A Comprehensive Analysis of Residual Antibiotics, Organochlorine Pesticides and Heavy Metals in Beef and Chicken

A Thesis Submitted for Partial Fulfillment of the Degree of Doctor of Philosophy (PhD) in Chemistry

Submitted by

Nargis Parvin
Registration Number: 35
Session: 2022-2023

A thesis submitted to
Organic Chemistry Research Laboratory
Department of Chemistry
University of Dhaka
Dhaka-1000

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APPROVAL SHEET

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Approved by

Dr. Abida Sultana
(Supervisor)
Associate Professor
Department of Chemistry
University of Dhaka
Dhaka-1000

Dr. Mohammad Shoeb
(Co-Supervisor)
Professor
Department of Chemistry
University of Dhaka
Dhaka-1000

Nargis Parvin
PhD Researcher
Registration Number: 35
Session: 2022-2023
Department of Chemistry
University of Dhaka
Dhaka-1000

DECLARATION

Experimental work described in the thesis has been performed by the candidate at the Organic Chemistry Research Laboratory, Department of Chemistry, University of Dhaka, Dhaka-1000 and at Central Analytical and Research Facilities (CARF), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205 under our joint supervision. The research work was conducted solely for the award of Doctor of Philosophy (PhD) and will not be presented for any other degree.

Dr. Abida Sultana
(Supervisor)
Associate Professor
Department of Chemistry
University of Dhaka
Dhaka-1000

Dr. Mohammad Shoeb
(Co-Supervisor)
Professor
Department of Chemistry
University of Dhaka
Dhaka-1000

Nargis Parvin
PhD Researcher
Registration Number: 35
Session: 2022-2023
Department of Chemistry
University of Dhaka
Dhaka-1000

ABSTRACT

Bangladesh is renowned for its agriculture-based economy. The farming of poultry and livestock is extremely popular in the country. Consumers in Bangladesh have a strong preference for beef and chicken meat, and these products are widely available. Toxic substances from various sources can permeate food animals through their feed and the environment, eventually making their way through the entire food chain via bioaccumulation and biomagnification. Additionally, these chemicals can enter the human body through the consumption of these foods, leading to significant public health concerns. This doctoral research focuses on identifying and measuring chemical pollutants such as leftover antibiotics, organochlorine pesticides, and heavy metals in beef and broiler chicken meat as well as liver samples, while assessing the health risks associated with each contaminant. Additionally, it aims to explore potential methods for reducing these contaminants. In this research, the presence of antibiotic residues (including tetracycline, oxytetracycline, chlortetracycline, amoxicillin, and patulin) in samples of beef meat, liver, and chicken meat, liver was examined utilizing reversed-phase High Performance Liquid Chromatography with a photodiode array detector (HPLC-PDA). Organochlorine pesticides and heavy metals were assessed using Gas Chromatography with an electron capture detector (GC-ECD) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), respectively. The beef and chicken meat and liver samples were extracted employing a modified version of the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method designed for antibiotics and organochlorine pesticides. A total of 180 biological samples, including beef meat, beef liver, and broiler chicken meat and liver, were collected for this study from local markets of Dhaka North and South City, Bangladesh.

A total of one hundred and twenty samples (with 30 each of beef meat, beef liver, chicken meat, and chicken liver) were examined for each antibiotic. The correlation coefficients (r^2) demonstrated a linear relationship, measuring 0.9978, 0.9980, and 0.9988 for oxytetracycline, tetracycline, and chlortetracycline, respectively, across six concentration levels. Matrix-matched calibration was performed for each matrix, resulting in linear correlation coefficients (r^2) of 0.9980, 0.9990, and 0.9981 for beef meat; 0.9985, 0.9984, and 0.9973 for beef liver; 0.9988, 0.9982, and 0.9993 for chicken meat; and 0.9980, 0.9985, and 0.9991 for chicken liver concerning oxytetracycline, tetracycline, and chlortetracycline, respectively. The intra-day and inter-day recovery tests for each of the tetracyclines (TCs) were conducted, with relative standard deviation (RSD%) remaining below 10%. The Limit of Detection (LOD) for oxytetracycline, tetracycline, and chlortetracycline were recorded at 1.11, 1.15, and 1.19 μg/kg, respectively, with the associated Limit of Quantification (LOQ) being 3.17, 3.84, and 3.96 μg/kg. Residual oxytetracycline was

found and measured in eight beef liver samples, with levels varying from 86.76 to 368.97 µg/kg, all below the Maximum Residue Limit (MRL) set by Codex. However, one beef liver sample surpassed the MRL established by the European Union (EU), registering at 368.97 µg/kg. Oxytetracycline was detected in three chicken meat samples (220.94, 153.45, and 101.32 µg/kg), tetracycline was present in two samples (715.00 and 698.88 µg/kg), and chlortetracycline was found in one sample (677.35 µg/kg). Four of the positive samples exceeded the Codex recommended MRL, while six positive chicken meat samples were above the MRL limit set by the EU. The linear correlation coefficient (r^2) for standard amoxicillin across six different concentrations was determined to be 0.9982. For amoxicillin in various samples, the respective linear correlation coefficients (r^2) were 0.9979 for beef meat, 0.9980 for beef liver, 0.9995 for chicken meat, and 0.9981 for chicken liver. The intra-day and inter-day recovery experiments were conducted for amoxicillin, yielding a relative standard deviation (RSD%) within 10%. The limit of detection (LOD) and limit of quantification (LOQ) for the validated method were found to be 0.55 and 1.84 µg/kg for standard amoxicillin, respectively. The Codex Alimentarius Commission and the EU have established a maximum residue level (MRL) of 50 µg/kg for amoxicillin in beef and chicken meat and liver. Residual levels of amoxicillin were identified in seven beef meat samples, with concentrations ranging from 4.89 to 9.36 µg/kg, and in fifteen beef liver samples, which varied from 10.39 to 89.47 µg/kg. Among the beef liver samples, two exceeded the MRL, with values of 53.46 µg/kg (BL10) and 89.47 µg/kg (BL14). The linear correlation coefficient (r^2) was determined to be linear at 0.9991 for standard patulin across six different concentrations. For patulin, the correlation coefficients (r^2) were found to be linear at 0.9984, 0.9983, 0.9980, and 0.9990 in beef meat, beef liver, chicken meat, and chicken liver, respectively. An intra-day and inter-day recovery study was conducted for patulin, and the RSD% remained within 10%. The limit of detection (LOD) for the proposed method was established at 0.18 and 0.60 µg/kg for standard patulin. Residual levels of patulin antibiotic (as well as mycotoxin) were identified in twenty-five beef samples, with concentrations ranging from 47.72 to 193.91 µg/kg, and in eighteen chicken meat samples, with levels from 16.94 to 310.53 µg/kg. Patulin was detected in six beef liver samples, with concentrations between 43.31 and 166.91 µg/kg, and in eleven chicken liver samples, with levels ranging from 14.75 to 52.88 µg/kg. The health risk for each antibiotic was assessed for adults, and the hazard index (HI) was found to be less than 1.

A total of one hundred and twenty samples (30 each of beef meat, beef liver, chicken meat, and chicken liver) were subjected to analysis for organochlorine pesticides (OCPs). To establish a standard calibration curve, six varying concentrations of a standard solution (comprising 20 OCPs) were injected into the gas chromatograph-electron capture detector

(GC-ECD). The correlation coefficients (r^2) showed a linear relationship, with values of 0.9999, 0.9994, 0.9990, 0.9988, 0.9980, 0.9997, 0.9991, 0.9994, 0.9993, 0.9996, 0.9983, 0.9978, 0.9995, 0.9985, 0.9990, 0.9997, 0.9981, 0.9991, 0.9994, and 0.9998 for alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, trans-chlordane, cis-chlordane, endosulfan I, 4, 4'-DDE, dieldrin, endrin, 4, 4'-DDD, endosulfan II, endrin aldehyde, 4, 4'-DDT, endosulfan sulfate, methoxychlor, and endrin ketone, respectively. Recovery experiments for intra-day and inter-day were conducted for 20 OCPs, and the relative standard deviation percentage (RSD%) remained within the acceptable limit of 20%. Out of thirty beef meat samples tested, alpha-BHC was detected in 11 samples (ranging from 1.01 to 62.49 µg/kg), gamma-BHC in 9 samples (with levels from 1.22 to 103.01 µg/kg), beta-BHC in 9 samples (from 1.13 to 8.94 µg/kg), and delta-BHC in 28 samples (with concentrations between 84.45 and 329.08 µg/kg). Heptachlor was found in 13 samples (ranging from 0.97 to 29.64 µg/kg), while aldrin was present in 20 samples (from 0.96 to 61.71 µg/kg). Heptachlor epoxide appeared in 28 samples (with levels from 57.87 to 304.25 µg/kg), trans-chlordane was found in 14 samples (from 0.86 to 7.90 µg/kg), and cis-chlordane in 18 samples (ranging from 0.61 to 6.22 µg/kg). Endosulfan I was detected in 26 samples (with concentrations between 1.23 and 22.86 µg/kg), and 4, 4′-DDE was found in 20 samples (ranging from 0.17 to 21.41 µg/kg). Dieldrin was present in 22 samples (from 0.47 to 36.50 µg/kg), endrin in 17 samples (ranging from 0.63 to 16.98 µg/kg), and 4, 4′-DDD was detected in 23 samples (with levels from 0.48 to 38.94 µg/kg). Endosulfan II was found in 27 samples (ranging from 1.61 to 187.29 µg/kg), and endrin aldehyde was present in 19 samples (from 0.98 to 24.70 µg/kg). Additionally, 4, 4′-DDT was detected in 15 samples (ranging from 0.24 to 76.81 µg/kg), endosulfan sulfate in 12 samples (with levels from 0.83 to 11.10 µg/kg), methoxychlor was found in 18 samples (ranging from 0.77 to 14.04 µg/kg), and endrin ketone was present in 4 samples (from 0.83 to 10.55 µg/kg), all measured in µg/kg. Among the thirty beef liver samples analyzed, alpha-BHC was detected in 28 samples (ranging from 17.40 to 340.42 µg/kg), gamma-BHC in 16 samples (from 1.75 to 15.79 µg/kg), beta-BHC in 16 samples (from 2.87 to 42.82 µg/kg), delta-BHC in 24 samples (from 2.24 to 26.41 µg/kg), heptachlor in 22 samples (ranging from 4.67 to 16.67 µg/kg), aldrin in 21 samples (from 0.66 to 20.93 µg/kg), heptachlor epoxide in 28 samples (ranging from 65.92 to 197.61 µg/kg), trans-chlordane in 16 samples (from 0.43 to 29.68 µg/kg), cis-chlordane in 10 samples (from 1.19 to 6.22 µg/kg), endosulfan I in 24 samples (ranging from 1.15 to 5.02 µg/kg), 4, 4′-DDE in 11 samples (from 0.10 to 3.38 µg/kg), dieldrin in 21 samples (ranging from 1.03 to 6.43 µg/kg), endrin in 20 samples (from 1.03 to 6.43 µg/kg), 4, 4′-DDD in 7 samples (ranging from 0.59 to 1.84 µg/kg), endosulfan II in 20 samples (from 0.22 to 25.55 µg/kg), endrin aldehyde in 26 samples (from 0.21 to 31.55 µg/kg), 4, 4′-DDT

in 9 samples (ranging from 3.08 to 17.75 µg/kg), endosulfan sulfate in 2 samples (from 0.04 to 0.05 µg/kg), and methoxychlor in 8 samples (ranging from 0.26 to 11.67 µg/kg).
In the thirty chicken meat samples, alpha-BHC was detected in 2 samples (at 1.00 and 2.87 µg/kg), gamma-BHC in 1 sample (at 4.94 µg/kg), delta-BHC in 27 samples (ranging from 5.91 to 201.65 µg/kg), heptachlor in 4 samples (ranging from 6.81 to 8.93 µg/kg), aldrin in 10 samples (from 1.60 to 33.93 µg/kg), heptachlor epoxide in 25 samples (ranging from 137.76 to 270.60 µg/kg), trans-chlordane in 13 samples (from 3.64 to 27.96 µg/kg), cis-chlordane in 7 samples (ranging from 0.44 to 2.24 µg/kg), endosulfan I in 25 samples (from 1.72 to 5.91 µg/kg), 4, 4′-DDE in 2 samples (at 0.41 and 1.69 µg/kg), dieldrin in 12 samples (ranging from 0.72 to 13.53 µg/kg), endrin in 10 samples (from 0.44 to 12.73 µg/kg), 4, 4′-DDD in 17 samples (ranging from 1.48 to 5.75 µg/kg), endosulfan II in 9 samples (from 0.97 to 56.96 µg/kg), endrin aldehyde in 16 samples (ranging from 0.93 to 5.03 µg/kg), 4, 4′-DDT in 7 samples (from 1.39 to 71.84 µg/kg), endosulfan sulfate in 6 samples (ranging from 0.21 to 3.14 µg/kg), and methoxychlor in 13 samples (from 1.59 to 5.95 µg/kg). In a study of thirty chicken liver samples, alpha-BHC was detected in 23 samples, with concentrations ranging from 2.12 to 159.13 µg/kg. Gamma-BHC was present in 3 samples, showing levels between 2.17 and 5.85 µg/kg, while beta-BHC was found in 10 samples, with values between 13.87 and 69.96 µg/kg. Delta-BHC was identified in 25 samples, displaying concentrations from 1.67 to 224.65 µg/kg. Heptachlor was detected in 3 samples, at levels ranging from 3.01 to 7.78 µg/kg. Aldrin appeared in 10 samples, with concentrations varying from 0.91 to 259.93 µg/kg. Heptachlor epoxide was found in 28 samples, with a range of 58.71 to 196.47 µg/kg. Trans-chlordane was present in 3 samples, with levels from 5.26 to 336.49 µg/kg, and cis-chlordane was identified in 4 samples, showing concentrations between 0.12 and 280.64 µg/kg. Endosulfan I was found in 26 samples, with values ranging from 1.23 to 22.86 µg/kg. The compound 4, 4'-DDE was detected in 9 samples (0.54 to 12.79 µg/kg), while dieldrin was present in another 9 samples, ranging from 0.84 to 9.09 µg/kg. Endrin was found in 9 samples, with concentrations between 1.24 and 40.29 µg/kg, and 4, 4'-DDD was detected in 10 samples, showing levels from 0.35 to 28.90 µg/kg. Endrin aldehyde appeared in 18 samples, with concentrations ranging from 1.12 to 307.65 µg/kg. 4, 4'-DDT was identified in 12 samples, at levels between 1.37 and 29.80 µg/kg, while endosulfan sulfate was found in 5 samples (0.97 to 204.26 µg/kg) and methoxychlor in another 5 samples, ranging from 0.72 to 5.68 µg/kg. Endrin ketone was present in 4 samples, with concentrations from 1.95 to 4.47 µg/kg. The health risks associated with these pesticides were assessed, revealing that the hazard indices for delta-BHC, heptachlor, aldrin, heptachlor epoxide, endrin, endrin aldehyde, and endrin ketone exceeded 1 for both adults and children.

A total of 120 biological samples were examined, comprising 30 each of beef meat, beef liver, chicken meat, and chicken liver for ten heavy metals. The samples underwent digestion with concentrated HNO_3 and H_2O_2. In the ICP-MS, four varying concentrations of a standard solution (a mix of the ten metals) were injected to establish calibration curves. A linear correlation coefficient (r^2) was determined for chromium (Cr), nickel (Ni), lead (Pb), cadmium (Cd), arsenic (As), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se), with values of 0.9988, 0.9989, 0.9990, 0.9991, 0.9991, 0.9994, 0.9991, 0.9994, 0.9998, and 0.9993, respectively. The Limits of Detection (LOD) and Limits of Quantification (LOQ) were calculated for each of the ten metals. Copper (Cu), manganese (Mn), cobalt (Co), zinc (Zn), and selenium (Se) were detected and quantified across all beef and chicken meat and liver samples. The concentration of Cu ranged from 2.82 x 10^{-5} to 1.24 x 10^{-4}, 0.09 to 0.23, 0.93 to 4.68, and 3.94 to 12.52; Mn levels ranged from 1.77 x 10^{-3} to 6.69 x 10^{-2}, 7.62 to 57.28, 0.005 to 0.05, and 0.04 to 0.10; Co concentrations were between 2.89 x 10^{-3} to 2.95 x 10^{-2}, 5.33 to 11.82, 0.03 to 6.28, and 2.95 to 9.18; Zn levels varied from 0.11 to 0.55, 81.03 to 203.27, 23.70 to 41.20, and 41.26 to 152.73, while Se was found in a range of 5.02 x 10^{-5} to 4.40 x 10^{-4}, 0.38 to 1.54, 0.48 to 0.81, and 1.12 to 2.54 in all beef meat, beef liver, chicken meat, and chicken liver samples, respectively. Cr concentrations were found to be between 2.63 x 10^{-5} to 3.20 x 10^{-4}, 0.06 to 0.20, 0.58 to 1.68, and 0.32 to 1.42 in 30 beef meat, 9 beef liver, 30 chicken meat, and 30 chicken liver samples. Ni levels varied from 2.43 x 10^{-5} to 9.86 x 10^{-5}, 0.04 to 0.92, 0.10 to 1.45, and 0.06 to 42.63 in 30 beef meat, 29 beef liver, 29 chicken meat, and 30 chicken liver respectively. Pb was found at concentrations ranging from 1.12 x 10^{-6} to 7.46 x 10^{-4}, 0.05 to 24.36, 0.05 to 1.71, and 0.03 to 5.28 in 30 beef meat, 29 beef liver, 26 chicken meat, and 22 chicken liver. Cd levels were detected between 4.82 x 10^{-6} to 5.61 x 10^{-4}, 0.04 to 1.17, 0.14 to 0.18, and 0.04 to 0.24 in 30 beef meat, 12 beef liver, 2 chicken meat, and 3 chicken liver respectively. As concentration ranged from 1.34 x 10^{-6} to 4.41 x 10^{-4}, 0.01 to 0.69, 0.005 to 0.52, and 0.005 to 10.79 mg/kg in 30 beef meat, 28 beef liver, 19 chicken meat, and 24 chicken liver samples respectively. The health risk assessment (EDI, THQ, HI, TCR) for heavy metals was conducted for both adults and children. Chemical pollutants can build up in the fatty tissues of beef and poultry, potentially changing the fatty acid profile and causing genetic mutations. Therefore, the fatty acid composition and overall fat content were examined using gas chromatography with a flame ionization detector (GC-FID). The average fat content (%) calculated was 1.57, 6.19, 0.78, and 2.95% for beef meat, beef liver, broiler chicken meat, and liver, respectively. This research underscores comprehensive evidence of the occurrence of antibiotic, pesticide, and heavy metal residues in raw beef and broiler chicken meat and liver in Bangladesh. Although most samples were within internationally accepted limits, several exceeded Codex and EU MRLs, posing potential public health concerns.

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List of Acronyms and Abbreviations

MRL	Maximum residual limit
ATP	Adenosine Triphosphatase
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PABA	Para-aminobenzoic acid
DHFA	Dihydrofolic acid
THFA	Tetrahydrofolic acid
WHO	World Health Organization
AOM	Acute Otitis Media
LRTI	Lower respiratory tract infection
OM	Otitis media
PID	Pelvic inflammatory disease
UTI	Urinary tract infection
URTI	Upper respiratory tract infection
GI	Gastrointestinal tract
CRD	Chronic Respiratory Disease
FMD	Foot-and-Mouth Disease
AMOX	Amoxicillin
PAT	Patulin
TLC	Thin Layer Chromatography
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
MS	Mass Spectrometry

ELISA	Enzyme-linked immunoassay
LFT	Liver Function Tests
GRACE	The Grace Communication Group
ACs	Acetylcholine
OCPs	Organochlorine pesticides
POPs	persistent organic pollutants
EC	Emulsifiable concentrate
WP	wettable powders
G	Granules
SP	soluble powders
A	Aerosols
GDP	Gross Domestic Product
UNEP	United Nations Environment Programme
DDT	Dichlorodiphenyl trichloroethane
HCH	Hexachlorocyclohexane
Mg-ATPase	Magnesium Adenosine Triphosphatase
Ca-ATPase	Calcium Adenosine Triphosphatase
BHC	Benzene Hexachloride
HMs	Heavy Metals
IARC	International Agency for Research on Cancer
PNS	Peripheral nervous system
CNS	Central nervous system
UL	Tolerable upper intake level
BSTI	Bangladesh Standard and Testing Institute
BAEC	Bangladesh Atomic Energy Commission
PHI	Public Health Institution
USEPA	United States Environmental Protection Agency
FAO	Food and Agricultural Organization
JECFA	Joint FAO/WHO Expert Committee on Food Additives

FAs	Fatty acids
IUPAC	International Union of Pure and Applied Chemistry
SFA	Saturated Fatty Acid
UFSA	Unsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
PHA	Polyhydroxyalkanoates
TAG	Triglycerols
DHA	Docosahexaenoic acid
DLS	Department of Livestock Services
BBS	Bangladesh Bureau of Statistics
C-18	octadecyl silica
PSA	Primary secondary amine
ACN	Acetonitrile
DI	Deionized water
RP	Reversed Phase
HPLC-PDA	High-Performance Liquid Chromatography with a Photo-diode-Array Detector
GC-ECD	Gas Chromatography with an electron capture detector
GC-FID	Gas Chromatography with a flame ionization detector
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
BCSIR	Bangladesh Council of Scientific and Industrial Research Laboratory
CODEX	Codex Alimentarius Commission
EU	European Union
USDA	The United States Department of Agriculture

USFDA	U.S. Food and Drug Administration
BFSA	Bangladesh Food Safety Authority
HIES	Household Income and Expenditure Survey Report
EDI	Estimated Daily Intake
HI	Hazard Index
ADI	Acceptable Daily Intake
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
PTFE	Polytetrafluoroethylene
LOD	Limit of Detection
LOQ	Limit of Quantification
FAME	Fatty Acid Methyl Ester

Chemical Symbols

°C	degree Celsius
µg	microgram
mg	miligram
g	gram
kg	kilogram
mL	mililitre
µg/L	microgram per litre
mL/L	Mililitre per litre
µg/kg	microgram per kilogram
mg/kg	miligram per kilogram

Chapter 1

INTRODUCTION

1. INTRODUCTION

1.1 General

Food is a fundamental necessity for human survival as it gives us energy, nourishment, and immunity against disease. Food is mostly made up of protein, carbohydrates, fats, and other nutrients that the body needs to provide energy and support growth and other critical functions. Digestion aids in the body's absorption and utilization of food, essential to nourishment. The primary source of food is plants, which convert light energy into chemical energy through a process called photosynthesis. Green plants employ light energy during photosynthesis to transform water, carbon dioxide, and minerals into organic compounds that are rich in energy and oxygen. Other animal species frequently use animals that consume plants as food supplies. Poor food quality, low nutritional density, and the presence of chemical and biological contaminants are the main causes of hunger, poverty, malnutrition, and illness.

Bangladesh is an agro-based nation with 170 million inhabitants and has 8.11 million hectares of arable land in 2021. However, the arable land reduced from 9.55 to 8.11 million hectares due to population increase, industrialization, and other infrastructural development during 1989 to 2021 [1]. In Bangladesh, the agricultural sector is crucial for people's livelihood, employment, and gross domestic product (GDP) contribution. Its contribution has reduced over the last decade, going from 17 % in 2010 to 12.6% in 2020. The agriculture sector is the backbone of our economy because it reduces poverty and guarantees food security. Despite the impact of the pandemic and climate change, the population continued to rise, rising from 147.6 million in 2010 to 164.7 million in 2020. Despite this, the sector has maintained its productivity and profitability [2]. The agriculture sector plays an important role in increasing productivity, ensuring sustainable food security and creating employment opportunities. According to the provisional calculation of BBS, the contribution of agriculture to the GDP in FY 2021-22 is about 11.50 percent [2]. However, given that it accounts for 20% of the GDP, agriculture is crucial to the nation's overall economic development. Over 70% of the population is employed in agriculture. The Green Revolution began in the 1960s, and increasing grain production through increased land use, especially on marginal land, was prioritized to ensure food security [3]. Animal meat that humans consume is rich in nutrients compared to plant diets and produces an environment favorable enough to support microbial growth. To ensure the safety and quality of meat consumption to preserve human health, the meat industry still faces several challenges, including the world economy's rapid expansion, trade and export

policies liberalization, shifting consumer demands, and changing lifestyles. Bangladesh and the rest of the world have experienced an increase in issues regarding food safety and quality. To ensure that all residents have access to safe food, the government of Bangladesh appropriately addressed the issue of food safety and quality. The Bangladesh Food Safety Authority (BFSA) was created to implement the Safe Food Law, which was passed by the government in 2013 [4].

