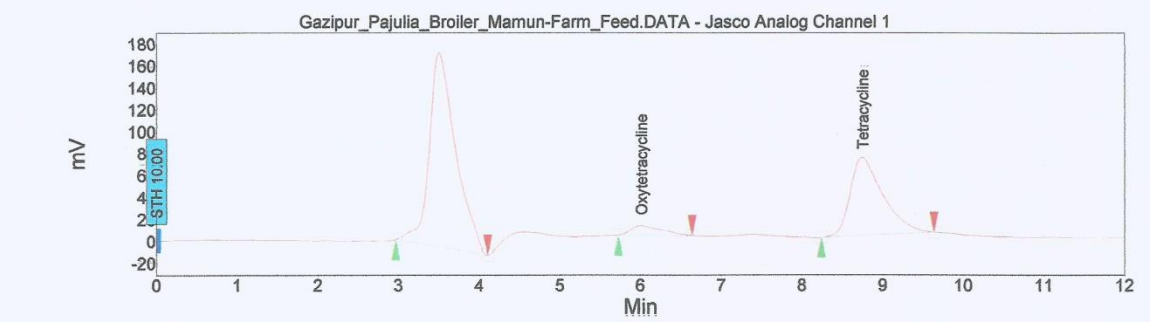
Figure 46. Chromatogram of TC and OTC in broiler feed (sample id GCBCAkF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.900	3.150	3.725	0.000	0.00	1932.4	601.4	100.000
Total						0.00	1932.4	601.4	100.000

Figure 47. Chromatogram of TC and OTC in broiler feed (sample id GCBCRoF**)**



Peak results :

Index	Name	Start	Time	End	Ret. time Offset	Quantity	Height	Area	Area %
		[Min]	[Min]	[Min]	[Min]	[ppb]	[mV]	[mV.Min]	[%]
1	UNKNOWN	2.964	3.508	4.110	0.000	0.00	177.2	67.4	64.488
2	Oxytetracycline	5.722	6.008	6.642	-0.092	15235.17	8.8	3.8	3.674
3	Tetracycline	8.241	8.750	9.640	0.250	130231.49	71.0	33.3	31.838
Total						145466.66	257.0	104.6	100.000

Figure 48. Chromatogram of TC and OTC in broiler feed (sample id GCBCMaF**)**