1.2 Food Contaminants

Food is susceptible to contamination in several kinds of ways, including when it is made and served in our homes, in restaurants, or by street sellers. Food contamination occurs when undesirable substances are added to food during the food chain, which can have a detrimental effect on human health. Food contamination can be classified into three categories: (i) chemical, (ii) biological, and (iii) physical.

(i) Food tainted by organisms or the compounds which they generate is referred to as biological contamination. This covers biological materials made by rodents, insects, microbes, and humans. Biological contamination is usually caused by bacteria and viruses, which can cause some of the most frequent food poisonings, such as *Escherichia coli*, L*isteria monocytogenes*, Norovirus and S*almonella spp*.

(ii) Food contamination caused by a foreign object is known as physical contamination. This can occur at any point during the manufacturing process and may involve plastic fragments, steel wool, or Band-Aids. A person who unintentionally swallows a foreign object may sustain injuries due to physical contamination. Physical contamination carries the additional risk that the foreign object may also be biologically contaminated.

(iii) Food that has been tainted by a natural or synthetic chemical agent is referred to be chemically contaminated. These pollutants expose people to a variety of harmful compounds, some of which are lethal, making them very dangerous. Furthermore, chemicals can contaminate food at any stage of the food production process, whether through industrial processes or pesticides that are carried from the soil the food is grown in. It is crucial to store chemicals apart from food in order to prevent chemical contamination. Depending on agricultural production, the environment, storage, packaging, processing, transit and sale, the pollutant may unintentionally contaminate food. Outside sources and food formation tend to represent the two causes of contamination, which are primary and secondary contamination, respectively [5]. Potential risk and its impact on human health could be the primary criterion for judging contamination. The main essential food

pollutants in this sense are veterinary medicine residues, pesticide residues, heavy metals, mycotoxin and other microbial toxins, radioactive isotopes, nitroso compounds, polycyclic hydrocarbon aromatic, halogen-containing organic compounds, etc. [6].

1.3 Sources of Food Contaminants

Food items provide a lot of nutrients that microbes need and can get contaminated. The primary contributors to contamination are insects, rodents, sewage, water, air, dust, machinery, and workers. In addition, sewage, living animals, the outside environment, and the insides of meat-eating animals can contaminate raw materials. Diseased animals are the source of further contamination in animal meals, though medical advancements have almost completely eradicated this source. Accidental chemical supply and food interaction can result in contamination from chemical sources. Ingredients may be a source of further chemical or microbiological contamination. Reduced contamination can be achieved by maintaining good housekeeping and cleanliness, safeguarding food while it is being stored, disposing of trash and litter properly, and avoiding contact with hazardous materials [7, 8].

1.4 Antibiotics

Antibiotics can kill or stop the growth of bacteria and can be produced by organisms or laboratories [9]. Even though antibiotics are beneficial for humans and animals due to their significant impact on health, overuse of them can have adverse consequences, particularly the emergence of resistant bacteria. Bangladeshi cattle and poultry farms frequently utilize amoxicillin, tetracycline, oxytetracycline, chlortetracycline and macrolide antibiotics as growth promoters as well as utilizing them for preventative and therapeutic purposes [10, 11].

The two primary sources of antibiotics in humans are pharmaceutical prescriptions (mostly for penicillins, macrolides, and fluoroquinolones) and compounds used in animal breeding (tetracyclines and sulfonamides). Maximum residual limits (MRLs) are now a part of food safety regulations due to the possible harm that antibiotics can pose to human health. Prolonged exposure has been connected to immune system degradation, renal complications, digestive disorders brought on by the loss of intestinal flora, and even cancerous effects [12-17].

1.5 History of Invention of Antibiotics

The first single-cell microbe was discovered in the 1670s by Dutch scientist Antonie van Leeuwenhoek. Further research on tuberculosis mycobacteria was conducted in 1882 by Louis Pasteur and Robert Koch. The cholera and typhus antimicrobial medicine was found by Rudolph Emmerich and Oscar Low in 1890, and the syphilis-causing bacteria was introduced by Paul Ehrlich in 1909 [18]. Scottish bacteriologist Alexander Fleming discovered in 1928 that a mold called Penicillium notatum had negatively impacted bacterial colonies developing on a culture plate. Ten years later, Australian pathologist Howard Florey, British biochemist Ernst Chain, and others identified the active component, penicillin, and demonstrated its potent antibacterial properties against a wide range of dangerous bacterial illnesses. In an effort to create semisynthetic forms of penicillin, scientists experimented with adding different chemical groups to the molecule's core towards the end of the 1950s. Thus, a variety of penicillins were made accessible to treat illnesses brought on by various bacteria, such as gonococci, pneumococci, staphylococci, streptococci, and the spirochaetes that cause syphilis. Mycobacterium tuberculosis was extremely sensitive to the antibiotic streptomycin, which was isolated from Streptomyces griseus in 1943.Streptomycin was shown to be highly efficient not only against tuberculosis but also against a wide range of other bacteria, such as the bacillus that causes typhoid fever. The compounds gramicidin and tyrocidin, which are made by Bacillus bacteria, were two further early discoveries. René Dubos, an American microbiologist of French descent, made the discovery in 1939. Although they were too poisonous to be used internally, they were useful in treating surface infections. The fungus Cephalosporium acremonium produces cephalosporins, which are related to penicillins and were first discovered by researchers in the 1950s. Scientists identified a class of antibiotics called quinolones in the ensuing ten years. Quinolones have been shown to be effective in treating urinary tract infections, infectious diarrhea, and a variety of other illnesses affecting tissues like bones and white blood cells by blocking DNA replication, an essential step in bacterial reproduction [19, 20].

Pasteur and Koch initially described the phenomenon as antibacterial activity, and French biologist Jean Paul Vuillemin coined the term "antibiosis" in 1877 [21, 22]. American biologist and Nobel laureate Selman Waksman later adopted the term "antibiotic" from antibiosis, which means "against life" [23].

1.6 Classification of Antibiotics

Antibiotics can be categorized based on origin, chemical structure, impact on activity, mechanism of action, route of administration, and spectrum of action (Table 1.1 and 1.2) [24, 25].

Table 1.1 Classification based on chemical structure

Class of Antibiotics	**Examples**
Aliphatic amine	Spermidine, trimethylamine-N-oxide (TMAO), methyhexamine
Aminoglycosides	Amikacin, gentamicin, neomycin, tobramycin, streptomycin, plazomicin
Alicyclic	Cycloheximide,
Aromatic (Nitrobenzene)	Chloramphenicol
Carbohydrate containing	Aminoglycosides, amikacin, kanamycin, streptomycin, tobramycin
Macrocyclic lactone	Azithromycin, clarithromycin, erythromycin
N-containing heterocyclic	β-lactam
N/O glycosides	Chromomycin
O-containing heterocyclic	Cycloserine
Pure saccharides	Streptozotocin
Peptide	Polymyxin, bacitracin, gramicidin
Quinolones	Fluoroquinolones
Other	Lincomycin

There are five basic mechanisms of antibiotic action against bacterial cells such as (i) inhibition of cell wall synthesis, (ii) inhibition of protein synthesis, (iii) alternation of cell membranes, (iv) inhibition of nucleic acid synthesis and (v) anti-metabolite activity. Antibiotics can be classified on basis of mechanism of action that is mentioned in Table 1.2.

Table 1.2 Classification based on mechanism of action

Class of Antibiotics	Examples
Aminoglycosides	Neomycin, streptomycin, spectinomycin
Amphenicols	Chloramphenicol, florfenicol, thiamphenicol
β-lactam	Amoxicillin, penicillin G, flucloxacillin
Carbapenems	Biapenem, doripenem
Fluoroquinolones	Ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin
Lincosamides	Clindamycin, lincomycin, pirlimycin
Macrolides	Azithromycin, erythromycin, spiramycin, tylosin
Nitrofuran	Furaltadone, furazolidone, nitrofurazone, nitrofurantoin
Polyketide	Amphotericin, patulin
Sulfonamides/ Sulfanilamide	Sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamerazine, sulfamethiazole, sufamethoxypyridiazine
Tetracycline	Chlortetracycline, doxytetracycline, oxytetracycline, tetracycline, omadacycline

1.7 Mechanism of Action of Antibiotics

Antibiotics use several modes of action to deliver their effects (Figure 1.1). Many antibiotics perform by preventing the production of bacterial cell walls; these substances are commonly known as β-lactam antibiotics. The bacterial cell wall is produced by partially assembling the wall's parts inside the cell, moving them over the cell membranes to the growing wall, combining them inside the wall, and then cross-linking the wall's material strands. Antibiotics that prevent the cell wall from synthesizing have a particular impact on a particular phase. This causes the organism's shape and cell wall to change, ultimately leading to the bacterium dying. Certain antibiotics, like erythromycin, clindamycin, aminoglycosides, and chloramphenicol, prevent bacteria from synthesizing proteins. Animal cells and bacteria use similar basic processes to create proteins, but the specific proteins engaged are different. Antibiotics that are selectively hazardous use these variations to attach to or block the bacterial proteins' ability to function, stopping the production of new bacterial cells and proteins. However, all antibiotics, regardless of their class or kind, function through one of the following processes: i). Inhibition of cell wall synthesis ii). Inhibition of protein synthesis iii). Inhibition of membrane function iv). Disruption of Metabolism v). Inhibition of nucleic acid synthesis (vi) inhibition of ATP Synthase [26].

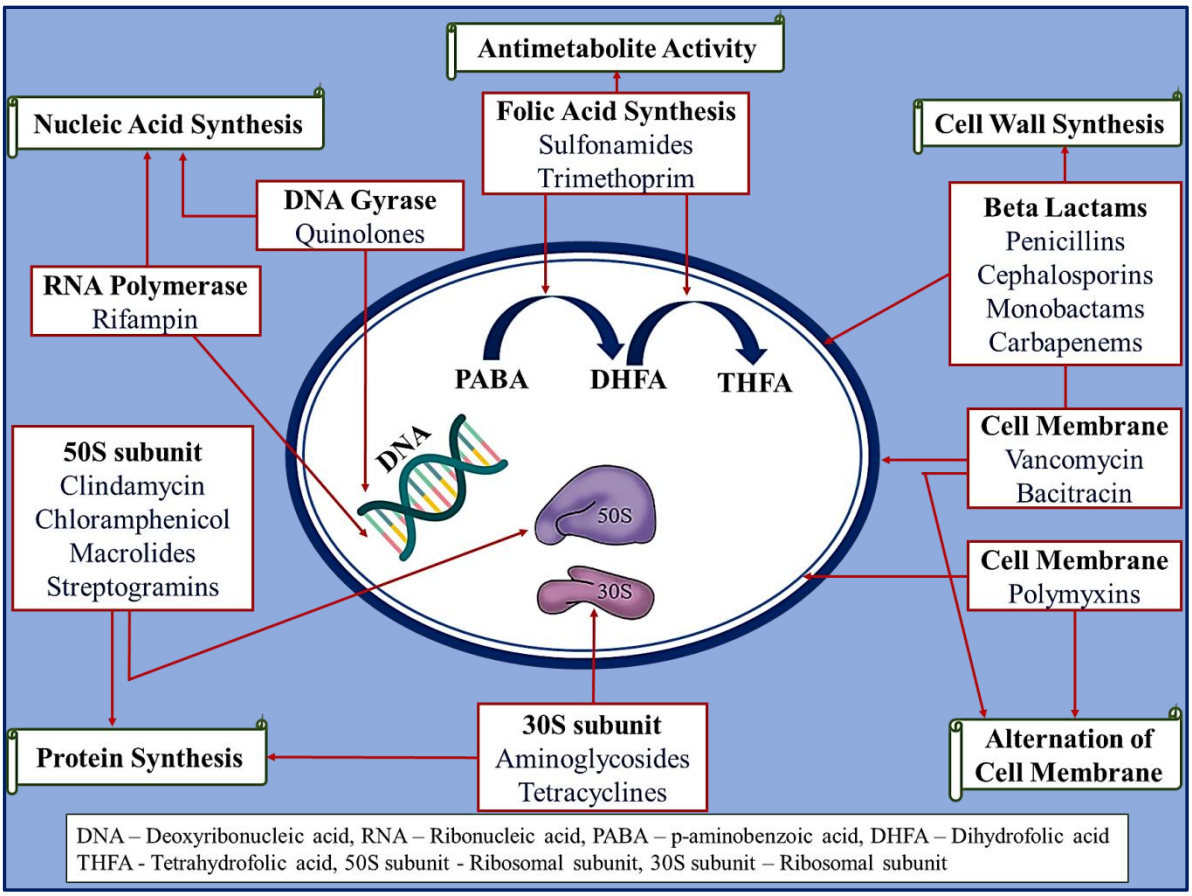


Figure 1.1 Mechanism of action of antibiotics

1.8 Medical Usages of Antibiotics

The class of medications known as antibiotics is crucial for treating bacterial infections. They are used by medical practitioners to treat a variety of illnesses, including skin infections, urinary tract infections, meningitis, pneumonia, and strep throat. Antibiotics are sometimes required for less serious infections that can be managed at home in certain individuals. A significant portion of the care given to many hospitalized patients who may be more critically ill due to an infection is the use of antibiotics. Antibiotics have numerous significant applications such as acne, bronchitis, conjunctivitis (pink eye), skin or soft tissue infection, streptococcal pharyngitis (strep throat), otitis media (ear infection), traveler's diarrhea, upper respiratory tract infection, urinary tract infections, bacterial meningitis, pneumonia, chlamydia, and sepsis. In certain instances, such as during surgery or certain types of dental care, they may also be utilized to avoid further infections [27, 28]. The usages of some universal antibiotics recommended by World Health Organization (WHO) are listed in Table 1.3.

Table 1.3 Usages of universal antibiotics

Class	**Antibiotics**	**Usage/Indications**	**Maximum Therapeutic Dosages**
Penicillin group	Amoxicillin	Pneumonia, streptococcal pharyngitis, AOM, UTI, skin infections, early or latent syphilis	500 mg/dose
	Ampicillin		2-3 g/day
	Amoxicillin + Clavonic acid		2.4 g amoxicillin/ 600 mg clavulanic acid
	Cloxacillin		4 g/day
	Penicillin		24 million units/day
Cephalosporins	Cefixime	Skin infection, cystitis, lyme disease, LRTI, OM, UTI, gastro-enteritis, sinusitis, bronchitis, meningitis, pharyngitis, gonorrhoea, pneumonia, surgical infection prophylaxis	400 mg/day
	Cephalexin		2 g/day
	Ceftriaxone		4 g/day
Macrolides	Azithromycin	bronchitis, RTI, OM, pneumonia, sinusitis, prophylaxis of surgical infection in bowel surgery, H pylori regimen, uncomplicated Genital infections,	2 g/day (500 mg/dose)
	Clarithromycin		1 g/day (500mg/dose)
	Erythromycin		4 g/day
Tetracyclines	Tetracycline	Conjunctivitis, sinusitis, or pneumonia, pharyngitis, urethritis, nongonococcal	3 g/day
	Doxycycline		200 mg/day
Quinolones	Ciprofloxacin	OM, PID, UTI, LRTI, Infected bites, sinusitis, gastro-enteritis, ear, nose and throat infections,gonorrhoea, skin infection	1 g/day
	Ofloxacin		1.6 g/day
Other antibacterials	Clindamycin	Antibiotic associated colitis, UTI, PID, Bacterial vaginosis, pneumonia, RTI, H pylori regimen, gastrointestinal tracts infection, pharyngitis	1.2-1.8 g/day
	Cotrimaxazole		3.8 g/day
	Metronidazole		4 g/day
AOM= acute otitis media, LRTI= lower respiratory tract infection, OM= otitis media, PID= pelvic inflammatory disease, UTI= urinary tract infection, URTI= upper respiratory tract infection			

1.9 Veterinary Usages of Antibiotics

Antibiotics are a vital tool for maintaining food safety, protecting animal health and welfare, and treating human and animal diseases. The use of antibiotics for any reason related to the care of livestock is known as "antibiotic use in livestock" and includes prophylactic therapy (prophylaxis), treatment when an animal becomes unwell, and metaphylaxis (treatment of a group of animals when at least one is diagnosed with a clinical illness). Veterinary drugs, which farmers or veterinarians administer at different stages in the life cycle of food-producing animals, are used to treat clinical diseases in livestock, including bird flu, cholera, chronic respiratory problems, typhoid, anthrax, pneumonia, gumboro, and others caused by bacteria. They are also used to control aggressive bacterial infections in a single animal or large group of animals [29]. Antibiotics are often given orally or by injection as a growth promoter in poultry and cattle farms to boost the output of milk and meat [30]. However, it might harm the well-being of animals and the quality of food, and abuse of this could lead to antibiotic resistance, which could be harmful to the environment, animal health, and human health. The usages of some universal antibiotics are listed in Table 1.4.

Table 1.4 Usages of some universal antibiotics in cattle and poultry

Antibiotics	**Cattle/Poultry**	**Infectious Diseases**
Amoxicillin trihydrate	Cattle, sheep, goat	GI tract diseases: Calf scour, salmonellosis, gastritis, enteritis, stomatitis and campylobacteriosis etc. Respiratory diseases: Pneumonia, shipping fever, bronchitis, calf diphtheria, sinusitis, pharyngitis and laryngitis etc. Urogenital tract diseases: Mastitis, metritis, pyometra, retained placenta, navel ill, cystitis, nephritis and MMA-syndrome etc. Musculoskeletal diseases: Skin and soft tissue infection, abscess, wounds, foot rot, arthritis, black quarter and eczema etc.
	Poultry	Pneumonia, infectious coryza, fowl cholera, colibacillosis, salmonellosis, necrotic enteritis, CRD, staphylococcosis, ranikhet and gumboro
Ampicillin sodium	Cattle, sheep, goat, buffalo	Pneumonia, footrot, enteritis, salmonellosis, calf scour, infected wound, fever, colibaciliosis, metritis, mastitis, pyelonephritis, black quarter, joint infection, kidney infection, haemorrhagic septicemia
Chlortetracycline hydrochloride	Cattle, calves, goats, sheep	Gastrointestinal and respiratory tract infections caused by chlortetracycline

		sensitive bacteria, like *Bordetella*, *Campylobacter, Chlamydia, E. coli, Haemophilus, Mycoplasma, Pasteurella, Rickettsia, Salmonella, Staphylococcus* and *Streptococcus spp*.
	Poultry	Respiratory and gastrointestinal tract infections caused by *E. coli*, *Bordetella, Chlamydia, Rickettsia, Campylobacter, Haemophilus, Mycoplasma, Pasteurella, Salmonella, Streptococcus* and *Staphylococcus spp*.
Ciprofloxacin	Cattle	Respiratory tract infections, gastro-intestinal infections, enterotoxaemia, skin bone and ear infections, anthrax, swine dysentery, uterine infections, udder infections, urinary tract infection.
	Poultry	C.R.D, C.C.R.D, mycoplasmosis, salmonelosis, colibacillosis, infectious coryza, pasteurellosis, streptococcosis, staphylococcosis etc. viral disease like gumboro, newcastle disease etc.
Doxycycline hydrochloride	Cattle, sheep, goat, buffalo	Primary and secondary respiratory infections caused by Pasteurella haemolytica. Pasteurella multocida, Actinobacillus spp. Bordetella bronchoseptica, Streptococcus, Mycoplasma spp.etc. It is also indicated in Pyoderma caused by Staphylococcus; in otitis media, Prostitis, Calf scour etc.
	Poultry	Prevention and treatment of Chronic Respiratory Disease (CRD), infectious coryza, colibacillosis, fowl cholera, fowl typhoid, infectious synovitis, mycoplasmosis and salmonellosis, chlamydiosis and necrotic enteritis etc.
Oxytetracycline	Cattle, Bultalo, Sheep and Goat	Anthrax, actinomycosis, anaplasmosis, black quarter, rinderpest, brucellosis, calf scour, calf diphtheria, dysentery, pyebnephritis, infectious enteritis, haemorrhagic septicaemia, malignant edema, pneumonia, navel ill, liver infection, abscess, pyometra, mastitis, metritis, peritonitis, kiptospirosis, infectious plauropneumonia, castration, paratyphoid, foot-rot, salmonellosis and secondary infections in FMD
	Poultry	Airsacculitis, chronic respiratory diseases, Colibacillosis (E. coli), infectious coryza

		(Haemophilus gallinarum), mycoplasmosis, infectious synovitis (Mycoplasma gallisepticum, Mycoplasma meleagridis, Mycoplasma synoviae) clostridial dermatitis (Clostridium septicum), necrotic enteritis (Clostridium perfringens, types A and C), pullorum disease (Salmonella pullorum) fowl cholera, coccidiosis etc.
Enrofloxacin	Poultry	Infections of G.I tract, respiratory tract, urinary and reproductive organs of poultry caused by gram-positive and gram-negative bacteria, Mycoplasma etc. e.g. Actinobacillosis, Brucellosis, CRD, *E. coli* infection (Colibacillosis), fowl typhoid, fowl cholera (Pasteurellosis), paratyphoid, mycoplasmosis, salmonellosais, coli septicemia, streptococcal and staphylococcal infection, secondary infection due to mixed bacterial and viral infection etc.
Tylosin Tartrate	Cattle	Gram positive (*Clostridium, Corynebacterium, Diplococcus, Staphylococcus aureus, Streptococcus, Erycepolothrix*,) and gram negative bacteria (*Mycobacterium, Proteus, Salmonella, Klebsiella, Shigella* etc.) and sinusitis, enteritis pneumonia etc.
	Poultry	particular action against mycoplasma gallisepticum S6, which is implicated in the CRD complex in chicken and infectious sinusitis in turkeys. The use of tylosin as feed additive to promote growth is safe.
Erythromycin + Sulfadiazine + Trimethoprim	Calves, foals	Respiratory and gastro-intestinal tract infections like bronchopneumonia, bronchitis, diarrhea, enteritis and scour.
	Poultry	Diseases caused by different gram positive and gram negative bacteria such as polluram diseases, fowl typhoid, fowl cholera, collibacilosis, septicemia, necrotic enteritis, nonspecific enteritis; respiratory tract diseases (CRD, bronchopneumonia), urinary and genital tract diseases and coccidiosis.
Sulfadiazine + Sulfadimidine + Sulfapiridine	Cattle	Haemorrhagic septicaemia, calf diphtheria, pneumonia, neval ill, dehorning, castration, urinary tract infection, matritis, arthritis, paratyphoid, distemper, secondary bacterial infection in case of FMD etc.

1.10 Antibiotic Resistance

Antimicrobial Resistance (AMR) results from bacteria, viruses, fungi, and parasites losing their ability to react to antimicrobial drugs. Drug resistance makes antimicrobial medications, including antibiotics, ineffective and makes treating infections challenging or impossible. This raises the risk of infection spread, serious illness, disability, and death. AMR is an organic phenomenon brought about by pathogen genetic alterations over time. Human activity, particularly the abuse and misuse of antibiotics for treating, preventing, or controlling illnesses in people, animals, and plants, has increased its appearance and spread [31, 32]. Microbes are either destroyed or, in the case that they have resistance genes, survive when exposed to an antibiotic. These survivors will continue to multiply and new generations will take over as the predominant kind in the ecosystem of bacteria very soon [33, 34]. The vast majority of bacteria divide every few hours, which enables them to evolve swiftly and rapidly adapt to changing environmental conditions. Mutations occur during replication, some of which may aid a single bacterium in surviving antibiotic exposure. Moreover, inappropriate and inadequate diagnostics can cause antibiotic resistance (Figure 1.2) [35-37].

Figure 1.2 Multiplex relationship of antimicrobial usage and resistance among humans, animals, and the environment

1.11 Adverse Effects of Antibiotics

Antibiotics are the most essential drugs for the treatment of bacterial infections. They are critical to the treatment of severe as well as lethal infections. On the other hand, using them may have negative consequences and increase bacterial resistance. Much less research on the adverse impacts of antibiotics has been published than on their beneficial effects. Before writing one, medical professionals must evaluate a prescription's prospective benefits against any potential risks. A moderate risk of damage is acceptable when a medicine has a big potential benefit. However, even a modest danger may be intolerable if the benefit is minimal [38]. For instance, quinolones are administered to treat diseases caused by gram-positive bacteria: *Staphylococcus saprophyticus* and gram-negative bacteria: *E. coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Citrobacter diversus*, *Enterobacter cloacae*) such as clinafloxacin, sparfloxacin, and lomefloxacin have this negative effect. Certain quinolones have been known to cause implications related to the central nervous system (CNS), such as headaches, sleeplessness, and dizziness, gastrointestinal disorders, phototoxic reactions on skin [39]. A disruption of the gut flora is often the cause of gastrointestinal side effects such as vomiting, nausea, stomach discomfort, diarrhea, bloating, and lack of appetite that can be caused by any antibiotic that has been evaluated. Secondary Candida species overgrowth is another common side effect of broad-spectrum antibiotics, particularly in diabetics. Amoxicillin or ampicillin, clindamycin, third-generation cephalosporins (including ceftazidime and cefotaxime), and fluoroquinolones are the main factors behind Clostridium difficile infections [40]. While some side effects occur while a patient is getting antibiotics in the hospital, almost half of the side effects occur after taking antibiotics that were recommended in the community, occasionally by specialists other than family physicians for complicated medical issues. Each category of medicines has unique persistent significant side effects [41-43]. The Hazardous consequences of some antibiotics have been evaluated in Table 1.5.

Table 1.5 Harmful consequences of some antibiotics

S/N	Antibiotics	Hazardous Consequences on Humans
1	Amoxicillin	Nausea, diarrhea, carcinogenic, mutagenic and teratogenic effects
2	Antimycobacterials, leprostatics	Neuritis, gastrointestinal effects, malaise, dizziness, drowsiness, headache, hallucinations
3	Aminoglycosides	Hypersensitivity reactions, blood pressure changes, gastrointestinal irritation Palpitations, ototoxicity confusion, agitation renal toxicity
4	Cephalosporins	Nephrotoxicity, pseudomembranous colitis, dizziness, lethargy, gastrointestinal disturbances, paresthesias, headache, superinfections
5	Fluoroquinolones	Bone marrow depression, dizziness, insomnia, headache, depression, photosensitivity, fever, rash, gastrointestinal effects
6	Lincosamides	Pseudomembranous colitiss, bone marrow depression, Gastrointestinal effects
7	Monobactams	Gastrointestinal effects, allergic reactions, hepatotoxicity
8	Penicillins/ penicillinase	Tongue superinfections, gastrointestinal effects, including sore mouth and furry, hypersensitivity reactions, including anaphylaxis
9	Macrolides	Hypersensitivity reactions, gastrointestinal effects, confusion, abnormal thinking, superinfections, pseudomembranous colitis
10	Tetracyclines	Nephrotoxicity, carcinogenicity, hepatotoxicity, allergic reactions, hypersensitivity reactions, dermatological reactions, gastrointestinal effects, superinfections, teeth (staining), bone damage,proximal and distal renal tubular acidosis, disruption of the natural microbiome of the intestines during the first trimester of pregnancy among other risks
11	Sulfonamides	Dermatological effects (including hypersensitivity and photosensitivity), bone marrow depression, Gastrointestinal effects, hepatotoxicity, headache, nephrotoxicity, vertigo, ataxia, depression, dizziness, seizures, carcinogenicity

1.12 Withdrawal Period of Antibiotics

A withdrawal period is a period of time required for an administered product to be metabolized by an animal and for the level of product concentration in the tissues to drop to a level that is safe, adequate, and permitted. The administration of antibiotics should be avoided before animal slaughter, and if withdrawal periods are advised, they should be properly adhered to. Using of long-acting and broad-spectrum antibiotics should be averted to stimulate animal growth in food animals. Antibiotic overdose and sub-therapeutic use should be avoided going forward. It is advised to use medications in food animals with short withdrawal times. Each antibiotic has a different withdrawal period from the others (Table 1.6). Therefore, ensuring suitable intervals for withdrawal is an important aspect of food safety [44-46].

Table 1.6 Withdrawal periods of some antibiotics

Antibiotics	**Withdrawal Periods before slaughter (days)**	
	Cattle	Poultry
Amoxicillin	25	3
Ampicillin	6	6
Bacitracin	0	0
Carbomycine	1	1
Ciprofloxacin	23	15-19
Chlortetracycline	10	1
Chloramphenicol	-	14
Dihydrostreptomycin	30	-
Erythromycin	3	2
Enrofloxacin	7	7
Furazolidone	28	28
Gentamycin	14	35
Monensine	-	5
Neomycin	1	5
Oxytetracycline	28	7
Penicillin G	10	5
Streptomycin	-	4
Sulfonaimde	-	4
Sulfamethazine	10	-
Sulfaqunoxaline	10	14
Tetracycline	5	5
Tylosin	21	5

Initially, education through veterinary professionals, literature, and regulatory agencies is essential to promote awareness among individuals and organizations. Implementing an approach for national regulation and monitoring of veterinary antibiotic usage, resistance, and residues is crucial. Applying rapid screening processes allows for analyzing, grading, and prohibiting products with residues that exceed the Maximum residual limit (MRL) [47, 48].

1.13 Reduction Process of Antibiotics

The reduction of antibiotic residue after various cooking methods may be clarified through the movement of antibiotic residues from the muscle to the boiling water and the loss of muscle fluids during roasting [49-53]. Cooking techniques such as boiling, frying, barbecuing, grilling, microwaving, roasting can only lower the concentrations of these drug residues to a safe level (Table 1.7). They cannot ensure that all drugs discovered in meat will be completely removed. Antibiotic residue exposure can be reduced by removing any fluids that emerge from the cooked edible tissues [51]. Educating people and organizations through veterinary professionals, literature, and government officials is the first and most important step in raising awareness. It is necessary to implement a system for national regulation and monitoring of veterinary antibiotic usage, resistance, and residues. Rapid screening approaches are used for the analysis, grading, and prohibition of products with residues that exceed the MRL [47, 48, 54].

Table 1.7 Impact of cooking methods on antibiotic residues

Sample Type	Number of samples	Oxytetracycline (µg/kg)			References
		Residue Level	After Cooking	Reduction %	
Beef	60	322.2	71.10 (B)	77.9	[55]
			93.7 (Br)	70.9	
Chicken Meat	45	244	91.29 (R)	37.41	[49]
			53.84 (B)	77.93	
			45.2 (M)	81.48	
			239 (F)	2.05	
Chicken Meat	120	446.16	69.04 (B)	84.52	[56]
			15.25 (G)	96.58	
			28.46 (F)	93.62	
Ciprofloxacin (µg/kg)					
Chicken Meat	45	388	301 (B)	22.4	[49]
			322 (R)	17.0	
			250 (M)	35.5	
			145 (F)	62.6	
Rabbit Meat	3	56.4	44.7 (B)	20.7	[57]
			49.18 (R)	12.8	
			34.9 (M)	38.1	
			19.34 (F)	65.7	
Ampicillin (µg/kg)					
Chicken Meat	120	253.7	47.6 (B)	81.2	[56]
			13.9 (M)	94.5	
			24.10 (F)	90.5	
Gentamicin (µg/kg)					
Chicken Thigh	3	2.56	1.64 (B)	35.9	[58]
			1.12 (M)	56.2	
			1.29 (F)	49.6	
Enrofloxacin (µg/kg)					
Chicken Thigh	3	27.2	19.5 (B)	-28.3	[59,60]
			8.3 (G)	-69.5	
			11.6 (F)	-57.4	
Tilmicosin (µg/kg)					
Chicken Thigh	3	1.45	0.92 (B)	36.5	[58]
			0.86 (M)	0.86	
			0.78 (F)	46.4	

1.14 Tolerance Limits of Antibiotics in Meat

Tolerance is defined as the tissue concentration below which an animal's edible tissues are deemed safe for human consumption, provided a marker remnant for the medication or chemical falls in the target tissue. Extensive toxicological investigations of potential human ingestion risk factors are the foundation upon which tolerances are developed. Utilizing antibiotics in food intended for human consumption must be prohibited if doing so could cause residues to accumulate in meat, milk, and eggs. A withholding period must be followed until the antibiotic residues are minimal or no longer detectable if the use of antibiotics is required, such as in the prevention and treatment of animal illnesses. Antibiotic use to enhance growth and feed efficiency, synchronize or regulate the reproductive cycle, and improve breeding performance frequently has adverse effects. Antibiotic residues in animal-derived food are a source of concern for two reasons: first, they may pose a direct hazard to human health; second, low levels of antibiotic exposure may alter micro-flora, lead to disease, and possibly develop resistant strains that could render antibiotic therapy ineffective in clinical settings. A withdrawal period is set up to protect people from eating food that has antibiotics added to it. Antibiotic use to enhance growth and feed efficiency, synchronize or regulate the reproductive cycle, and improve breeding performance frequently has adverse effects. Antibiotic residues in animal-derived food are a source of concern for two reasons: first, they may pose a direct hazard to human health; second, low levels of antibiotic exposure may alter micro-flora, lead to disease, and possibly develop resistant strains that could render antibiotic therapy ineffective in clinical settings. A withdrawal period is set up to protect people from eating food that has antibiotics added to it. The amount of time needed for the residue of toxicological concern to reach a safe concentration as determined by tolerance is known as the withdrawal time. It is the period of time between when an animal is taken off medication and when it is legally allowed to be slaughtered. The veterinarian and livestock producer bear a great deal of responsibility for monitoring the drug withdrawal phase before slaughter to ensure that there is no unlawful accumulation of drug residue in meat, milk, or eggs [56, 60]. The tolerance limits of some typical antibiotics are listed in Table 1.8 [61, 62].

Table 1.8 Tolerance Limits of antibiotics in meat (mg/kg)

S/N	Antibiotics	Tolerance Limit in Meat (mg/kg)
1	Amoxicillin	0.01
2	Ampicillin	0.01
3	Chlortetracycline	0.2
4	Cloxacillin	0.01
5	Enrofloxacin	0.01
6	Erythromycin	0.1
7	Flunixin Meglumine	0.01
8	Meloxicam	0.01
9	Monensin	0.01
10	Oxytetracycline	0.2
11	Sulphadiazine	0.01
12	Tetracycline	0.2
13	Tylosin	0.1
14	Tilmicosin	0.15
15	Trimethoprim	0.01

1.15 Tetracycline

Tetracycline is any category of broad-spectrum antibiotic compounds that are semi-synthetically synthesized from those selected chemical compounds or obtained naturally from various species of *Streptomyces* bacteria. These substances have a common basic structure (Figure 1.3). They are only effective against multiplying microorganisms because they impede a bacterium's capacity to generate specific essential proteins [63].

Tetracyclines is employed in the diagnosis, management, and treatment of numerous infectious disorders. Tetracycline, chlortetracycline, oxytetracycline (Table 1.9), and demeclocycline are naturally occurring medications in this class. Lymecycline, methacycline, minocycline, rolitetracycline, and doxycycline are examples of semi-synthetic tetracyclines. Tigecycline is the sole agent belonging to the glycylcycline category. Finally, eravacycline, sarecycline, and omadacycline are members of a more recent class of tetracyclines [64]. These antibiotics are effective in treating a variety of illnesses, including rickettsial infections, ehrlichiosis, anaplasmosis, leptospirosis, amebiasis, actinomycosis, nocardiosis, brucellosis, melioidosis, tularemia, chlamydial infections, pelvic inflammatory disease, syphilis, traveler's diarrhea, early Lyme disease, acne, legionnaire's disease, and Whipple disease. These include Vibrio vulnificus,

Mycobacterium marinum, Mycoplasma pneumoniae, Borrelia recurrentis, *Staphylococcus aureus* (including methicillin-resistant *S. aureus*), Meningococcal prophylaxis and susceptible strains of vancomycin-resistant enterococcus [65]. Additional conditions for which tetracyclines are indicated include pityriasis lichenoides chronica, rheumatoid arthritis, panniculitis, scleroderma, bullous dermatoses, sarcoidosis, Kaposi sarcoma, pyoderma gangrenosum, hidradenitis suppurativa, Sweet syndrome, and cardiovascular diseases (abdominal aortic aneurysm and acute myocardial infarction) [63]. The half-life of elimination of tetracycline is 8-11 hours [66].The half-life of elimination of oxytetracycline and chlortetracycline are 6-8 hours [67] and 5.6 hours [68].

Table 1.9 Molecular formula and weight of three tetracycline

Tetracycline	Oxytetracycline	Chlortetracycline
Molecular formula: $C_{22}H_{24}N_2O_8$	Molecular formula: $C_{22}H_{24}N_2O_9$	Molecular formula: $C_{22}H_{23}ClN_2O_8$
Molecular weight: 444.44	Molecular weight: 460.44	Molecular weight: 478.88

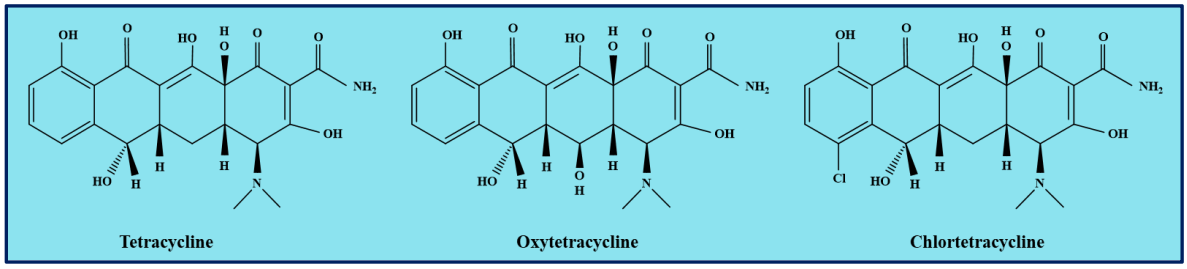


Figure 1.3 Chemical structure of tetracycline, oxytetracycline and chlortetracycline

In livestock, antibiotics are widely used for preventive, therapeutic, metaphylactic, growth-promoting, and food effectiveness purposes. The common use of antibiotics in animals is to promote growth, and regulatory initiatives aim to reduce the amount of antibiotics used in aquaculture, cattle, and poultry. The producer or farmer might combine relatively modest amounts of antibiotics with the feed to promote growth and enhance nutritional efficacy [67]. Tetracyclines are a class of drugs that are widely used because of their wide range of action and inexpensive price when compared to other antibiotics. Although there are currently more than 20 tetracyclines on the market, the ones used most frequently in veterinary medicine include tetracycline, chlortetracycline, oxytetracycline, and doxycycline [69]. Tetracyclines are commonly employed in livestock feed at subtherapeutic levels in many other nations for objectives other than therapeutics, such as aquaculture and the promotion of swine and poultry growth. Since data implies that the usage of antibiotics as growth

promoters can have detrimental impacts such as promoting bacterial resistance, allergic reactions in humans and animals, changes in environmental microflora and microbial communities, and other adverse consequences, the application of antibiotics has long been associated with beneficial aspects, particularly a boost in absorption of nutrients efficiency and commercial revenue for farmers [70, 71]. Tetracyclines are employed for treating various infections in pets and animals used for food production. First-generation tetracyclines are typically utilized in food-producing species, such as horses, while second-generation tetracyclines are preferred for usage in pets. Animal respiratory infections, skin and soft tissue infections, peritonitis, metritis, and other intestinal infections are among the therapeutic indications. Infections in honeybees and aquatic species and are also included. Antibiotics are given to groups of food animals concurrently through feed or drinking water to treat or prevent disease, as this makes administration easier [72]. In several nations, tetracyclines are still permitted as growth promoters [73]. Tetracyclines have demonstrated efficaciousness against a variety of pathogens, including Ornithobacterium rhinotracheale, Pasteurella, Clostridium, and Mycoplasma. Though they have also been used to promote growth, tetracyclines are no longer utilized for this purpose, particularly in European nations due to concerns about the emergence of bacterial resistance [74]. The maximum residue level of tetracyclines in edible tissues of cattle and poultry regulated by Codex Alimentarius Commission (CAC/MRL 2-2015) of July 2015, Commission Regulation (EU) No 37/2010 of 22 December 2009 and Bangladesh Food Safety Authority (BFSA) of 10 July 2017 (Table 1.10). The acceptable daily intake (ADI) of tetracycline is 0-30 µg/kg body weight [75-77].

Table 1.10 MRL Levels of tetracyclines in cattle and poultry

Antibiotics	**Species**	**Tissue**	**MRL (µg/kg) (Codex)**	**MRL (µg/kg) (EU)**	**MRL (µg/kg) (BFSA)**
Tetracycline Oxytetracycline Chlortetracycline	Cattle	Muscle	200	100	200
		Liver	600	300	600
		Kidney	1200	600	1200
		Milk	100	100	100
	Poultry	Muscle	200	100	200
		Liver	600	300	600
		Kidney	1200	600	1200
		Egg	400	200	400

1.16 Mechanism of Action of Tetracyclines

Any cell needs to be able to synthesize proteins. This requires the use of ribosomes, which are responsible for translating an mRNA code into functional proteins. This happens on ribosomes containing the 40S and 60S subunits in eukaryotes. In prokaryotes, like bacteria, ribosomes containing the 30S and 50S subunits are used in the production of proteins. The ribosome transfers RNA (tRNA), which has an amino acid charge, attaches itself to the mRNA template at these locations. The subsequent elongation and synthesis of cellular proteins is facilitated by the binding of each tRNA laden with an amino acid. Tetracyclines impede the binding of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex by selectively inhibiting the 30S ribosomal subunit. A cell can no longer sustain normal functioning and cannot proliferate or divide further if this mechanism is stopped. This kind of tetracycline impairment is what characterizes them as "bacteriostatic." Bacterial species resistant to tetracycline medicines are becoming increasing concern. Bacterial genes resistant to tetracyclines are frequently located on transferable elements such as transposons or plasmids. Two well-established mechanisms of resistance have been identified: modifications to efflux pumps or ribosomal protection proteins. The first method permits the ribosomes to continue synthesising proteins even when the medication is present at high intracellular concentrations. To prevent cell death, the latter mechanism uses different subtypes of transmembrane pumps to force solutes-in this case, antibiotics-out of the cell [78-81].

1.17 Amoxicillin

Amoxicillin ($C_{16}H_{19}N_3O_5S$) is a penicillin family antibiotic and a member of the aminopenicillin class. Amoxicillin was first used in medicine in 1972, although it was discovered in 1958 [82, 83]. Amoxil was authorized for use in medicine in the UK in 1977 [84] as well as in the US in 1974 [85]. It is included in the World Health Organization's (WHO) list of essential medications [86, 87].

Amoxicillin (Figure 1.4) belongs to the antibiotic class β-lactam specially Aminopenicillins [88]. Antibiotics with a beta-lactam ring in their chemical structure are known as β-lactam or beta-lactam antibiotics. Penicillins, cephalosporins and cephamycins (cephems), monobactams, carbapenems, carbacephems and Beta-lactamase inhibitors are among the antibiotic derivatives included in this category [88-90]. The most commonly used class of antibiotics, β-lactams, function by preventing the bacterial organism from synthesizing its cell walls. The high molecular weight penicillin-binding proteins, including penicillin-binding protein 1, are hindered competitively by amoxicillin.

Glycosyltransferase and transpeptidase processes that result in the cross-linking of D-alanine and D-aspartic acid in bacterial cell walls are caused by penicillin-binding proteins. The bacteriocidal effect results from bacteria upregulating autolytic enzymes and being unable to form and repair the cell wall in the absence of penicillin-binding proteins [91, 92].

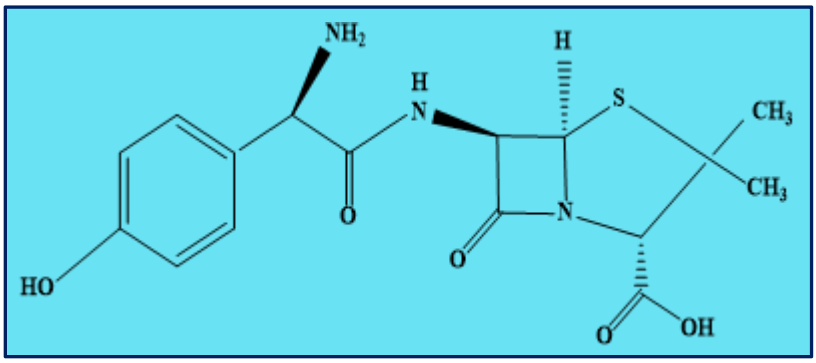


Figure 1.4 Chemical structure of amoxicillin

Treatment for susceptible bacterial infections of the skin, skin structure, genitourinary tract, ear, nose, and throat should be limited to amoxicillin [93, 94]. Acute bacterial sinusitis, community-acquired pneumonia, lower respiratory tract infections, acute bacterial otitis media, skin and skin structure infections, and urinary tract infections are all treated with amoxicillin and clavulanic acid. Helicobacter pylori infections are treated with omeprazole and amoxicillin [95]. Approximately 60% of the dose of amoxicillin is eliminated in the urine within 6 to 8 hours of administration, with a half-life of 61.3 minutes [91].

Amoxicillin is a beta-lactam antibiotic with a broad spectrum of pharmacological activity that is effective against both Gram-positive and Gram-negative bacteria. Oral administration of amoxicillin results in a faster absorption rate compared to naturally occurring penicillins, and it is stable in the gastrointestinal tract. Amoxicillin's pharmaceutical and physicochemical qualities make it a popular antibiotic in both human and veterinary medicine, utilized for treating and preventing bacterial infections of the skin, gastrointestinal tract, respiratory system, and kidneys [96]. Penicillinases or bacterial β-lactamases decompose amoxicillin. A penicillinase inhibitor called clavulanic acid is frequently used with amoxicillin in humans. However, this combination is not extremely prevalent in veterinary usages. Amoxicillin is utilized in a wide variety of domestic and commercial food animals, such as cattle, pre-ruminating calves (including veal calves), sheep, pigeons, cats, dogs, horses, and pigs and goats. Diseases of the urogenital tract include MMA syndrome, metritis, pyometra, retained placenta, navel illness, cystitis, and nephritis; respiratory conditions include shipping fever, bronchitis, sinusitis, pharyngitis, laryngitis, pneumonia, and calf diphtheria; gastrointestinal tract conditions include

campylobacteriosis, enteritis, gastritis, salmonellosis, calf scours, etc.; musculoskeletal disorders include eczema, black quarter, arthritis, sores, abscesses, foot rot, and infections of the skin and soft tissues. To prevent post-operative infections with viruses or parasites, as well as secondary bacterial infections. Treatment for the following infectious illnesses with amoxicillin is recommended in poultry (especially broilers) such as staphylococcosis, ranikhet and gumboro, pneumonia, colibacillosis, salmonellosis, necrotic enteritis, CRD, infected coryza, etc. Amoxicillin is also utilized to treat fish diseases including gill rot, dropsy, fin rot, tail rot, and white and red spot [97].

A broad-spectrum penicillin antibiotic termed amoxicillin is used to treat specific bacterial illnesses in poultry. Amoxicillin works against certain Gram-negative bacteria and Gram-positive bacteria that are susceptible to penicillin. Certain species of Staphylococci, Clostridium, and alpha- and beta-hemolytic streptococci are among the gram-positive spectrum of action. In addition, amoxicillin performs adequately against especially *Salmonella strains*, *Pasteurella multocida*, and *Escherichia coli*. To prevent pasteurellosis (fowl cholera) from bites or scratches, amoxicillin is most frequently administered to birds that have just been attacked by a predator [98]. Amoxicillin is prescribed to chickens to treat diseases of the respiratory, urogenital, and alimentary tracts that are susceptible [99].

Amoxicillin is an antibiotic that belongs to the β-lactam class and has a wide range of bactericidal activity. The mechanism of action of β-lactam antibiotics involves structural mimicry of the D-alanine-D-alanine motif found in peptidoglycans within bacterial cell walls. They prevent the formation of isopeptide bonds, the process by which bacterial transpeptidases catalyze peptidoglycan cross-linking. Peptidoglycan is a crucial part of the bacterial cell wall and is important for maintaining osmotic stability as well as shielding the bacteria from external stress. The breakdown and reconstruction of cell walls are essential for the development and division of bacteria. PBPs, which are crucial peptide cross-linking enzymes required for peptidoglycan formation, are the main target of β-lactam antibiotics. A stable acyl-enzyme complex is produced when a serine nucleophile targets the lactam carbonyl in the presence of a β-lactam. This process compromises the bacterial cell wall's integrity, impairing the organism's ability to grow and divide and eliminating its defense against osmotic or tensile stress. L, D-transpeptidases are a significant mechanism of action that is unique to carbapenem β-lactamS. For instance, the production of L, D transpeptidase covalent adducts is responsible for faropenem's effectiveness against mycobacteria [100].

Amoxicillin is employed to treat respiratory tract infections in cattle, such as shipping fever and pneumonia caused by *M. haemolytica*, *P. multocida*, *Staphylococcus spp.*, *Haemophilus spp.*, and *Streptococcus spp.* including acute necrotic pododermititis (foot rot)

induced by Fusobacterium necrophorum [101]. Furthermore, amoxicillin is administered to treat bacterial enteritis in pre-ruminating calves due to *E. coli*. Amoxicillin is recommended for the treatment of respiratory tract infections in goats brought on by P. multocida, H. somnus, and Mannheimia haemolytica, among other microorganisms. Still, it is not approved for the treatment of penicillinase-producing S. aureus [102].

Amoxicilloic acid and amoxicillin piperazine-2,5-dione, often known as digetopiperazine, are the two main metabolites of amoxicillin. Although the parent component's antibacterial activity has been reduced in these metabolites, amoxicilloic acid may have allergenic qualities [97]. After adding 1 ml of 0.1 M HCl solution to 1 ml of amoxicillin solution (25 mg/mL in dimethyl sulphoxide, Figure 1.5 illustrates the breakdown of amoxicillin to its major metabolites, amoxicilloic acid and amoxicillin piperazine 2',5'-dione, as well as two minor inactive metabolites [103].

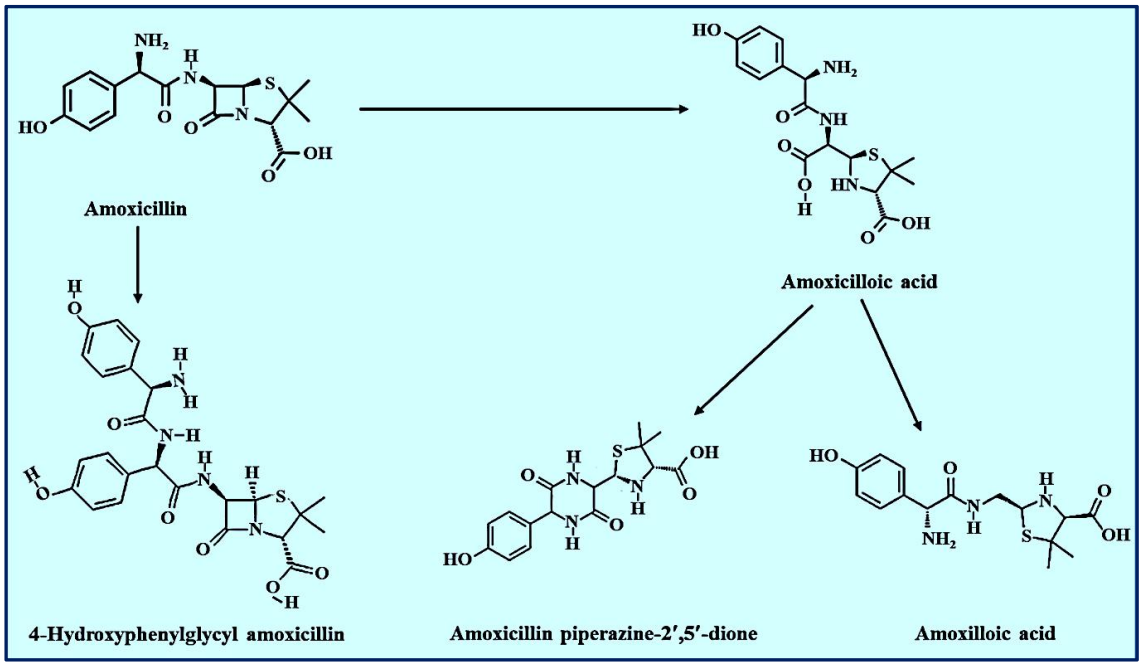


Figure 1.5 Fundamental metabolic route of amoxicillin

1.18 Patulin

The main producers of mycotoxin patulin (4-hydroxy-4*H*-furo{2,3-*C*}pyran-2{6*H*}-1) (clavacin; PAT), (molar mass 154.12 and molecular formula $C_7H_6O_4$), are *Penicillium*, *Aspergillus*, and *Byssochlamys* species. Patulin is a polyketide lactone that is quite tiny in size and can be extracted as white or colorless crystals. In the presence of water, it is soluble in ethanol, methanol, acetone, ethyl-or amyl-acetate, and less soluble in diethyl ether and benzene. Although it can be broken down by heating in sulfuric acid, it remains stable in acidic solutions [104]. It was discovered that patulin (Figure 1.6) significantly increased on fruits such as grapes, pears, and apples.

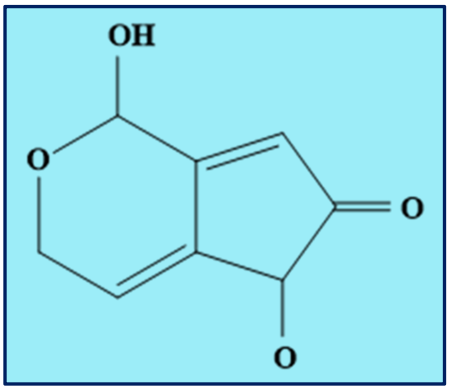


Figure 1.6 Chemical structure of patulin

A serious health risk and financial threat are connected to the significant PAT producer, Penicillium *expansum*, which belongs to the genus Penicillium. Blue mold rot and other common post-harvest diseases are caused by mycotoxin. Among fruits, apples are among the most edible foods susceptible to PAT mycotoxin. Patulin was isolated and employed as a powerful anti-microbial agent against gram-positive and gram-negative bacteria in 1943 [105]. A crucial fruit concentrate, patulin was used in a variety of juices, compote combinations, commercial apple-based beverages, and baby meals [106]. The fact that patulin increases health risks due to its mutagenic, carcinogenic, neurotoxic, genotoxic, immunotoxic, and gastrointestinal impacts on both human and animal health has garnered attention worldwide. Because patulin has antiviral, antiprotozoal, and antibacterial qualities, it was used to treat nasal infections and common colds in the 1960s. Because of its harmful effects on both human and animal health, it was later designated as a real mycotoxin [107, 108]. Patulin has the potential to cause cancer, but there is currently insufficient data to substantiate its carcinogenicity, according to the International Agency for Research on Cancer, which classifies it as a Group 3 chemical [109, 110]. Further research on several mammalian cell types, including mouse lymphoma L5178Y cells and Chinese hamster lung fibroblast V79 cells, demonstrated the genotoxicity and mutagenicity of PAT [111].

PAT is known to cause chromosome abnormalities, micronuclei production [112], and DNA strand breaks [113]. According to Schumacher (2006), PAT is thought to primarily cause cell death by quickly generating covalent adducts with sulfhydryl groups and more slowly with amino functionalities of proteins and glutathione. Therefore, the cytotoxicity and genotoxicity of PAT are increased when the glutathione production inhibitor buthionine sulfoximine reduces the amount of glutathione in the cells [114]. Based on research on animals, 50% of PAT is absorbed in the stomach, processed in the kidneys or liver, and then eliminated through the urine. The formation of covalent bonds with sulfhydryl compounds, which causes apoptosis, oxidative stress, and decreased cell viability, defines PAT's harmful effects [l15].

Mycotoxins are substances that are secondary metabolites produced by filamentous fungi including Fusarium, Penicillium, and Aspergillus, and can seriously harm both humans and animals [116]. Food and feed are contaminated by fungi that produce mycotoxin, negatively affecting the economy and human health. Food crops, such as almonds, cereals (barley, corn, wheat, rice), coffee, cotton seeds, fruits, lentils, peanuts, pistachios, walnuts, spices (ginger, paprika, pepper), and meat, are in danger of mycotoxin contamination worldwide [117]. Almost 1.3 billion metric tons of food are wasted yearly due to mycotoxin contamination, which accounts for one-third of the world's food production. Additionally, mycotoxins have been linked to allergies, cancer, and organ damage. Depending on the level that people are exposed to and their mutagenic and teratogenic properties, the severity of their consequences will vary [118, 119].

Animals exposed to PAT have been demonstrated to lose weight, eat less, die at higher rates, and develop skeletal abnormalities in mice [120]. The evaluation of the risks patulin poses to human health is based on several investigations carried out over the previous 50 years. In humans, patulin causes different amounts of acute, chronic, and cellular damage [121]. Patulin consumption can cause a variety of negative side effects, such as convulsions, agitation, vomiting, damage to the kidney and gastrointestinal tissues, edema, ulceration, hyperemia, distension of the gastrointestinal tract, intestinal hemorrhage, degeneration of epithelial cells, inflammation of the intestines, and dyspnea [122]. The regulatory agencies have set a maximum suggested concentration of 50 μg L–1 (50 ppb) for apples and their products, based on the data that is currently available [123-125]. The Joint WHO/FAO Committee on Food Additives (JECFA) has set the acceptable daily consumption at 0.4 μg/kg body weight/day [126].

Adhering to regulated storage conditions, applying appropriate farming practices, and maintaining clean food processing techniques are all necessary to comply with legal

restrictions for mycotoxins. Regular product analysis is required because of the intricate pathways of contamination. Although mycotoxins are poisonous, it is essential to detect them and make sure they are present within permissible levels [123].

1.19 Pesticides

Pesticides are chemical substances, either natural or manufactured, mostly employed in agriculture to protect plants against weeds and pests. They are made to eliminate weeds, control plant diseases and pests, and destroy germs that contaminate agricultural goods. Pesticides have long been used to combat parasites and diseases that are vectors. Insects, rodents, other animals, weeds, fungi, and microorganisms like bacteria and viruses can all be considered pests. Pests can ruin food that has been stored and lower productivity, which lowers both the quality and amount of food produced. Eighty percent of all pesticide applications are composed of insecticides, and these are the most widely used variety ("Pesticides", GRACE Communications, 2018) [127]. Pesticides are described by the Food and Agriculture Organization [128] as:

Any material or combination of materials meant to prevent, eradicate, or manage any pest, such as pests that spread disease to humans or animals, undesirable plant or animal species, or any material that impacts or otherwise gets in the way of food production, processing, storage, transportation, or marketing, wood and wood products, animal feed, or materials that can be given to animals to control insects, arachnids, or other harmful organisms in or on their bodies.

Agricultural productivity, including food crops and other crops, is enhanced by pesticides, which also shield crops from diseases and pests. However, depending on their nature and concentration, pesticides may cause more or less harm to harmful environmental contamination once they have escaped their intended target [129]. Since chemical pesticides are persistent and in human tissue, they are all essentially poisonous and a long-term threat of concern for the humans and environment. Although practically all pesticides that can kill pests also can be harmful to human health, there is rising concern about the appropriate application of these chemicals and the risks they pose to consumers, the environment, and farmers who use them. Laws mandate that pesticide use be properly regulated and that MRL not be surpassed. An accurate and appropriate analysis method is required to determine the amount of residue in food products. To eliminate information gaps and provide a system to alert consumers of potential issues, measuring the amount of residual pesticides in food and agricultural commodities is crucial [130].

1.20 Categorization of Pesticides

Pesticides can be categorized based on what sort of pests or conditions they are effective against. Certain insecticides are exclusively effective against a single species of infection or pest. A significant percentage of pesticides lack specificity, are not very selective, or both. When applied, these non-selective insecticides can damage or even kill various insects, microbes, animals, and plant species. Pesticides can also be categorized based on how they function. By systemic action or by interaction, this could happen. For contact insecticides to be effective, potentially hazardous organisms must come into direct contact with them. The spray mist can penetrate the crop more effectively and kill the pests by contact provided it is finer. Systemic insecticides cling to plant surfaces, seep into them, and then spread throughout the entire plant. They can linger in the soil, protecting seeds in colder regions. To select the most optimal pesticide product, farmers must be aware of the types of chemicals compatible with particular circumstances [131]. Pesticides can be categorized according to their toxicity, mechanism of action, functional groups, and chemical classes. Typically, the active components of pesticides are classified as either inorganic (such as minerals including copper sulfate, ferrous sulfate, copper, lime, sulfur, etc.) or organic (containing carbon). Natural organic pesticides are derived from naturally occurring sources, while synthetic organic pesticides are manufactured artificially in factories by chemical synthesis [132, 133].

i) Pesticides can be classified on basis of the degree of biodegradability:

Biodegradable: Those that microorganisms and other living organisms can metabolize down into innocuous molecules are considered biodegradable.

Consistent: Those that can take months, or even decades to decompose are persistent.

ii) Pesticides can be categorized based on their functionality (Table1.11) [134, 135].

Table 1.11 Classification of pesticides based on functionality

Pesticide Type	**Function/Uses**	**Example**
Bactericides	employed to restraint bacteria	chlorine, fluorine, and iodine, and chlorine compounds including sodium hypochlorite, chloramines, chlorine dioxide
Herbicides	utilized to eradicate unwanted weeds or plants	2,4-D, imazaquin, dicamba, glyphosate, and sethoxydim
Insecticides	capable to destroy or kill six-legged insects	aldrin, dieldrin, diazinon, parathion
Fungicides	prevent or hinder fungal diseases such as molds, blotches, rusts, mildew, scabs and rots	carboxin and thiabendazole
Larvicides	a chemical that kills larvae	methoprene, temephos, oils, and pyriproxyfen
Rodenticides	eradicate rodents such as rats and mice	phosphorus paste, barium carbonate salt, and powders such as calcium cyanide, thallium sulfate, white arsenic, strychnine, strychnine sulfate, and zinc phosphide
Insect growth regulators	stop insect pests from growing and reproducing.	fenoxycarb, hydroprene, methoprene, pyriproxyfen
Antimicrobials	microorganisms of medicinal and veterinary significance	Non-public health products: Iodophors Public health products: antiseptics and germicides, disinfectants, sterilizers (sporicides), sanitizers
Algaecides	algae developing on various surfaces, such as patios	copper(II) sulfate, hydrated lime, barley straw, benzalkonium chloride, dichlone, dichlorophen, endothal, fentin
Biopesticides	naturally occurring substance that can be insecticides, fungicides or herbicides	citronella, garlic, jatropha, lemongrass oil, neem, onion, tobacco
Wood preservatives	pesticides that repel fungi, insects, and other diseases away from wood	chromated arsenicals, creosote, pentachlorophenol, propiconazole, triadimefon, cid copper chromate (acc), acq (alkaline copper quaternary), borates, copper napthenate
Treated seeds	seeds treated with a fungicide, insecticide, or both to stop fungal diseases and soil insect pests from causing harm	carboxin + thiram + metalaxyl, captan + PCNB + metalaxyl, carboxin + thiram, carboxin + captan (used for fungicide treated seed)

Acaricides /Miticides	eight-legged mite pests are significant in agriculture, landscape design, veterinary care, medicine, and forestry	avermectins, carbamates, chlorinated hydrocarbons, , formamidines organophosphorous compounds, pyrethroids
Molluscicides	significant slugs and snails for forestry, agriculture, and landscape	metaldehyde. niclosamide. metal salts such as iron(iii) phosphate, aluminium sulfate, and ferric sodium EDTA
Plant growth regulators	modify a plant's growth, for example, by causing or delaying blossoming	auxins, abscisic acid, cytokinins, chlormequat, daminozide, ethylene, gibberellins, Jasmonic acid, paclobutrazol
Pheromones	draw in and seize male insects; frequently species-specific	aggregation pheromones, alarm pheromones, trail pheromones, sex pheromones

(iii) Pesticides can be categorized based on their chemical nature/composition (Table 1.12).

Table 1.12 Classification of pesticides based on chemical nature [136]

No.	Class	Chemical Name
1	Organophosphates	Abate, Bidrin, Caumphos, Dimethoate, Dimefox, Dichlorovas, Demetox, Diptrex, Enitrothion, Fenthion, Oxydemeton-methyl, Phorate, Mipafox, Malathion, Methyl Parathion, Phosphomidon, Ronnel, Trichlorofan
2	Organochlorines	Aldrin, BHC, Chlordane, Chlorobenziate, Dieldrin, DDT, DDD, DDE, Dicofol, Eldrin, Endosufan, Lindane, Methoxychloro Heptaclor, Isodrin, Isobenzan, Toxaphene, Chloropropylate
3	Carbamates	**Thio** (Cycloate, Diallate, Vernolate, Monilate, Pebulate, Butylate, Thiourea, Trillate) **Dithio** (Amoban, Ferban, Dithane M- 45, Methan, Thiram, Naban, Maneb, Polyran, Zineb, Ziram) **Methyl** (Carbaryl, Carbanolate, Prupoxur, Dimethan, Dimetilan, Isolan, Carbofuran, Pyrolan, Aminocarb, Aldicarb)
4	Pyrethroids	Cypermethrin, Dimethrin, Allethrin, Tetramethrin, Bonthrin, Fenevelerate, Cyclethrin Furethrin, Alphamethrin, Decamethrin, Ptrethrin
5	Phenoxy alkanoates	2,4 5 T(2,4 5 Trichloro Phenoxy acetic acid), 2,4-D (2,4 Dichloro phenoxy acetic acid) Dichloroprop, Erbin, Mecoprop, Sesone
6	Phenyl amides	**Acylanalide** (Alachlor, Bromuron, Butachlor, Dicryl, Propanil, Propachlor, Solan, Karsil) **Carbanilates** (Barban, Carbetamide, Chlororprofan,

		Chloroxuron, Diuron, Flumeturon, Fenuron, Prophan, Phenyl Urea, Monuron, Neburon) **Toluidines** (Acetamide, Benefin, Dipropanil, Oryzalin, Isopropanil, Nitralin,Diphenamid, Trfluralin)
7	Benzoic acid	Dicamba, Dichlorobenil, Chloroambin, Tricamba, Neptalan, Bromoxynil
8	Dipyrids	Diaquat, Paraquat,
9	Phtalimides	Diflotan, Captan, Folpet
10	Triazines	Atrazine, Atratone, Ametryn, Chlorazine, Cynazine, Cyprazine, Metribuzin, Propazine, Turbutryn, Simazine, Simetryn
11	Others	Aluminium phosphide, Calcium arsenate, Cacodylic acid, Ethyl mercuric Phosphate, Floroacetate, Pentachlorophenol, Phenyl mercuric acetate, Methyl mercuric chloride, Sodium arsenate, Lead arsenate, Zinc phosphide

1.21 Formulation

The word "formulation" refers to a pesticide's physical state and specifies the application method. Furthermore, to its toxicity, pesticide form has a significant role in how well it works to control weeds, plant diseases, and pests. In addition, it enhances a chemical's qualities for safe handling, storage, application, and handling. Typical phrases are: There are two methods to apply a liquid: (i) mixing it with an oil basis to create an emulsion that is diluted with water (emulsifiable concentrate, EC); (ii) mixing a liquid with water to create a suspension in a spray tank (flowable), iv) the chemical agent is combined with a fine powder that appears to be dust and combined with water (wettable powders, WP); v) the active ingredient is added to an edible or attractive substance and is frequently employed to control snails, slugs, rodents, and ground-dwelling insects (baits; B). iii) the effective ingredient is made into coarse particles with inert material like fired clay particles (granules, G). and vi) soluble powders (SP), characterized by the ingredients that are dissolved in water. Household pests are also treated with pesticides; these are very low-concentrate solutions that are typically sprayed as a fine mist or spray and are supplied in aerosol cans for mosquitoes (aerosols, A). The active components are added to talc or fine, inert powder (dust, D) [137, 138].

1.22 Usage of Pesticides in Bangladesh

Bangladesh is an agricultural republic where the national economy is heavily dependent on the agricultural sector. The principal source of income is agriculture for almost eighty percent of the population in this country, who reside in agrarian regions [139]. The most significant industry, accounting for over 13.02 percent of the country's GDP (Gross Domestic Product) and employing about 40.60 percent of the labor force, is agriculture [140]. Bangladesh has an ideal climate along with fertile soils that allow for the year-round production of a wide variety of crops. The effectiveness of the agricultural sector has a big influence on a lot of things, like reducing poverty, food security, nutritional achievement, national development, creating jobs, and income inequality [141].

In Bangladesh, the use of chemical inputs like pesticides has increased to increase agricultural productivity and output. Because vegetables and other crops and plants are susceptible to insect and disease attacks, pesticides are frequently utilized on them. Bangladeshi farmers produced vegetables by applying insecticides in the form of liquid, powder, and granules, coupled with a tiny amount of fungicides, acaricides, rodenticides, and herbicides. 77% of farmers reported pesticide use in crops to have occurred at least once (37% integrating once, 31% administering twice, and the remainder spraying three to five times). During each growing cycle, farmers sprayed these vegetables 17–150 times [142]. Most farmers in Bangladesh apply pesticides frequently because they are unaware of the genuine requirements or effectiveness [143]. Over 90% of pesticides are used excessively, carelessly, and unnecessarily because people are unaware of the risks associated with using them [144]. That is why improper usage of pesticides causes residues in the vegetables, contaminating fresh vegetables with dangerous pesticides. Consequently, food security has become a serious public health concern [145].

The farmers of Bangladesh employ a variety of pesticides. Organophosphorus, pyrethroids, carbamate, organochlorine, nereistoxin substitute group, neonicotinoids, and other pesticides were among the commonly employed ones. To be more precise, the most frequently encountered pesticides were malathion, dimethoate, diazinon, and chlorpyrifos. The most used insecticide in Bangladesh for managing insects and mites on crops is organophosphorus (OPs). They have a wide range of activities and are incredibly effective [146].

1.23 Toxicity of Pesticides

The term "toxicity" implies a poison's capacity to have negative consequences. These side effects might range in severity from mild headaches to serious convulsions, coma, or even death. As soon as appropriate medical care is sought promptly, the majority of toxic consequences are treatable and do not result in irreversible harm. On the other hand, some poisons result in long-lasting damage [147]. Lethal dose 50 and lethal concentration 50, or LD_{50} and LC_{50}, respectively, are the primary metrics used to express the hazardous consequences. An extended duration of pesticide exposure can be used to quantify chronic toxicity in a test organism. Almost all poisons impair normal metabolic processes, toxicity can arise in as many ways as there are bodily functions. According to Bolognesi and Merlo (2011), [148] and Khoshnood (2024) [149], pesticides vary in their level of toxicity to both target and non-target organisms. Since many pesticides have cumulative effects, they move through food chains and circulate in ecosystems, where they can accumulate in a variety of living things [150]. To ensure for evaluation of the harmful effects of herbicides, some biological subjects—individuals, species, or communities are favored as models. Different routes exist for pesticides to enter the body based on species, metabolic quirks, and toxin susceptibility [151]. As an illustration, pesticides can affect the immune system, reproduction, development, and endocrine and neurological systems. Consequently, there is widespread concern worldwide over the toxicity of pesticide exposure to creatures that are not intended targets [149]. The central nervous system, muscarinic receptors on the parasympathetic nervous system target organs, nicotinic receptors in skeletal muscle tissue, and elevated acetylcholine levels at ganglionic synapses of the sympathetic and parasympathetic nervous systems are the sources of carbamate toxicity [152]. Since organophosphate pesticides can enter the body through the skin, integumentary system, and respiratory system due to inhalation, or direct ingestion, poisoning is the main outcome of acute exposure to these substances. Inhalation is the most common way that organophosphate insecticides present therapeutically. The effects of acute organophosphate poisoning can also be produced by chronic exposure. On the other hand, persistent exposure can cause memory loss, speech impediment, poor coordination, and poor judgment in people. Chronic exposure to organophosphates has also been linked to peripheral polyneuropathy and has been known to induce flu-like symptoms such as nausea, vomiting, malaise, and weakness. Exposure to certain organophosphates has been associated with a potential risk of cancer. According to a report from the International Agency for Research on Cancer, pesticides such as diazinon, tetrachlorvinphos, malathion, and parathion are categorized as possible carcinogens [153]. The characteristics of the species, metabolic irregularities, and toxin susceptibility of the organism, pesticides may enter the body through several routes. However, once an organism has absorbed a chemical,

it must be able to cope with it in order to neutralize or eradicate its harmful effects through conjugation, biotransformation, isolation, and/or excretion into the environment, or a combination of these processes [154].

1.24 Persistence of Pesticides in the Environment

Environmental ecotoxicological research has gained significant importance in recent decades due to the regular release of pesticides into the environment, which is a concern from the perspective of environmental protection. Even with acceptable agricultural practices, they may create significant ecological changes and disrupt the ecosystem's equilibrium status. Although pesticide-related issues are typically associated with forestry or agriculture, they can also be found in large amounts in urban wastewater that builds up from weed treatment along roads or rail lines, as well as from gardens, parks, and urban woodland areas. The risk evaluation based on ambient levels is complicated by the diverse ways pesticides might affect organisms. In targeted organisms, detrimental effects are frequently hard to identify because many only show up after extended exposure. Destructive processes could already be irreversible when the results eventually become apparent. Ecosystems can be considered as significantly altered as a result of the persistent nature of many pesticides in the environment as a result of their careless or uncontrolled usage in agriculture and other human endeavors. Pesticides circulate and accumulate in a variety of biological entities because of their cumulative qualities; some of these organisms are even utilized as model subjects for evaluating the harmful effects of pesticides [149, 151].

1.25 Health Impacts

Pesticides can enter the human body via four basic routes: the skin, oral, optical, and respiratory systems. Pesticides can vary in their level of toxicity depending on the type of contact, such as oral, cutaneous, or respiratory (inhalation). Despite the fact that the chemical of concern is harmful, the risk of pesticide pollution usually increases with dosage (concentration) and basic duration [155]. Pesticide exposure can occur directly from work-related activities, domestic chores, and agricultural practices, but it can also be transmitted through diet. According to [156], the primary pathways via which humans are exposed to pesticides are the food chain, water, air, soil, fauna, and flora. Although the circulatory system distributes pesticides throughout the human body, they can also be released through the skin, urine, and exhaled air [157].

Pesticide poisoning can cause ranging from a minor skin rash to a coma or even death. Various chemical families or classes produce various kinds of symptoms. Additionally, the sensitivity of each person to varying concentrations of these substances varies. Users and handlers of pesticides need to be aware of the typical symptoms and indicators of pesticide poisoning due to possible health risks [158]. The intensity of the poisoning and the entry point determines when symptoms start to appear. If the material has been consumed, gastric symptoms including vomiting, diarrhea, nausea, fatigue, headache, intestinal disorders, and cramping in the stomach start early [159]. Furthermore, Advanced poisoning instances may include breathing difficulties, convulsions, altered heart rate, and coma, all of which may be fatal. Respiratory failure may arise from the involvement of the respiratory muscles. As the pesticide is absorbed through the skin, symptoms related to the stomach, intestines, and respiratory system typically manifest simultaneously. Convulsions may be the initial sign of poisoning in youngsters [160]. Farmer health has been impacted for years by the numerous hazardous substances found in pesticides. The effects on farm laborers are severe, and even after receiving treatment for exposure, they may still experience health problems years later. Infertility in men and women, birth deformities, miscarriages, neurological disorders like Parkinson's disease and amyotrophic lateral sclerosis, and illnesses resembling dementia are among the long-term consequences of pesticide exposure [161]. Inappropriate use of pesticides can have a number of adverse impact on human health, including acute intoxication, chronic diseases such as Alzheimer's disease [162], Parkinson's disease [163], neurotoxicity [157, 159], infertility [160, 164], leukemia [165], and diabetes, as well as various cancers (brain, breast, prostate, bladder, and colon) [166, 167]. Significant public health issues, particularly in developing nations, are the acute and long-term health impacts of dietary exposure to agricultural pesticides. Chemical pesticides have the potential to be cytotoxic, mutagenic, and carcinogenic to humans [168]. Farmers are exposed to chemical poisoning and run the danger of experiencing long-term symptoms, as does everyone in the nearby communities. Concerns over the long-term effects of pesticides on human health have been raised since neurotoxicity has been linked to the onset of neurological illnesses (Figure 1.7). According to a WHO and United Nations Environment Programme (UNEP) report, worldwide, three million people are poisoned and 200,000 die due to exposure to pesticides, predominantly in developing countries [169].

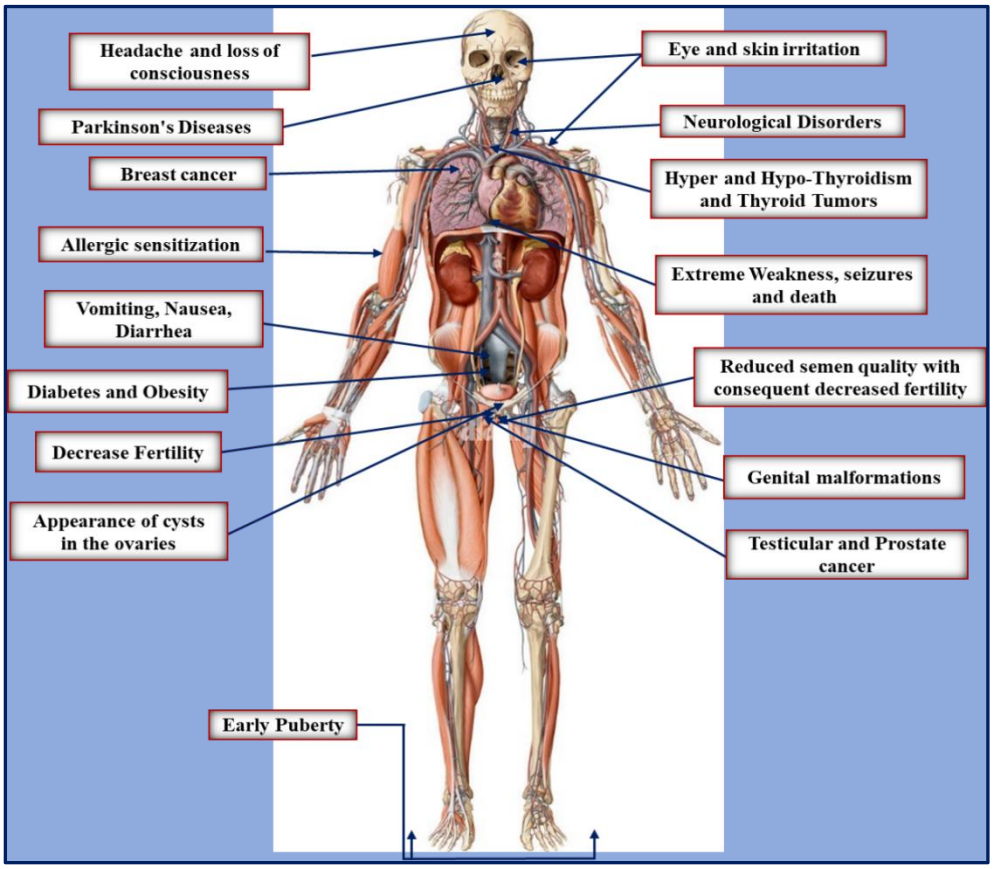


Figure 1.7 Health effects of pesticides

1.26 Description of Pesticides of the Present Research

1.26.1 Organochlorine Pesticides

Organochlorine pesticides are recognized as synthetic hydrocarbon components that have chlorine substituted in them. Dichlorodiphenyl trichloroethane (DDT), aldrin, numerous isomers of endosulfan, and the widely used lindane are a few examples of organochlorine pesticides. OCP was structurally divided into five groups: (I) DDT (dichlorodiphenyltrichloroethane) and the homologous compounds DDD (dichlorodiphenyldichloroethane) and DDE (dichlorodiphenyldichloroethylene), (II) hexachlorocyclohexane (HCH), such as lindane (III) cyclodienes, which include endosulfan, heptachlor, and chlordane, (IV) toxaphene and (V) Mirex and Chlordecone [170]. The chemical structures of the twenty pesticides of this present study are shown in Figure 1.8. Aldrin and toxaphene had respective field half-lives of 365 and 9 days and other OCPs, such as DDD, DDT, and DDE, have half-lives of 15 years [171]. The majority of OCPs often have acute toxicity at concentrations higher than those deemed environmentally feasible. This means that death might occur gradually in the natural world

and is frequently attributed to chronic disease or general waste. Because they are persistent and lipophilic, most OCP can cause long-term storage in fatty tissues before being released into the circulatory system in adverse environmental settings. OCP can be inhaled by humans, as well as through food, drink, and skin absorption. Food is the main way that people are exposed to OCP since these compounds bioaccumulate in fish and other animals that people eat. Long-term and regular skin absorption of cosmetics containing OCP is another way of exposure [172].

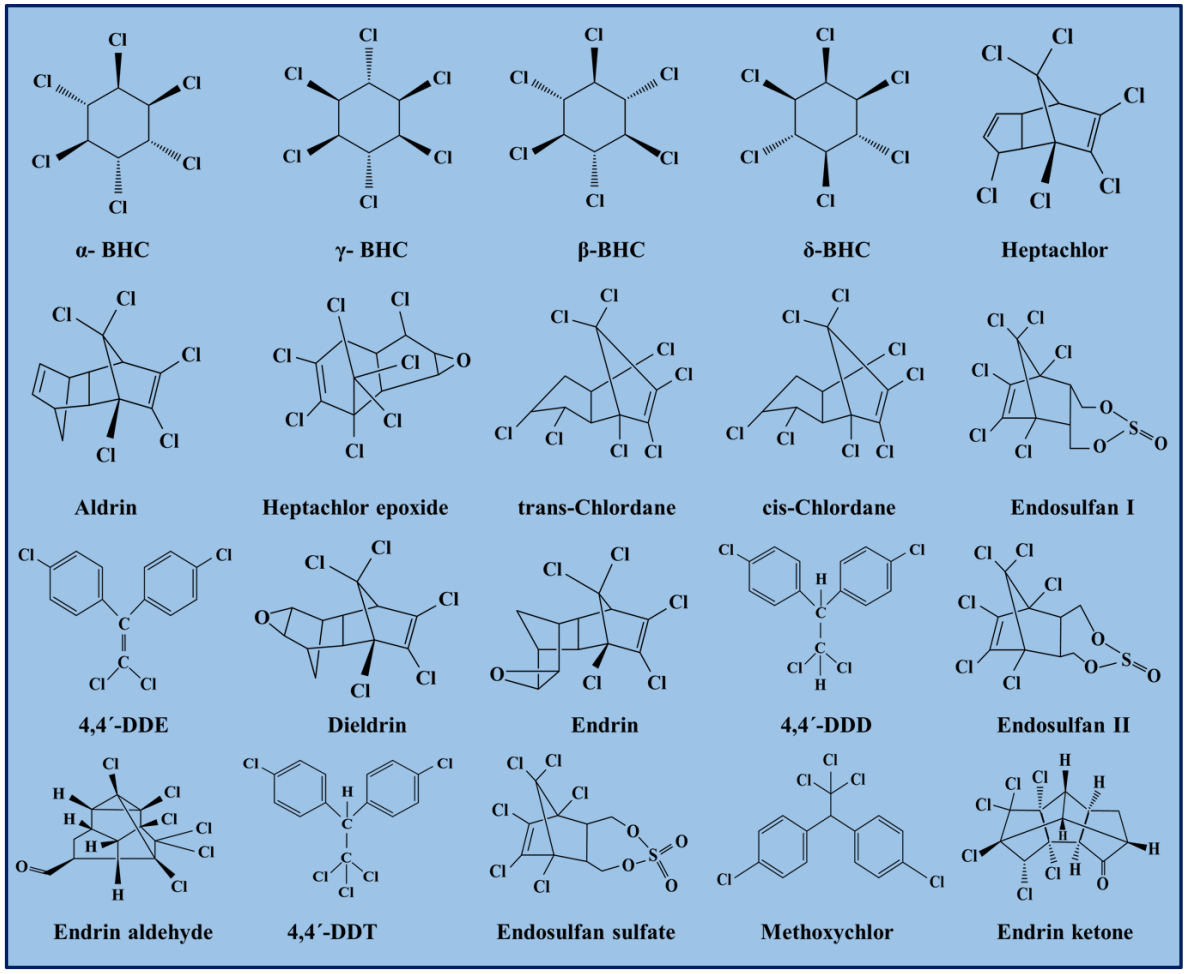


Figure 1.8 Chemical structure of organochlorine pesticides

The Stockholm Convention has prohibited the usage of several organochlorine (OC) pesticides and categorized most of them as a risk to the environment due to their significant potential for bioaccumulation and persistence [173]. However, the restriction is useless because they are still utilized in many underdeveloped countries. Pollution of the land, water, and air has increased due to the growing usage of pesticides for high agricultural productivity. Because pesticides have a long half-life, are highly lipophilic, bioaccumulate, and have the potential to travel long distances, their increased use for high agricultural production has resulted in increased pollution of water, air and soil. The health of the environment and ecosystem services are being negatively impacted by excessive usage or abuse of pesticides. Many aquatic and terrestrial animals have been documented to be impacted by pesticides. Pesticides hurt ecosystem life, including bacteria, plants, fish and invertebrates [174, 175]. Numerous disorders can arise from human exposure to OCPs, even at low exposure levels. It can have detrimental effects on the immune systems and endocrine as well as and reproduction in addition to the hazards of neurotoxicity, genotoxicity and carcinogenesis [176]. OCPs accumulate in lipophilic human body parts (Figure 1.9), particularly in fatty tissues and other lipid-rich tissues, as a result of their lipophilicity and high persistence [177].

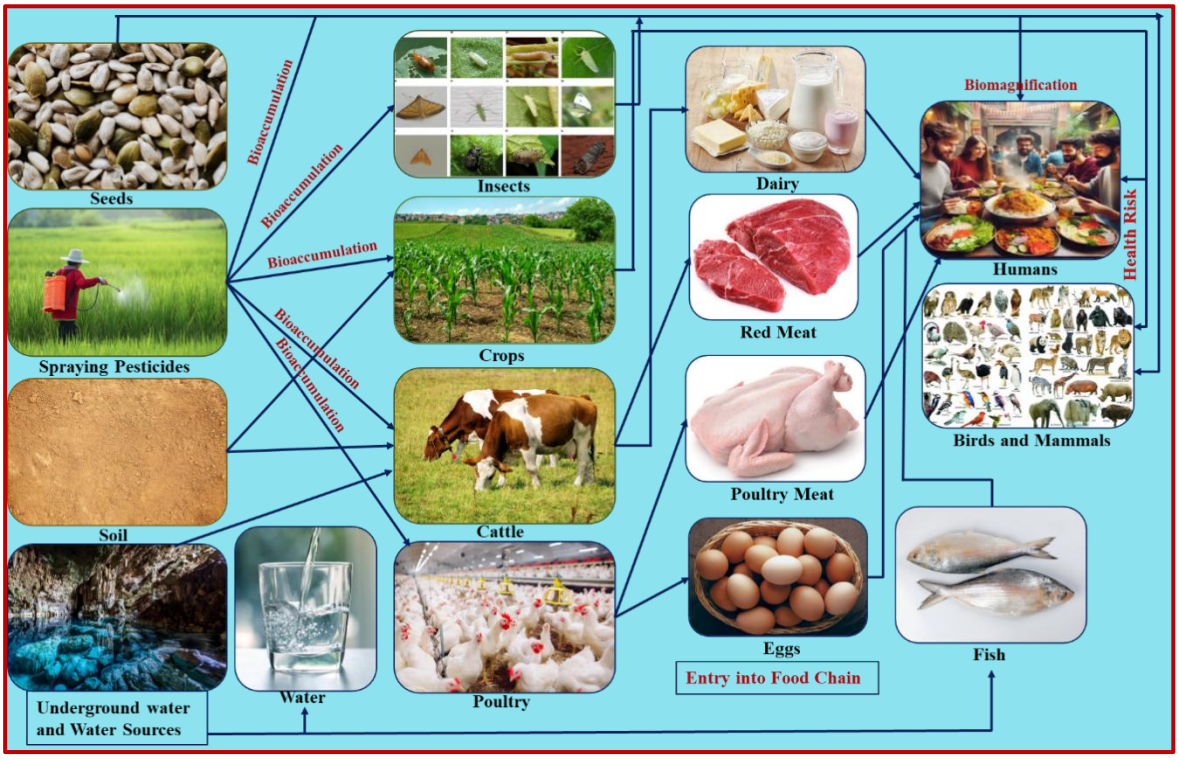


Figure 1.9 Pathway of entry of pesticides into the human body

1.26.2 Chemistry, persistence and hazard classification of OCPs

Organochlorine pesticides have four basic characteristics: high lipid solubility, low polarity, low aqueous solubility, and high persistence. Following pesticide applications, the disposal of contaminated trash in landfills, and the release of these chemicals from industrial facilities, organochlorine pesticides can find their way into the environment. Both volatile and stable, some of them have the ability to stick to surfaces like soil and air, increasing their likelihood of being very persistent in the environment. They are also known to expose humans and animals to long-term exposure. The chemical names, structures, toxicity, applications, and persistence in various environmental media of the main organochlorine pesticides are listed in detail in Table 1.13. Their chemical structures are similar, displaying aromatic or aliphatic rings that have had chlorine replaced. These substances have similar structures, which means they have similar physicochemical properties including toxicity, persistence, and bioaccumulation. Persistence, which is defined as a half-life longer than two months in water or six months in soil sediment, is one fundamental quality they all have in common. OC compounds range in persistence from high persistence, with half-lives of up to 10-15 years, to moderate persistence, with half-lives of about 60 days. Dichlorodiphenyltrichloroethane (DDT), a moderately toxic pesticide with a half-life of two to fifteen years, is the most widely used pesticide in agricultural practice [178].

Table 1.13 Chemical structure, use, toxicity and persistence of OCPs

Chemical Name and Formula	**Usage**	**Toxicity LD_{50}**	**Persistence in Environment**	**WHO Classification based on rat oral LD_{50}**
BHC ($C_6H_6Cl_6$)	Acaricide Insecticide Rodenticide	**Rat** Oral: 4000 mg/kg **Guinea pigs** Oral: < 3000 mg/kg **Rat** Oral: 10,000 mg/kg	High Persistence Half-life: 3 – 6 years	Acute hazard is unlikely
Heptachlor ($C_{10}H_5Cl$)	Insecticide	**Mouse** Oral: 30–68 mg/kg **Rat** Oral: 40– 220 mg/kg Dermal: 119–320 mg/kg **Rabbit** Dermal: 2000 mg/kg **Guinea pigs**	High Persistence Half-life: 2 years	Highly – Moderately hazardous

		Oral: 116 mg/kg Dermal: 1000 mg/kg		
Aldrin ($C_{12}H_8Cl_6$)	Insecticide	**Mouse** Oral: 44 mg/kg **Rat** Dermal: 100 mg/kg Oral: 39 to 60 mg/kg **Dog** Oral: 65–95 mg/kg	Moderate Persistence Half-life: 4–7 years	Highly hazardous
Heptachlor epoxide ($C_{10}H_5Cl_7O$)	Insecticide	**Rats and Mice** Oral: 39 to 144 mg/kg Dermal: 119-250 mg/kg/day	High Persistence Half-life: 2-4 years	Moderately hazardous
Chlordane ($C_{10}H_6Cl_8$)	Insecticide	**Mice** Dermal: 153 mg/kg Oral: 145– 430 mg/kg **Rat** Dermal: 530–690 mg/ kg Oral: 200 to 700 mg/kg **Rabbit** Dermal: 780 mg/kg	High Persistence Half-life: 10 years`	Moderately hazardous
Endosulfan ($C_9H_6Cl_6O_3S$)	Insecticide	**Rabbits** Dermal: 200–359 mg/kg **Rat** Oral: 18 to 220 mg/kg Dermal: 74 mg/kg **Ducks** Oral: 33 mg/kg	Moderate Persistence Half-life Alpha Isomer: 35 days Beta Isomer: 150 days	Highly hazardous
4,4′ DDE ($C_{14}H_8Cl_4$)	Insecticide	Rat Oral: 800–1240 mg/kg	High Persistence Half-life: 10 years	Slightly hazardous
Dieldrin ($C_{12}H_8Cl_6O$)	Insecticide	**Mouse** Oral: 38–77 mg/kg **Rat** Dermal: 50–120 mg/kg Oral: 46 mg/kg **Rabbit**	Highly Persistence Half-life 9 months	Highly hazardous

		Oral: 45–50 mg/kg **Cow** Oral: 25 mg/kg **Dog** Oral: 56–120 mg/kg **Duck** Oral: 381 mg/kg		
Endrin ($C_{12}H_8Cl_6O$)	Avicide Insecticide	**Mouse** Oral:1.37g/kg Intravenous: 2300 g/kg **Rat** Oral: 3 mg/kg Dermal: 15 mg/kg **Rabbit** Oral: 60–94 mg/kg **Goat** Oral: 50 mg/kg	Moderate Persistence Half-life 1 day to 12 years	Highly hazardous
4,4′ DDD ($C_{14}H_{10}Cl_4$)	Insecticide	**Rat** Oral: 4000 mg/kg	High Persistence Half-life: 5–10 years	Acute hazard is unlikely
Endrin aldehyde ($C_{12}H_8Cl_6O$)	Insecticide	**Rat** Oral: 500 mg/kg	Moderate Persistence Half-life: 10 years	Moderately hazardous
4,4′ DDT ($C_{14}H_9Cl_5$)	Acaricide Insecticide	**Mice** Oral: 150–300 mg/kg **Rat** Dermal: 2510 mg/kg Oral: 113–130 mg/kg **Gunia Pigs** Oral: 300 mg/kg **Rabbit** Oral: 400 mg/kg	High Persistence Half-life: 2–15 years	Moderately hazardous
Endosulfan sulfate ($C_9H_6Cl_6O_4S$)	Insecticide	**Rat** (Male &Female) 34.5 and 12.6 mg/kg	High Persistence Half-life: 9 months to 6 years	Highly hazardous
Methoxychlor ($C_{16}H_{15}Cl_3O_2$)	Insecticide	**Mice** Oral: 2000 mg/kg **Rat**	High Persistence Half-life:	Acute hazard is unlikely

		Oral: 5000–6000 mg/kg **Monkey** Oral: 2500 mg/kg	< 120 Days	
Endrin ketone ($C_{12}H_8Cl_6O$)	Insecticide	**Rat** Oral: 10 mg/kg	High Persistence Half-life: 14 years or more	Highly hazardous

Although DDT is no longer permitted in many nations, the majority of developing nations still use it illicitly. Although DDT is no longer permitted in many nations, the majority of developing nations still use it illicitly. This also holds for endosulfan, a highly dangerous insecticide used in the manufacture of cashew that has a half-life of fifty days and considerable persistence [179]. The Stockholm Convention has prohibited the use of several OC chemical compounds and categorized the majority of them as environmental hazards due to their significant potential for bioaccumulation and persistence. However, the restriction is useless because they are still in use in many underdeveloped nations.

1.26.3 Biochemical Effects of OCPs

The primary cause of organochlorine poisoning is central nervous system stimulation). As reported by [180], cyclodines, like the GABA antagonists endosulphan and lindane, block the release of neurotransmitters by preventing calcium ion influx and Ca- and Mg-ATPase. The etiological link between organochlorine pollution and Parkinson's disease has been revealed by epidemiological research (Table 1.14).

Table 1.14 Biochemical effects of major organochlorine pesticides

Chemical name	**Organism**	**Biochemical effects**	**References**
Chlordane	Human	Tremor, convulsions, mental confusion and incoordination	[181]
	Mice	Liver cancer, reduced fertility,	
	Seals	Trauma, meningoencephalitis, cancer	[182]
BHC and DDE	Human	Psoriasis, eczema, leucoderma, cyst in hands, itching, skin rashes	[183]
DDT	Human	Nausea, confusion, headache, dizziness, vomiting, fatigue, prickling sensation of the mouth,	[184]

		lethargy, incoordination, hyperexcitability, tremors in the extremities, anorexia, nervous tension, anemia, anxiety and muscular weakness	
	Mice	Margination and formation of lipospheres, liver changes including hepatocellular hypertrophy, liver tumors	[185]
	Birds	Eggshell thinning	[186]
	Fish	Affecting membrane function and enzymes	
	Salmons	Impaired behavioral development	
Aldrin and Dieldrin	Human	Immunological, developmental, neurotoxic, tumourigenic, reproductive, and genotoxic effects, aplastic anemia, nausea, vomiting, and muscle twitching	[187]
	Rat, Mouse, rabbit dog and guinea pig	Loss in body weight, depression, convulsions, salivation, hyperexcitability, prostration increased irritability, and death	
Endosulfan and Endosulfan sulfate	Human	Affects semen quality, sperm count, spermatogonial cells, genotoxic effects, sperm morphology and other defects in male sex hormones DNA damage and mutation. Adverse effects on humoral and cell-mediated immune system, decreases the white blood cell count and macrophage migration.	[188, 189]
	Rats	Chromosomal abnormalities, immunosuppression, neurological disorders, mental retardation, congenital birth defects, impaired learning and memory loss and glomerulonephritis	[190]
Methoxychlor	Sea Urchins	Fertilization and early development of eggs	[191, 192]
	Rats	Reduced fertility	
Endrin, Endrin aldehyde and Endrin ketone	Human	Nausea, vomiting, nonmalignant respiratory system disease, tonic-clonic contractions and seizures, cardiovascular toxicity, death	[193, 194]

	Rat	Neurological, renal, respiratory hepatic, and gastrointestinal effects, cardiac and respiratory effects, fatty degeneration, developmental effects, musculoskeletal effects and death	[195]
	Mice	Hepatic necrosis, and inflammation, decrease maternal body weight	[195, 196]
	Rabbit	Renal, respiratory hepatic, and gastrointestinal effects, cardiac and respiratory effects,	[195]
Heptachlor and Heptachlor epoxide	Human	Systemic, genotoxic, and carcinogenic effects, cancer, death, cardiovascular diseases	[197, 198]
	Rat, mice	Increased heart weight, death	[199]

1.27 Heavy Metals (HMs)

A particular group of metals and metalloids known as heavy metals have a relatively high density and are hazardous even at parts per billion (ppb) concentrations [200]. The elements that possess an atomic number larger than 20 and an atomic density greater than 5 g cm−3 are represented as heavy metals and are required to display metal qualities. Heavy metals are known as trace metals when they are found in small concentrations. There are two categories of heavy metals: essential and non-essential metals. For living things to exist and survive, trace metals (Cu Fe, Mn, and Zn) must be present in the body in minute concentrations. There are two categories of heavy metals: essential and non-essential metals. For creatures that live to exist and survive, trace metals (Co, Cu, Mn, Se, and Zn) must be present in the body in minimal concentrations. Natural sources of trace metals include soil, rocks, and water. Exposure to heavy metals, plants, animals, or humans can have extremely toxic and detrimental impacts. The main ways that heavy metals enter the environment are through the burning of fossil fuels, the use of disinfectants and antiseptics, the disposal of industrial waste, worn-out batteries, and inadequate farming methods. The production of agriculture, human health, and the purity of water are all impacted by trace heavy metals [201]. The development of industry has been a major factor in the heavy metal pollution.

Several biochemical and physiological processes have been indicated to depend on heavy metals, including copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), nickel (Ni), magnesium (Mg), molybdenum (Mo), manganese (Mn), zinc (Zn) and selenium (Se). Numerous deficiency illnesses or syndromes are brought on by an inadequate intake of certain micronutrients [202]. Phase association, temperature, adsorption, sequestration, and

other physical variables all impact their bioavailability [203]. It is also impacted by complexation kinetics, lipid solubility, octanol/water partition coefficients, and chemical variables that affect speciation at thermodynamic equilibrium. Other significant factors are biological elements including trophic relationships, species traits, and physiological or biochemical adaptation [204]. Diseases or anomalous circumstances result from either a surplus or an inadequate quantity of an essential heavy metal. However, different categories of creatures, such as plants, animals, and microbes, may have distinct essential heavy metal lists. This implies that a heavy metal can be necessary for one class of species but not necessary for another. The complicated relationships that heavy metals have with many types of organisms [205].

Other metals that are identified as non-essential because they have no known biological functions, such as aluminum (Al), arsenic (As), antinomy (Sb), bismuth (Bi), barium (Ba), cadmium (Cd), germanium (Ge), gallium (Ga), gold (Au), lead (Pb), indium (In), nickel (Ni), lithium (Li), platinum (Pt), silver (Ag), mercury (Hg), strontium (Sr), thallium (Tl), titanium (Ti), tin (Sn), tellurium (Te), uranium (U), and vanadium (V) [206]. Animals and plants employ heavy metals for physiological and biochemical processes. They are vital components of numerous essential enzymes and are involved in many different oxidation-reduction processes [202].

In several parts of the world, the consumption of meat and meat products is crucial for human nutrition because they are known to contain trace elements. Ecosystems are becoming more and more concerned about the effects of human pollution. Ecosystems on land and in water are continuously exposed to heavy metals originating from man-made pollution sources. Heavy metal contamination poses a significant risk due to its toxicity, ability to bioaccumulate, and ability to biomagnify within the food chain [207].

1.28 Sources of Heavy Metals

There are two types of heavy metal sources in the environment such as natural and anthropogenic (Figure 1.10). Natural sources of trace metals (Table 1.14) include rocks, water, and soil. Volcanic eruptions and the weathering of rocks that contain metals are two examples of the natural or geological origins of heavy metal pollution. Anthropogenic contributions (Table 1.15) to the environmental concentration of heavy metals have increased as a result of global trends in industrialization and urbanization [208]. Heavy metals in the environment are anthropogenically caused by mining, industry, and agriculture. When certain elements are extracted from their relevant ores and mined, these heavy metals are released. Through dry and wet deposition, heavy metals that are discharged into the atmosphere during mining, smelting, and other industrial activities find

their way back to the ground. Heavy metals accumulate in the environment when wastewater such as residential sewage and industrial effluents are discharged. The ignition of fossil fuels and chemical-based fertilizers both add to the anthropogenic load of heavy metals in the environment [209]. In urbanized and metropolitan areas, higher quantities of environmentally significant heavy metals have been found in the plants and soils near highways. Emissions from burning coal and other fuels are significant human-caused sources of heavy metal pollution [210]. Heavily metal-containing materials are produced by humans when fossil fuels are employed in residences, industries, and transport. One of the primary source human sources of heavy metals like Cd, Cr, Pb, and Zn is traffic from vehicles [211].

Table 1.15 Natural and anthropogenic sources of heavy metals

HMs	Natural Sources	Anthropogenic Sources	References
Cr	Ultramafic and mafic parent rocks, basaltic rocks, granite rocks, polluted soil, air, water	Refractory steel, drilling muds, leather processing and finishing, tanning industries, electroplating cleaning agents, and catalytic manufacture and in the production of chromic acid, metal planting, cooling tower, water treatment, hide tanning and until recently, wood preservation, fertilizers, smoking, and food	[212, 213]
Ni	Ultramafic and mafic parent rocks, derived from weathering of rocks and soils, wind-blown dust, volcano activities and forest fires	Diesel oil and fuel oil, combustion of coal, incineration of waste and sewage, electroplating agents	[214, 215]
Pb	Limestone and dolomites also contribute to lead content in the soil; shale, mainly black shale, is also a Lead source in the soil; argillaceous rocks, acidic igneous rocks and sedimentary rocks	Lead can be spread in the soil by the mining and smelter sites, paint, gasoline additives, smelting, automobile demolition, and pesticide application; Pb can be released in the soil from manufacture/ industrial effluent; following items containing Lead (traditional or folk remedies, candy/food packaging, batteries, leaded crystal glassware, ceramic glazes, cosmetics, solders, hair colors, jewelry, firearms and ammunition, antique fishing sinkers, tire weights, imported children's toys); burning coal and oil, domestic sewage effluent, and	[216, 217]

		burning of waste	
Cd	Cd can be naturally found in Black shale, volcanic activity also is the primary natural source of Cd in the soil and atmosphere, Parent material, marine sedimentary rocks, and phosphates	Extraction and refining of non-ferrous metals, manufacture and application of phosphate fertilizers, burning of fossil fuel, Incineration, domestic sewage, and disposal of waste, tannery industry, electroplating, spent rechargeable as well as the household batteries 6. Cd can be added to the soil by batteries, paint, stained glass, and paper ink that are common in MSW	[215-217]
As	Volcanic activity, weathering of rocks, geothermal waters, and forest fires	animal feed, glass and ceramics, herbicides, pesticides, wood preservatives, metallurgical operations, use of polluted water in food preparation and the irrigation of crops, cigarettes	[218, 219]
Mn	Combination form of manganese oxides, hydroxides, silicates and carbonate minerals (Pyrolusite, Psilomelane, Cryptomelane, Manganite, Rhodonite, Rhodochrosite, Spessartine), manganese nodules, soil, air, deposited dust, water, and food	mining, iron/steel making, ferro/silico-Mn alloy and dry alkaline battery production, and welding	[220, 221]
Co	Ultramafic and mafic parent rocks, minerals such as cobaltite, skutterudite and erythrite, copper and nickel mines. Cuprum nickel, iron, and silver mining, deep-sea nodules. food such as red meat, milk, fish, cabbage, figs and turnips	production of Co alloys, burning of coal or oil,	[222, 223]
Cu	Cu is naturally found in different parent rocks and can be abundant in basic igneous rock (basalts), The abundance of Cu also can be found naturally in shale-clay and black shale	Non-ferrous metal production, copper smelters, and steel production, municipal incinerators, residue of copper mining, sewage sludge, mineral fertilizers, and pesticides, valorizing and application of bio-solids add cupric to the soil, cupric contamination	[224-226]

		of agricultural land can also result from cupric-based fungicides	
Zn	Sedimentary rocks and acidic granitic rocks, black shale and clayey sediments, sandstone, limestone, and dolomite, food (plant and animal)	Mining activities, steel and zinc production facilities, combustion of coal and fuel, waste disposal and incineration and use of fertilizers and pesticides containing zinc	[226, 227]
Se	Weathering of selenium-containing rocks to soils and volcanic eruptions, erosion of rocks, shale soils, food (plant, animal)	Burning coal and oil, burning fossil fuels, incineration of garbage, tires and paper	[228, 229]

Figure 1.10 Natural and anthropogenic sources of heavy metals

1.29 Pathways of Entrance of HMs into Human Body

Pollution from heavy metals is one of the most important ecological issues. Arsenic (As), copper (Cu), cobalt (Co), chromium (Cr), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), manganese (Mn), selenium (Se) and zinc (Zn) are the main heavy metal contaminants found in the environment [230]. Due to their extreme toxicity, longevity, and ability for bioaccumulation, heavy metals have significant ecological implications. Heavy metals can enter the human body through three different channels, as depicted in the atmosphere, soil, and water (Figure 1.11). Fertilizer application, sewage sludge discharge, improper soil improvement, mining, and neighboring automotive exhaust all cause a lot of heavy metals to end up in agricultural soils where they are eventually adsorbed by crops and absorb into human bodies. Applying "farmyard manure" is still a common practice in most rural regions, and it is one of the major factors influencing the amount of heavy metals in the soil. As everyone is aware, mineral additions are frequently used in animal feeds to meet nutritional needs and enhance animal growth. Livestock requires minerals including Cu, Zn, Fe, Cr, Mn, and Co. Livestock are exposed to non-essential trace elements like cadmium, mercury, arsenic, lead, and other heavy metals because mineral additions used in animal feed have low purity. Animals only absorb a small portion of the heavy metals in feed; the majority are expelled as manure, which is then utilized as "farmyard manure," altering the concentration of heavy metals in soil used on farms [231]. The most serious issue with agriculture soil pollution is heavy metal contamination because to its long half-life, irreversibility, low transfer quantity, high toxicity, powerful concealment, intricate chemical makeup, and potent ecological reaction [232].

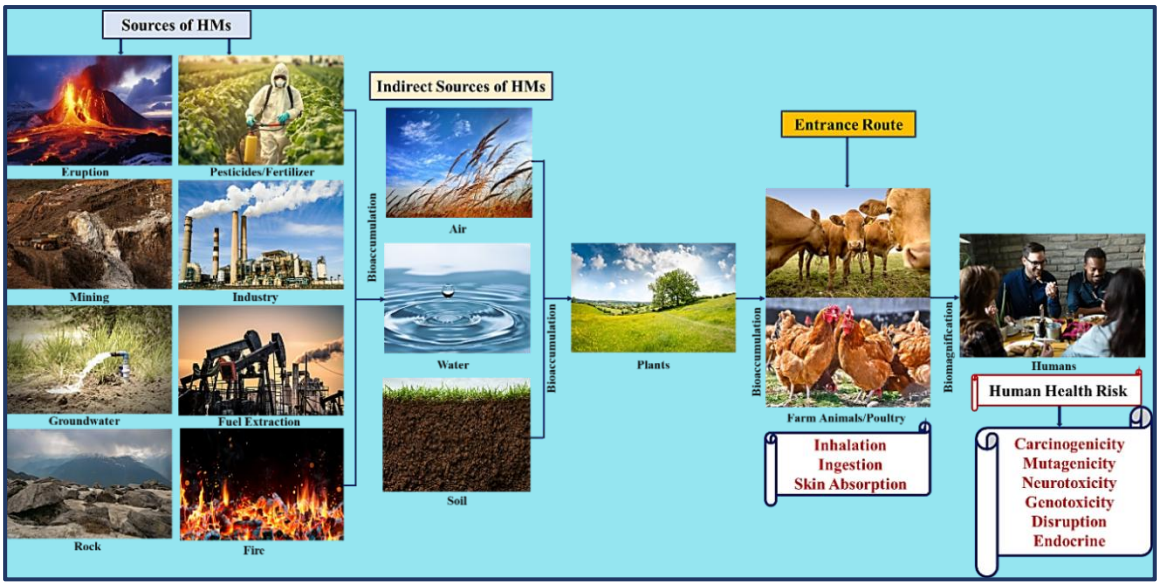


Figure 1.11 Pathways of the entrance of heavy metals into the human body (1)

Phosphate fertilizers are especially significant because of the heavy metals in commercial chemical fertilizers (Figure 1.12). The two primary routes by which hazardous heavy metals from phosphate fertilizers enter the human body are depicted below [209].

Figure 1.12 Pathways of the entrance of heavy metals into the human body (2)

1.30 Toxicity of Heavy Metals

Toxicity is the capability of a chemical molecule or compound to adversely impact vulnerable areas or cells in the human body or other live biological systems, such as plants, animals, or ecosystems, is known as chemical toxicity. The term "response" refers to the poisonous or detrimental reaction resulting from a chemical exposure. The term "dose" refers to the quantity of a substance to which the body is exposed. Dose-response relationships are used to quantify the extent of harm that biological systems may sustain. A chemical can enter the body through the gastrointestinal tract, skin, lungs, or mucous membranes in a fatal or illness-causing amount. The disposal process may become more difficult if there are several access points [233]. Depending on the dose and duration of exposure, certain heavy metals have been reported to be carcinogenic, mutagenic, and teratogenic to humans as well as other different species (Table 1.16). Heavy metals have an impact on both human health (Figure 1.13) and wildlife [234] .

Table 1.16 Adverse effects of predominant heavy metals on human

Heavy Metals	Adverse Effects	References
Chromium	Dermatitis, allergies, ulcers, respiratory, gastrointestinal, neurologic, reproductive problems and and cancers	[235, 236]
Nickel	Lung fibrous, cardiovascular difficulties, renal illnesses, and degenerative changes in heart muscle and brain, lung, liver, and kidney tissues lead to cancer of the respiratory system and lungs, which in turn leads to sarcoma of bone, connective tissue, and muscles.	[236, 237]
Lead	Lead colic, lead palsy and lead encephalopathy Aanemia (less Hb), hypertension, kidney damage, miscarriages, learning difficulties, disruption of nervous systems, fertility problems, cardiovascular problems, renal dysfunction and hepatic lesions, brain damage, infertility, intellectual disorders	[238, 239]
Cadmium	Lung, prostate, pancreas, and kidney cancers, skin irritation, organ system toxicity such as cardiovascular, reproductive, renal, skeletal, urinary, central and peripheral nervous, and respiratory systems, Parkinson, Alzheimer and Huntington's diseases	[239, 240]
Arsenic	Birth defects, gastrointestinal damage, kidney damage, skin irritation, and liver, bladder, renal and lung cancers, severe vomiting, diarrhea, death	[218, 219]
Manganese	Parkinson disease such as postural deficiencies, bradykinesia, shuffling gait, mask-like facies, micrographia, and speech difficulties, neurotoxicity, cytotoxicity, cardiovascular and pulmonary diseases; cardiotoxicity, hepatotoxicity, and increased mortality in infants	[241-243]
Cobalt	Allergic reactions, lung and heart diseases, skin inflammations and asthma, endocrine, cardiovascular, metabolic, central and peripheral nervous system, gastrointestinal and hematologic effects (acute), diseases of the pulmonary system, including occupational asthma and hard metal disease (Chronic)	[244, 245]
Copper	Can affect renal and metabolic functions, abdominal pain, hematemesis, Wilson disease, jaundice, melena, severe thirst, anorexia, vomiting associated with erosive gastropathy and diarrhea, depression, fatigue, irritability, excitation, and difficulty focusing, rhabdomyolysis, cardiac and renal failure, methemoglobinemia, intravascular	[246, 247]

	hemolysis, hepatic necrosis, Pink disease (infantile acrodynia), encephalopathy, and ultimately death	
Zinc	Nausea, vomiting, epigastric pain, lethargy, and fatigue, respiratory dysfunction, gastric distress, headaches, dizziness, and loss of appetite, copper deficiency anemia	[248, 249]
Selenium	Nail discoloration, brittleness, and loss, hair loss, nausea, vomiting,, irritability, fatigue, foul breath odor (often described as " garlic breath "), stomach discomfort, headache, and rash	[250, 251]

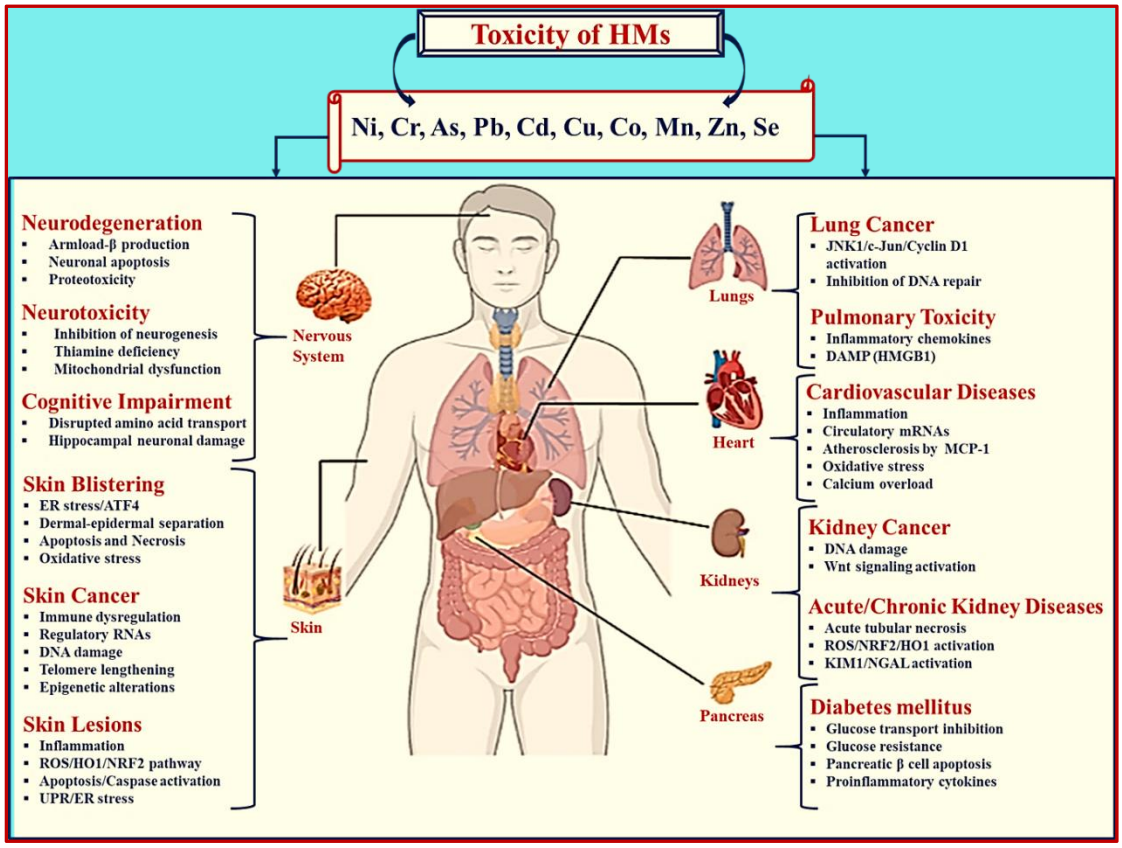


Figure 1.13 Toxicity of heavy metals

1.31 Regulations of Heavy Metal Pollution in Bangladesh

Bangladesh has conducted heavy metal risk evaluations in food items based on WHO and FAO safety guidelines [252]. Moreover, there is no integrated safety protocols have been produced for evaluating maximum tolerated limits (MTLs), even though numerous research has intended to measure the amounts of heavy metals in samples collected in Bangladesh [253]. Several government organizations, including the Bangladesh Standard and Testing Institute (BSTI) and the Bangladesh Atomic Energy Commission (BAEC), are

in charge of regulating food safety, including the contamination of food items with hazardous heavy metals.

In terms of toxicity (pesticides, heavy metals, mycotoxins, etc.) in Bangladesh's common fresh and processed foods, the Bangladesh Food Safety Authority (BFSA) was established based on the Bangladesh Food Safety Act, 2013 to certify the safety of food. Furthermore, an accredited laboratory has been organized by the National Food Safety Laboratory of Public Health Institution (PHI) to scan pesticides and heavy metals in commercially available food items [254]. The adoption of environmentally friendly and sustainable management techniques for sites contaminated by heavy metals is thought to be the main obstacle to heavy metal research in Bangladesh.

The obstacles of the inadequate proclamation of the current legislative framework due to unconsciousness among the stakeholders, and the insufficiency of a consolidated and reference analytical protocol for comparative evaluation of the toxic heavy metals in prevalent foodstuffs and environmental matrices. There are many National and global regulatory bodies and respective institutions involved in regulatory acts and legislative monitoring, and safety regulations of heavy metal contamination including United States Environmental Protection Agency (USEPA; 2010, 2015) [255, 256], World Health Organization (WHO; 1996, 2007) [257, 258], Food and Agricultural Organization (FAO/WHO; 2006, 2011) [259, 260], Joint FAO/WHO Expert Committee on Food Additives (JECFA; 2003) [261], Food Safety (Contaminants, Toxins and Harmful Residues) regulation 2017 and Food Safety Act 2013 [254].

The global demand for a sustainable supply of novel protein sources has been aided by the scarcity of protein sources and the high price of red meat. The authorities continue to be concerned about the occurrence, existence, and management of hazardous metals in meat globally. While some potentially toxic metals are naturally found in the environment, others have been introduced by mining, urbanization, industrialization, and animal husbandry. Mercury (Hg), Lead (Pb), Arsenic (As), and Cadmium (Cd) have been found in numerous studies to be the main hazardous metal pollutants found in meat from wildlife. Lead (Pb) is one of these lethal metals that naturally exists in the environment in soil, rocks, and the hydrosphere. Lead from contaminated plants may also be enriching significantly to the bioaccumulation of lead in the edible tissues of animals that graze in these areas [261].

1.32 Fatty Acids (FAs)

Fatty acid is a crucial part of lipids, the fat-soluble parts of living things found in microbes, plants, and animals [262]. A fatty acid typically consists of an even number of carbon atoms arranged in a straight chain, with hydrogen atoms at both ends and throughout the chain, and a carboxyl group (COOH) at the other end. It is an acid because of the carboxyl group (carboxylic acid). The length of the chain allows us to categorize fatty acids into four groups: short-chain (<6C), medium-chain (6C–12C), long-chain (13C–21C), and very long-chain (>22C). Most fatty acids found in nature have an even number of carbon atoms, and their chains typically range in length from 12 to 22 cations. They can also be classified as saturated (with no double bonds) or unsaturated (with one or more double bonds). Two other configurations for unsaturated fatty acids are cis, where the vinylic carbon H atoms are on the same side of the double bond, and trans, where the vinylic carbon H atoms are on the opposite side. Apart from the IUPAC nomenclature, fatty acids can also be identified by their popular names (e.g., oleic acid); by their numerical characterization (e.g., 18:0, which denotes an acid with 18 carbons and no degree of unsaturation); and, if relevant, by the location of the double bond. ω or n denotes a degree of unsaturation to the terminal methyl group, whereas Δ represents the position of the double bond to the carboxyl group [263]. The chemical structure of fatty acids investigated in present study has been shown in Figure 1.14 -1.16.

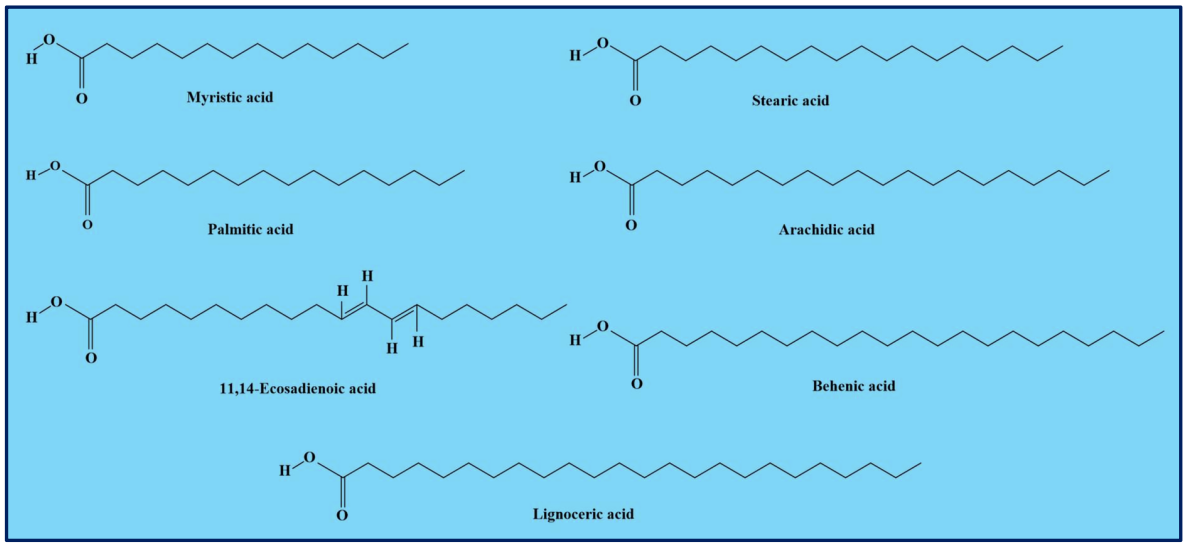


Figure 1.14 Chemical structure of analyzed fatty acid (1)

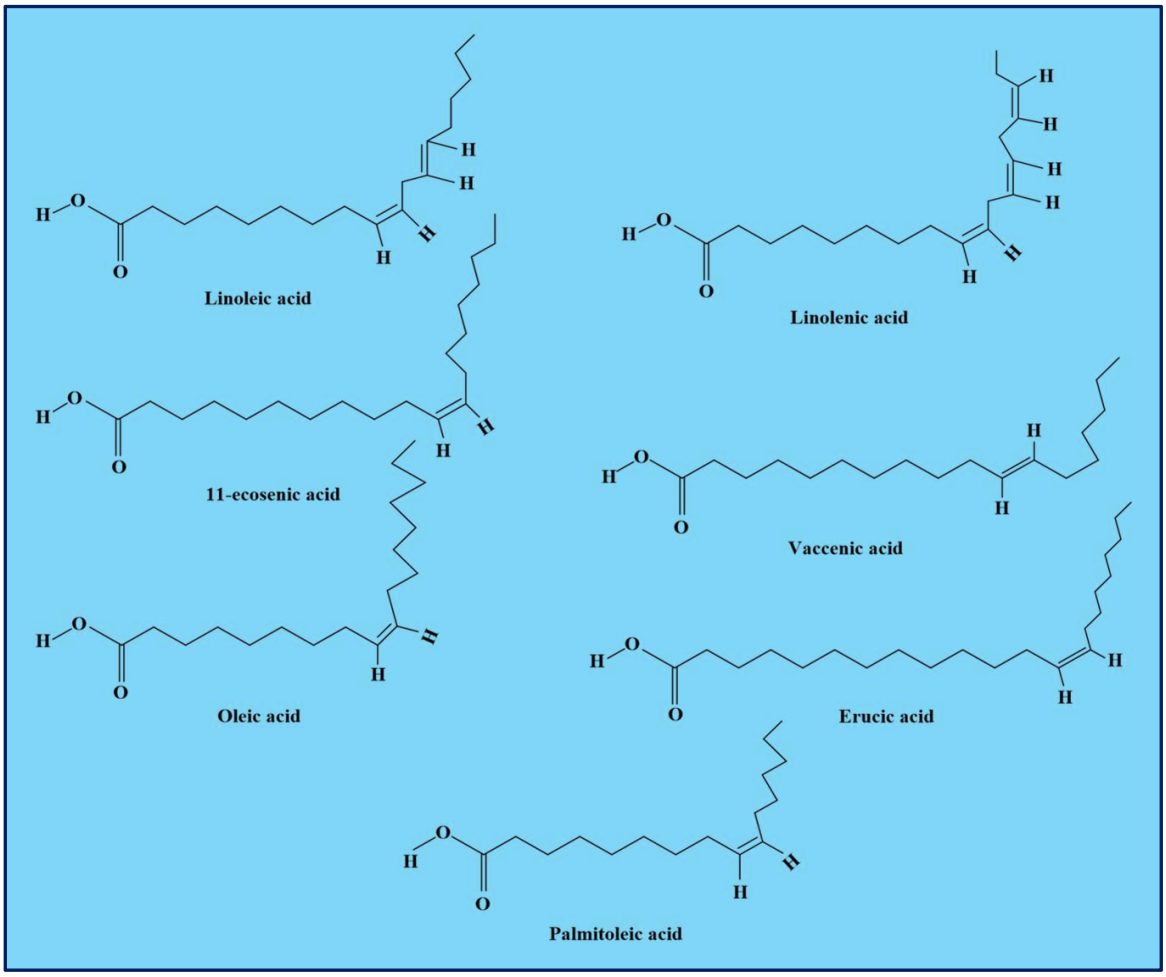


Figure 1.15 Chemical structure of analyzed fatty acid (2)

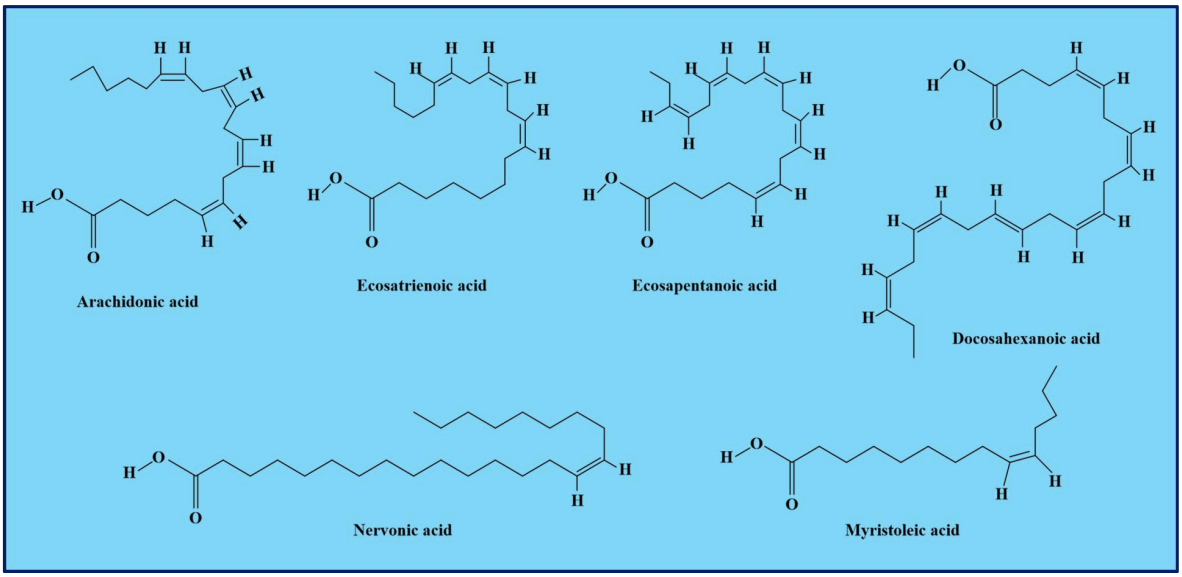


Figure 1.16 Chemical Structure of analyzed fatty acids (3)

1.33 Classification of Fatty Acids

(i) Fatty acids can be classified into three types based on their degree of saturation or unsaturation in the carbon chain [264]. Classification of fatty acids based on nomenclature and function, and the nature of chain has been shown in Table 1.17 and Figure 1.17.

Table 1.17 Classification of fatty acids on basis of degree of saturation or unsaturation in the carbon chain

Types of Fatty Acid	Characteristictics	Examples
Saturated	contains no C=C double bonds. They have the formula $CH3(CH2)_n$ COOH, for different n.	palmitic acid, myristic acid, stearic acid
Unsaturated	contains one or more C=C double bonds. Trans and cis isomers can be produced via C=C double bonds.	oleic acid (cis- Δ^9), elaidic acid (trans- Δ^9), vaccenic acid (trans-Δ^{11})
Monounsaturated	contains one C=C double bonds	linolenic acid, palmitoleic acid, erucic acid
Polyunsaturated	contains more than one C=C double bonds.	arachidonic acid, 11-14 eicosadienoic acid
Trans Fatty	created by adding hydrogen molecules to unsaturated fats to make them self-stable. Trans fats are generally unhealthy and the Food and Drug Administration (FDA) regulates their use in food products.	vaccenic acid (trans- Δ^{11}), rumenic acid (cis- Δ^9,trans-Δ^{11}), elaidic acid

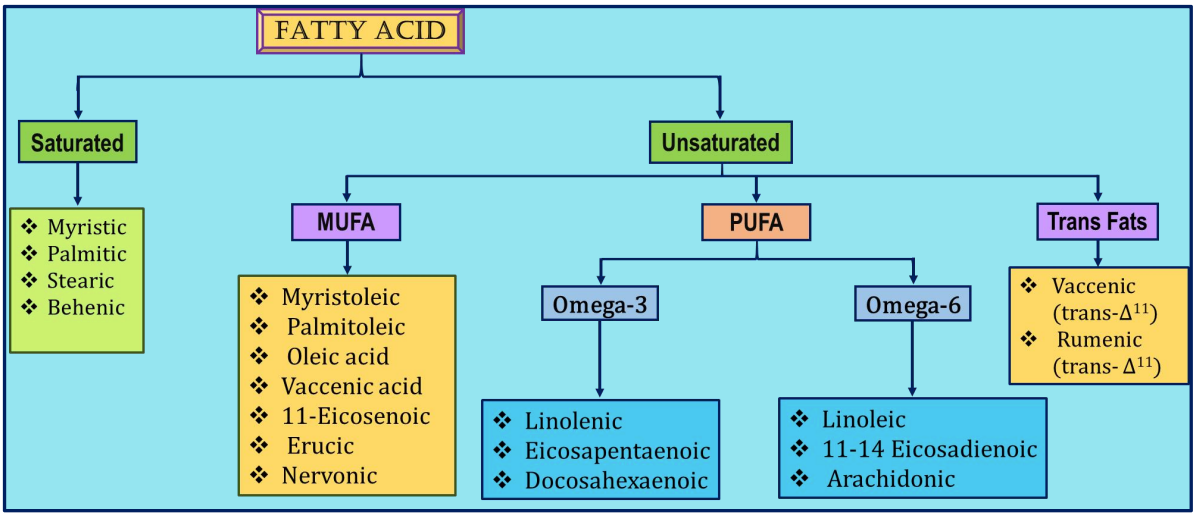


Figure 1.17 Classification of Fatty Acids based on the nature of chain

(ii) These are categorized based on the fact that animals can synthesize them or not, whose insufficiency can be compensated with food supplements [265].

Essential fatty acid: The polyunsaturated fatty acids (PUFAs) that are required for good health but cannot be produced by the body are referred to as essential fatty acids (EFA) and must be obtained from diet. EFA are classified into two families: omega-3 (ω-3) and omega-6 (ω-6).

Non-essential fatty acid: The body is capable of synthesizing most of the fatty acids it needs from food are referred to as nonessential fatty acids.

(iii) Fatty acids can be classified based on their carbon atoms [265]

Short-chain fatty acids: less than 6 carbon atoms

Medium-chain fatty acids: 8 to 12 carbon atoms.

Long-chain fatty acids: 13 to 21carbon atoms

Very long-chain fatty acids: 22 carbon atoms and up.

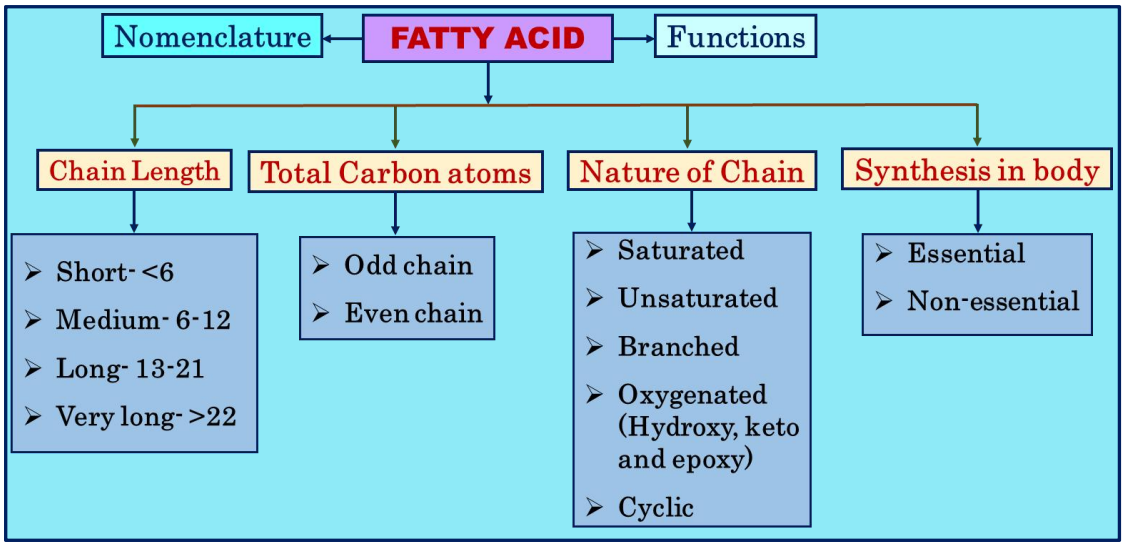


Figure 1.18 Classification of Fatty Acids based on their nomenclature and function

1.34 Properties of Fatty Acids

(i) The pKa values of fatty acids show a little variation in their acidity.

(ii) Esterification and acid-base reactions are two processes fatty acids go through like other carboxylic acids.
(iii) Longer-chain fatty acids do not significantly affect the pH of an aqueous solution because their solubility in water decreases with increasing the chain.

(iv) Fatty acid conjugate bases, such as oleate, exist at pH values close to neutral.

(v) The chemical transformation of unsaturated fatty acids leaduto-os to axidation. The presence of trace metals accelerates the process and needs oxygen consumption or air.

(vi) Ozonolysis- Unsaturated fatty acids can be broken down by ozone. The production of azelaic acid from oleic acid involves this process [264].

1.35 Production of Fatty Acids

Industrial Process

(i) The process of hydrolyzing triglycerides and extracting glycerol is the standard industrial method for producing fatty acids. Phospholipids are an additional source. Certain fatty acids are produced by hydrocarboxylation of alkenes.

(ii) The production of hyper-oxygenated fatty acids occurs during specific industrial processes used to make topical skin creams. The process involves adding or completely submerging peroxides into fatty acid esters while gaseous oxygen bubbles at regulated temperatures and is exposed to UV light [265].

In Animals

Fatty acids are primarily formed from carbohydrates in the liver, adipose tissue, and the mammary glands of animals during lactation.

1.36 Functions of Fatty Acids

(i) **Fatty acid as a modulator of membrane properties**:

a) Bacteria and prokaryotes modify their fatty acid composition to preserve membrane fluidity when temperatures fluctuate.
b) The phospholipid bilayer that makes up the eukaryotic cell membrane regulates cellular communications, manages the movement of chemicals to and from the cell, and shields the cell from the external environment [266].

(ii) **Materials for energy storage and supply**:

a) Esters, wax, polyhydroxyalkanoates (PHA) and triglycerols (TAG) are neutral storage lipids made from fatty acids.
   Normal brain and retinal growth and function depend on omega-3 polyunsaturated fatty acids.
b) Docosahexaenoic acid (DHA) participates in anti-inflammatory signaling, modifies synaptic activity and functions as a neurotrophic modulator [266].

(iii) **Biological markers**:

a) There is structural variety among fatty acids, and certain fatty acids are unique to both eukaryotic cells and certain taxa of bacteria.
b) Therefore, a suitable biomarker is the presence of specific fatty acids and their ratio.
c) Food web energy fluxes are monitored using the fatty acid markers.
d) A prokaryotic organism can be recognized by its fingerprint of fatty acids [266].

1.37 Dietary Sources of Fatty Acids (Beef and Chicken meat)

Consuming meat has many different negative effects on one's health because it is a very complicated matrix. It is high in fats with a variety of fatty acid compositions and proteins with necessary amino acids [267]. Therefore, meat provides a healthy source of energy, unsaturated fatty acids (UFA), high-quality proteins, heme iron, zinc, and vitamins. Beef fat also contains saturated fatty acids (SFA), which may have an adverse impact on human health. Red meat consumption has been linked in recent years to an increased risk of obesity, cancer, and coronary heart disease, however it should be noted that epidemiological study findings are not always reliable [268]. Animal breeds and species revealed differences in the fatty acid composition [269], as did the kind of muscle and meat cut [270], the animal's food [271], the amount of fat [272], and sample handling, particularly heat treatment [270]. Globally, chicken and beef are the most common meat varieties [273]. For chicken breast and beef ribeye, the fat content in these unprocessed meat varieties ranges widely, from 2.62 g to 22.07 g/100 g, respectively [274]. Out of the two varieties of meat, beef had the highest percentage of SFA, accounting for roughly 46–47% of total fatty acids [275] and chicken meat, particularly breast, had the lowest level of SFA, with a percentage of 27–30%. From a nutritional perspective, poultry is very beneficial since it has the largest percentage of UFA, particularly PUFA (polyunsaturated fatty acids), which is found in chicken flesh (56–61% of total fatty acids, including 26–30% of PUFA) [276]. Compared to chicken meat, beef have a substantially lower amount of UFA (particularly PUFA) and a lower PUFA/UFA index value. However, depending on the meat cut, the MUFA (monounsaturated fatty acid) share in both forms of meat is relatively high, ranging from 39–48% for beef [275]. PUFA is good for human health, but it also renders meat very prone to oxidation, which lowers the product's quality and ultimately compromises the health of the consumers. The rate of lipid oxidative stability is influenced by heat or oxygen exposure, as well as the degree of unsaturation of fatty acids. This can lead to a change in the composition of fatty acids, with a decrease in UFA content and an increase in SFA level. Dominguez et al. (2019) [277] noted in their review that the unsaturation of fatty acids rather than the total fat content of meat was the primary cause of meat's sensitivity to the oxidation process. The food industry's task is to preserve meat quality in light of UFA stability over the course of shelf life, especially under aerobic conditions. To achieve this, a variety of technological techniques were used, including packaging in a vacuum or in a modified atmosphere [278].

1.38 Correlation between residual chemical contaminants (antibiotics, organochlorine pesticides and heavy metals) and total fat/ composition of fatty acids

The fat and fatty acid composition of organisms, including humans, can be greatly impacted by residual chemical pollutants such as antibiotics, organochlorine pesticides and heavy metals. These pollutants have the potential to cause health problems, change lipid metabolism, and impact the types and levels of fatty acids [279]. Adipose tissue fat deposition is encouraged by certain antibiotics, while the expression of genes related to lipid metabolism may be impacted by others, which could result in changes to the distribution and storage of fat. The composition of the gut microbiota, which is essential for lipid metabolism and nutritional absorption, can potentially be impacted by antibiotics [280, 281]. OCPs have a tendency to build up in adipose tissues since they are lipophilic, which might cause problems with lipid metabolism. They have the ability to change the fatty acid composition of tissues, which may have an impact on cellular activity and membrane fluidity. Studies indicate that OCPs have the ability to alter the amounts of different fatty acids, particularly those involved in energy metabolism, and to cause lipid peroxidation [282]. The composition of fatty acids can potentially be affected by heavy metals. Heavy metal exposure has been shown to raise saturated fatty acid content while lowering unsaturated fatty acid concentration. Furthermore, lipid metabolism pathways may be impacted by heavy metals, which may result in changes to fat storage and deposition. Research indicates that fatty acids may have an impact on heavy metal adsorption, underscoring the intricate relationships between these compounds [283, 284]. Many of these contaminants, especially heavy metals and OCPs, have a tendency to bioaccumulate in the food chain, with concentrations rising with higher trophic levels. This implies that higher concentrations of these contaminants may be present in species at the top of the food chain, such as humans. These contaminants can alter the content of fat and fatty acids, which can have a number of adverse health implications. These consist of: [280-285]

(i) **Cardiovascular Diseases**: Switch in the ratio of saturated to unsaturated fats, in particular, can have an impact on cardiovascular health.

(ii) **Metabolic Disorders**: Obesity and type 2 diabetes are two metabolic disorders that can be exacerbated by disturbances in lipid metabolism.

(iii) **Effects on the Nervous System**: Long-term exposure to some contaminants can harm the nervous system and cause neurological conditions.

(iv) **Elevated Cancer chance**: Certain contaminants are recognized as carcinogens and can raise the chance of developing specific types of cancer.

(v) **Reproductive Obstacles**: The health of the reproductive system may be impacted by particular contaminants.

(vi) **Hormonal Imbalances**: Hormonal imbalances may result from certain contaminants interfering with endocrine function.

There are serious health consequences associated with the complicated relationship between the content of total fat and fatty acids and residual chemical pollutants. To reduce human exposure to these pollutants and safeguard the public's health, it is essential to comprehend these interactions [286].

1.39 Objectives of The Research

Bangladesh is primarily an agricultural nation, with a population of 170 million spread over 56,000 square miles. The economy largely relies on agriculture, while small industries such as garments and leather are growing and making contributions to the country's Gross Domestic Product (GDP) [1]. The nation is also susceptible to natural disasters, including cyclones, tornadoes, floods, monsoons, and heavy rainfall, making it vulnerable to climate change. The amount of arable land is diminishing daily due to these natural calamities and the ongoing process of urbanization [2]. To satisfy the food demand of such a large population, a significant amount of pesticides and fertilizers are applied to agricultural crops. Chemicals, including antibiotics, are also employed in the farming of beef cattle, broiler chickens, and aquaculture [29, 30]. The government is currently ensuring food security while also prioritizing food safety, quality, and nutritional value. Animal meat plays a crucial role in fulfilling human nutritional requirements. Meat is rich in protein and provides a complete set of essential amino acids. Various sources of meat exist, including animals (cow, buffalo, goat, lamb, pig) and poultry (hen, duck, quail, pigeon, turkey) across the globe, including in Bangladesh. The livestock population in Bangladesh is estimated to consist of 25.7 million cattle, 0.83 million buffaloes, 14.8 million goats, 1.9 million sheep, 118.7 million chickens, and 34.1 million ducks according to the 2021 DLS Report from the Department of Livestock Services (DLS). Bangladesh produces 87.10 Lakh Metric Tons of meat yearly, surpassing the demand of 76.08 Lakh Metric Tons, with beef making up the largest portion (49.8% of total meat) according to the Livestock Economy at a Glance 2022-23 from the Department of Livestock Services (DLS). Poultry comprises about 20% of protein consumption and 35.25% of overall meat supply in Bangladesh, as reported by DLS in 2021. The daily consumption rates for beef are 14.7g/day body weight in urban areas and 10.2g/day in rural regions, while for chicken, the rates are 33.1g/day and 17.2g/day in urban and rural areas, respectively, with other meats at 1.0g and 0.9g/day body weight, based on the BBS report, 2022 [292]. It is widely

recognized that beef and chicken are highly favored and appetizing non-vegetarian foods among the people of Bangladesh. Therefore, this research focuses on beef and chicken due to their availability, affordability, popularity, and high consumption rates among Bangladeshi consumers.

The main sources of protein are animal-derived foods such as meat, milk, and eggs. Meat from livestock is considered one of the primary food sources globally and provides a rich supply of protein, minerals, and various nutrients [29]. These food items may contain synthetic chemicals commonly found in everyday life. This is primarily a consequence of intensified production practices, which routinely involve the use of pesticides, antibiotics, and other inputs. Ensuring high-quality food for human consumption hinges on the quality of inputs used in the production of animals. Antibiotics are applied in cattle and poultry farms to manage bacterial infections and promote growth on a global scale, including in Bangladesh. The reckless and unregulated use of antibiotics can result in their accumulation in edible tissues like muscles, liver, and kidneys, potentially entering the human body. The emergence of antibiotic resistance can occur due to the proliferation of susceptible bacteria, complicating the treatment of bacterial infections [32]. Furthermore, human consumption of food containing antibiotics has been linked to various adverse health effects, such as hypersensitivity, gastrointestinal disorders, neurological conditions, and tissue damage [42, 43]. Pesticides are often applied indiscriminately, leading to numerous negative health impacts, including acute poisoning and chronic illnesses like neurotoxicity, Parkinson's disease, Alzheimer's disease, leukemia, infertility, and diabetes [161]. Antimicrobial substances, heavy metals, chemicals, and pesticide residues have all been identified as health risks in animal-derived food products and the environment. Contaminants may accumulate in fatty tissues, which can alter the fatty acid profile of meat [279]. While the residues of veterinary drugs in food are the main concern for many consumers, there are several other potential environmental contaminants that could also pollute products from various sources. These contaminants include pesticides, persistent organic pollutants, toxic heavy metals, and other newly identified hazardous substances. Certain chemicals found in trace amounts in beef and chicken meat can have serious harmful effects, displaying properties that disrupt endocrine function, cause cancer, are toxic to the immune system, are genotoxic, or are teratogenic [47]. Food safety is a critical issue in Bangladesh. The Bangladesh Food Safety Authority has established several regulations to ensure food safety based on the Codex Alimentarius Commission guidelines [4]. Nevertheless, the detrimental effects of residual pesticides and antibiotics, along with excessive heavy metal levels in beef and chicken meat, remain a significant concern in Bangladesh. Findings from this study can contribute to establishing maximum residue levels (MRL) and developing regulations to ensure food safety in Bangladesh. However,

there is insufficient data regarding the prevalence of chemical contaminants in beef and broiler chicken meat. As a result, it is essential to examine the current levels of chemical contaminants (residual antibiotics, organochlorine pesticides and heavy metals) and analyze the fatty acid composition in beef and broiler chicken meat samples, as well as assess antibiotic resistance and its harmful effects on public health. The goals of this research are:

1. To develop and validate extraction and reproducible analytical methods for analyzing various classes of antibiotics (such as tetracyclines, β-lactam class antibiotics like amoxicillin, and macrolides like patulin) in beef and chicken meat and liver samples using a reversed-phase high performance liquid chromatography (HPLC) paired with a photo diode array detector (PDA).

2. To identify and quantify the residual levels of tetracycline, chlortetracycline hydrochloride, oxytetracycline hydrochloride, amoxicillin, and patulin in beef and chicken meat and liver samples.

3. To assess the fluctuations of residual antibiotics across four different matrices and evaluate the potential health risks for humans in Bangladesh while comparing findings with international research.

4. To validate an optimized method (in both extraction and analytical phases) for detecting and quantifying residual heavy metals in four distinct matrices using inductively coupled plasma mass spectrometry (ICP-MS), while assessing the potential health risks for consumers in Bangladesh.

5. To validate a developed method (encompassing both extraction and analytical methods) for detecting and quantifying residual organochlorine pesticides in beef and chicken meat and liver samples via gas chromatography with an electron capture detector (GC-ECD), and to perform a health risk assessment for consumers in Bangladesh.

6. To develop and validate a method (extraction and analytical methods) for analyzing and quantifying fat content and fatty acid composition in beef and chicken meat and liver samples using gas chromatography with a flame ionization detector (GC-FID).

7. To determine the correlation between residual chemical contaminants (antibiotics, organochlorine pesticides and heavy metals), and total fat and composition of fatty acids.

Chapter 2

EXPERIMENTAL

2. EXPERIMENTAL

2.1 Materials and Methods

2.1.1 Chemicals and Solvents

Analytical grade anhydrous magnesium sulfate (Scharlaw, Spain), sodium chloride (Darmstadt, Germany), oxalic acid, extra pure (Scharlaw, Spain) disodium hydrogen phosphate dihydrate (Scharlaw, Spain), anhydrous sodium acetate (Sigma-Aldrich, Japan) anhydrous sodium sulfate, were used for this analysis. Primary secondary amine (PSA) and octadecyl silica (C-18), a bulk sorbent from Agilent USA, were used in the cleaning-up steps. Analytical grade acetone, methanol, dichloromethane, ultra-pure n-hexane from Merk, Germany, and HPLC grade acetonitrile (ACN) and methanol were obtained from Sigma-Aldrich, Germany. HPLC-grade water (DI water) was used as a constituent of the mobile phase, free from cations, anions, and hydrocarbons. Hydrogen peroxide (30 % H_2O_2; EMSURE, Germany) and nitric acid (69%, Sigma-Aldrich; USA) were used to digest meat and liver samples. Acetic acid (glacial 98 %) and phosphoric acid were used to fix the pH of the buffer solution. Hydrochloric acid (HCl, fuming, ≥ 37%) was purchased from Sigma-Aldrich, Germany and used to saponify meat samples to analyze fatty acids.

2.1.2 Certified Standards

The analytical standards of tetracycline (99 % purity), oxytetracycline hydrochloride (99 % purity), chlortetracycline hydrochloride (99 % purity), amoxicillin (99 % pure), and patulin were purchased from Sigma Aldrich and Dr. Ehrenstofer, Germany. The analytical standards of the Organochlorine Pesticides (OCPs mix, 20-compounds) and Fatty Acid Metyle Ester (FAME mix, 20-compounds, 100 mg) were purchased from Restek, USA, respectively.

2.1.3 Glass and Plastic Apparatus

Pipettes and volumetric flasks used in the current experiment were calibrated by Bangladesh Standard and Testing Institute (BSTI). Mobile phase vacuum filtration apparatus set (1000 mL), LC vial, GC vial, round bottom flask, Teflon tube (50 ml), graduated test tube, funnel, separating funnel, conical flask, beaker, plastic syringe, nylon filter (Restek, USA) and micropipette were also used in different steps of the analysis.

2.1.4 Minor Equipment

A four-digit analytical balance (Shimadzu, Japan), a digital balance (Model: KERN 440, max 400 g, USA), a water purification system (Automatic Plus 1+2 GRUF, Wasserlab, Spain), rotary vacuum evaporators (Model: EV400H-V, 20-260 rpm, Lab Tech, USA, and Heidolph, Germany), a vortex mixer (Model: VM-2000-C, 3000 rpm, Taiwan), an oven (Salvis G-1020, Bergkirchen, Germany), a sonicator (Hwashin Tech, Korea), a freeze dryer (Labconco, Kansas, USA), a digital balance (Model: KERN 440, max 400 g, USA), and a hot plate (Environmental Express, USA) were employed in the experiment. Two centrifuges (Cowbell, Narang Scientific Industries, Haryana, India) (Model: SIGMA 2-16 P, Benchtop, 15,000 rpm, Germany) for centrifugation and a pH meter (Hanna Instrument, Italy) to measure pH were utilized in this experiment.

2.1.4.1 Carbolite Furnace

Different inorganic compounds like anhydrous sodium sulfate are activated by heating. All glassware was heated in a carbolite furnace (Model: GSM 11/8 Hope Valley, S336RB, England) for about 3 hours (Figure 2.1).

Figure 2.1 Carbolite Furnace

2.1.4.2 Analytical Balance

Analytical balance (FR-200, NDO-450 ND, Japan) was used to take all measurements. The measuring pan of an analytical balance is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation (Figure 2.2).

Figure 2.2 Analytical balance

2.1.4.3 Oven

Different inorganic compounds like anhydrous sodium sulfate are activated by heating at above 100°C temperature. Glass apparatus was heated at above 50°C to clean these properly. All glassware was heated in Oven at 50ºC for about 3 hours (Figure 2.3).

Figure 2.3 Oven

2.1.4.4 Homogenizer

After cleaning and chopping the meat samples, they were required to make them homogenous. Meat samples were homogenized and blended using a normal kitchen blender (Miyako Chopper, Japan), (Figure 2.4).

Figure 2.4 Homogenizer

2.1.4.5 Vortex Mixer

All shaking was carried out with the help of a vortex machine (Model: VM-2000-C, 3000 rpm, Taiwan), (Figure 2.5).

Figure 2.5 Vortex Mixer

2.1.4.6 Centrifuge Machine

The samples during the extractions were centrifuged using a benchtop centrifuge by Sigma, Germany (Model: Sigma 2-16P), (Figure 2.6).

Figure 2.6 Centrifuge (15000 rpm)

2.1.4.7 Electric Centrifuge Machine

The supernatants (organic layer) were centrifuged at 2000 rpm for clean-up during the extractions (Figure 2.7).

Figure 2.7 Centrifuge (Cowbell, 8 Tests, 2000 rpm)

2.1.4.8 Rotary Vacuum Evaporator

A rotary vacuum evaporator (Model: EV400H-V, 20-260 rpm, Lab Tech, USA) evaporated the solvent from the solution under reduced pressure. Since many organic compounds are volatile and easily decomposed at high temperatures, hence the evaporation was carried out at 40 °C under reduced pressure. It was ensured that the water bath temperature did not rise over 40 °C (Figure 2.8).

Figure 2.8 Rotary Vacuum Evaporator

2.1.4.9 Nitrogen Evaporator

The cleaned-up extracts of pesticides evaporated under a nitrogen gas stream using a N_2-evaporator (Figure 2.9).

Figure 2.9 Nitrogen Evaporator

2.1.4.10 Ultrasonic Bath

Sonication is the act of applying sound energy to agitate particles in a sample, for various purposes. It is used for the production of nanoparticles such as nanoemulsion, nanocrystals, liposomes, and wax. It is also used to speed dissolution by breaking intermolecular interactions and to provide the energy for certain reactions to proceed (Figure 2.10). Again, it is used to remove dissolved gases from liquid (degassing) by sonicating the liquid.

Figure 2.10 Ultrasonic Bath

2.1.4.11 Reflux System

Reflux system is used for the condensation of vapors which then return to the system from which it is vapored. It is used to conduct a reaction at a specific temperature without the loss of solvent. The main purpose of refluxing a solution is to heat a solution in a controlled manner at a constant temperature (Figure 2.11).

Figure 2.11 Reflux System

2.1.4.12 Freeze Dryer

The samples are dried by freeze dryer (Labconco, Kansas City, USA). All freeze-drying was done to remove water and traces of organic solvents. The aqueous samples were first frozen in round-bottomed flasks in a methanol refrigerator at -30 to -35℃ and finally, the materials were subjected to freeze-drying operation (Figure 2.12).

Figure 2.12 Freeze Dryer

2.1.4.13 pH Meter

pH meter was used for the measurement of pH (Figure 2.13).

Figure 2.13 pH Meter

2.1.4.14 Hot Plate

All meat samples were digested on a hot plate in a fume hood chamber for the analysis of heavy metals (Figure 2.14).

Figure 2.14 Hot Plate

2.1.4.15 Water Purification System

Deionized (DI) water was obtained from a water purification system for the preparation of meat samples for the analysis of residual antibiotics and pesticides. It was also used for preparing solvent for HPLC (Figure 2.15).

Figure 2.15 Water Purification System

2.1.4.16 Solvent Filtration Apparatus

All solvent was filtered by using the solvent filtration apparatus (Model: AH0-3315, FilterSys TM, 47 mm, 1000 mL funnel w/4 L vacuum flask, Phenomenex, UK) for the HPLC (Figure 2.16).

Figure 2.16 Solvent Filtration Apparatus

2.1.5 Major Equipment

2.1.5.1 High-Performance Liquid Chromatography

A reversed-phase High Performance Liquid Chromatography (HPLC; RF 1200, Prominence, Shimadzu, Kyoto, Japan), consisting of dual pumps, degassing unit (DGU-20A5R, USA), a rheodyne manual sample injector (20 µL), and a photodiode-array detector (SPD-M20A, 230 V) in the range of 200-800 nm (wavelength). The LC system was fitted with a C-18 column (Phenominex, Luna C-18, 250 × 4.60 mm i.d., particle size: 5 μm) was used to analyze antibiotics (Figure 2.17).

Figure 2.17 HPLC-PDA (Prominence)

A reversed Phase High-Performance Liquid Chromatography (Model: Prominence-i, LC-2030C 3D Plus, Shimadzu, Kyoto, Japan), consisting of dual pumps, an auto-injector, an auto-sampler and a photodiode-array detector (PDA) in the range of 200-800 nm (wavelength). The LC system was fitted with a C-18 column (Shimadzu, Shim-pack GIST C-18, 250 × 4.60 mm i.d., particle size: 5 μm). This instrument was used to analyze antibiotics (Figure 2.18).

Figure 2.18 RP-HPLC (Prominence-i, LC-2030C 3D Plus)

2.1.5.2 Gas Chromatography with Electron Capture Detector

A Gas chromatography with an electron capture detector (ECD) was used to analyze organochlorine pesticides. A GC-ECD (Shimadzu, Nexis GC-2030), with an auto-injector and auto-sampler was used for a particular analysis. A high-polarity SH-CLP II (5 % Diphenyl – 95 % Dimethylpolysiloxane, low bleed, 30 m x 0.32 mm x 0.25 µm, Shimadzu, Japan) fused silica column was used for chromatographic determination of the OCPs (Figure 2.19).

Figure 2.19 Shimadzu GC-ECD 2030

2.1.5.3 Gas Chromatography with Flame Ionization Detector

The fatty acid methyl esters (FAME) were analyzed using a Shimadzu GC-2030 Plus, Nexis gas chromatography with a flame ionization detector unit (Shimadzu, Kyoto, Japan). A high-polarity SH-FAME (Crossbond Carbowax Polyethylene Glycol, 30 m x 0.32 mm x 0.25 µm, Shimadzu, USA) fused silica column was used for chromatographic determination of the FAMEs (Figure 2.20).

Figure 2.20 Shimadzu GC-FID (Nexis GC-2030)

2.1.5.4 Inductively Coupled Plasma Mass Spectrometry

Heavy Metals were analyzed by an Inductively Coupled Plasma Mass Spectrometry (NexION 2000 ICP-MS, Perkin Elmer, USA) from the Central Laboratory, Bangladesh Council of Scientific and Industrial Research Laboratory (BCSIR), (Figure 2.21).

Figure 2.21 Inductively Coupled Plasma Mass Spectrometer (NexION 2000)

2.2 Treatment of Chemicals and Apparatus

2.2.1 Cleaning of Glass Apparatus

All necessary glass equipment was washed with soap, rinsed with distilled water, and then sterilized with acetone. Every piece of glassware was baked for the entire night at 300ºC, cooled, and then covered with aluminum foil for storage. All of the glass equipment was cleaned with the appropriate solvent before use.

2.2.2 Activation of Anhydrous Magnesium sulfate and Sodium sulfate

Magnesium and sodium sulfate were anhydrous ingredients that were stored in a desiccator until they were needed after being activated by heating them to 300ºC for eight hours in a furnace.

2.2.3 Evaporation

All evaporations were performed using a rotary vacuum evaporator with a water bath at a temperature of a maximum of exceed 40ºC under reduced pressure. Small volumes of organic solvents were evaporated while the test tube was in a block heater using a nitrogen gas stream.

2.3 Method Validation

2.3.1 Preparation of Primary Standard Solutions

The primary standard solutions were prepared in 10 mL volumetric flask by dissolving separately certified reference standards (10 mg) of tetracycline antibiotics (oxytetracycline, tetracycline, chlortetracycline) in HPLC grade 10 mL methanol and beta-lactam antibiotic, amoxicillin in HPLC grade methanol to prepare 1000 mg/L stock solution, respectively. Patulin (5 mg) was dissolved in ethyl acetate to prepare a 100 mg/L stock solution. Pesticide standard (mix-20 component, 100 mg) was dissolved in 100 mg n-hexane and fatty acid methyl ester standard (mix-20 component, 100 mg) in 100 mL HPLC grade dichloromethane to make 1000 mg/L standard solution, respectively. Every prepared solution had a label with the name of the standard, the concentration, and the preparation date. Permanent blank ink was used to mark the meniscus of the solutions, which were then kept in a refrigerator at -20°C away from the sample storage area until they were needed again.

2.3.2 Preparation of Secondary and Working Solutions

The primary standard solutions were taken from the refrigerator to reach room temperature and checked the meniscus of the layer. Then 1.0 mL of the primary stock solution was diluted with 9.0 mL of corresponding solvent in a 10 mL volumetric flask to prepare 100 µg/L and 10 µg/L as secondary standard solutions. Then, 1 µg/L solution was prepared from 10 µg/L These solutions were labeled indicating the name of the standard, concentration and date of preparation. The meniscuses of the solutions were marked with permanent ink and stored in a refrigerator (-20°C) away from the residual antibiotic and pesticide laboratory. Working solutions were made from secondary standard solutions by serially diluting the corresponding diluent for each type of standard daily.

2.3.3 Calibration Curves for Standards

The HPLC and GC were conditioned until a smooth baseline was obtained. To condition the HPLC system, the mobile phase (acetonitrile: water) was passed through it in several ratios until a smooth baseline was achieved. The instruments were conditioned for approximately 30 minutes at particular parameters of a documented procedure to equilibrate the column. The instruments were conditioned at specific parameters of a reported method to equilibrate the column, for about half an hour. The calibration curves of the Tetracyclines (TCs) were prepared by diluted standard solutions of 50, 100, 150, 200,

250, and 300 µg/L from the middle standard solution 1 mg/L. Then serially diluted standards from lower to higher concentrations were injected gradually into RP-HPLC coupled with a PDA detector to get the area. The calibration curve was then obtained by plotting the areas of the standards against their concentrations (µg/L). The specifics of the correlation coefficients (r^2) for the linearity demonstrate that the calibration curves were linear across the range of the confirmed concentrations. The Codex guidelines recommended an r^2 value of 0.95. The calibration curves were made using Microsoft Excel 2010 software.

2.3.4 Selectivity, Sensitivity and Linearity

In order to evaluate selectivity (or specificity), standard mixtures of TCs, blank matrices, and blank matrices spiked with the mixture of TCs simultaneously were analyzed, and their retention times were recorded.

The limits of detection (LODs) and limits of quantification (LOQs) for each TC standard in the matrix were determined in order to evaluate the instruments' sensitivity.

A standard mixture was injected into HPLC-PDA at five to six distinct concentration levels, covering the anticipated range of TCs that might be present in the samples, in order to generate calibration curves for each TCs and assess linearity.

2.3.5 Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is the smallest concentration from which the presence of an analyte can be deduced, and the limit of quantification (LOQ) is the lowest concentration from which it is possible to quantify the analyte with a reasonable degree of statistical certainty [287, 288]. The LOD was determined by injecting serially diluted mixtures of standard TC solution in HPLC-PDA. For LOD, the peak area of each standard was considered 3 times higher than the baseline noise i.e., the signal-to-noise ratio was 3:1. In contrast, the LOQ were determined with a signal-to-noise ratio of ten.

2.3.6 Identification and quantification by RP-HPLC/GC

A particular substance in a sample and its reference standard solution both produce peaks in GC and HPLC at retention periods that are comparable when analytical circumstances are the same. In addition to cleaned sample extracts, the certified standard solutions were injected into GC or HPLC instruments using the same parameters. The residue found in the

samples was identified by comparing the retention times of the various peaks in the sample with the retention times of the approved standard compounds. The standard calibration curve was used to determine the concentration of the associated analytes for quantification, considering that the peak area fell in the middle of the curve (Equation 1).

$$\mathbf{Y = mX \pm C \ldots \ldots \ldots \ldots \ldots (1)}$$

Here,

y = Peak area

m = Slope of the calibration curve

x = Concentration

c = Intercept

$$\mathbf{Concentration = \frac{(Peak\ Area - intercept) \times DF}{Slope \times Sample\ Weight} \ldots \ldots \ldots \ldots \ldots (2)}$$

Here, DF= Dilution factor

2.3.7 Control

To ensure precise recovery of experimental findings, untreated or control samples were employed. These samples had previously been confirmed to be free from any antibiotic or pesticide residues. Moreover, to assess the matrix effect of the analytical technique, three control samples were spiked with a known amount of antibiotics and pesticides. These samples subsequently underwent the relevant extraction and clean-up procedures. Additionally, to ensure logical consistency in the analysis, a reagent blank was conducted using the identical extraction method and clean-up procedure. This included using only solvents and reagents without any actual samples.

2.3.8 Recovery Experiment

The samples were prepared in triplicate at two or three different concentration levels to conduct recovery experiments on clean control samples. To enable the antibiotics to penetrate the matrix, the spiked samples were permitted to equilibrate for 30 minutes before extraction. After following the relevant extraction and cleanup procedures, the processed spiked samples were analyzed. The recovery for each analyte was determined using the recovery (%) formula provided below.

$$\textbf{Recovery } \% = \frac{C_{\text{spiked std.}} - C_{\text{unspiked std.}}}{C_{\text{added std.}}} \times 100\% \ldots\ldots\ldots\ldots\ldots (3)$$

2.3.9 Dietary Exposure Assessment

The estimated daily intake (EDI), as calculated by Moudgil et al. 2019 [289], was used to determine the daily exposure to OTC, TC, CTC, amoxicillin and patulin of the human body from edible sections of beef meat and liver, broiler chicken meat and liver.

$$\textbf{EDI } (\mu g/kg \textbf{ weight per day}) = \frac{C \times DIF}{BW} \ldots\ldots\ldots\ldots\ldots (4)$$

Where, BW is the average adult body weight (60 kg), DIF is the daily food intake (in grams per day), and C is the average antibiotic content in beef and broiler chicken meat and liver (in micrograms per gram). The antibiotic's maximum mean concentration was taken into account when calculating EDI. The average daily intake (ADI) for Amoxicillin and Patulin are 0.07 and 0.4 µg/kg bw per day, whereas the ADI for oxytetracycline, tetracycline and chlortetracycline are 30, 30 and 30 µg/kg bw per day, according to FAO/WHO [290, 291].

According to the Household Income and Expenditure Survey Report from 2022, a Bangladeshi adult weighing 60 kg consumes 14.7 and 33.1 g of beef and chicken meat per day, respectively [292]. It was estimated that 3 g of liver tissue were consumed daily per person in Bangladesh [293]. A hazard index (HI) was computed using the formula in order to forecast the long-term health impacts.

$$\textbf{HI} = \frac{\textbf{EDI}}{\textbf{ADI}} \ldots\ldots\ldots\ldots\ldots (5)$$

Beef and broiler chicken meat is considered safe for human consumption if its HI value is less than 1. However, HI > 10 indicates an unacceptable risk for consumption, while 1 ≤ HI < 10 warns of a risk but not an emergency [294].

The maximum residue limits (MRLs) and regulations issued by the Codex Alimentarius were used to assess the health risks associated with antibiotic residues. The ADI values were also obtained from the Codex Alimentarius. The detection frequency was calculated as the number of samples detected/total number of samples × 100. The monitoring sample

test results (concentration) were compared with the MRLs and used to determine whether the antibiotic residues in the sample exceeded the MRLs standard. The exceeding MRLs standard rate was calculated as the number of samples detected/total number of samples × 100 [295].

2.4 Analysis of Residual Tetracyclines in Chicken Meat Samples

2.4.1 Samplings

A total of thirty broiler chicken meat samples were randomly obtained from various supermarkets and local markets in the North and South Dhaka City of Bangladesh during January and February 2019. The broiler chickens were subsequently rinsed with clean water and placed into plastic bags. The uniform portions (thigh and breast) of the chicken muscles were stored in zip-locked plastic bags and labeled accordingly. These samples were then kept at -20°C in the freezer until they were analyzed (Table 2.1).

Table 2.1 List of collected broiler chicken meat samples

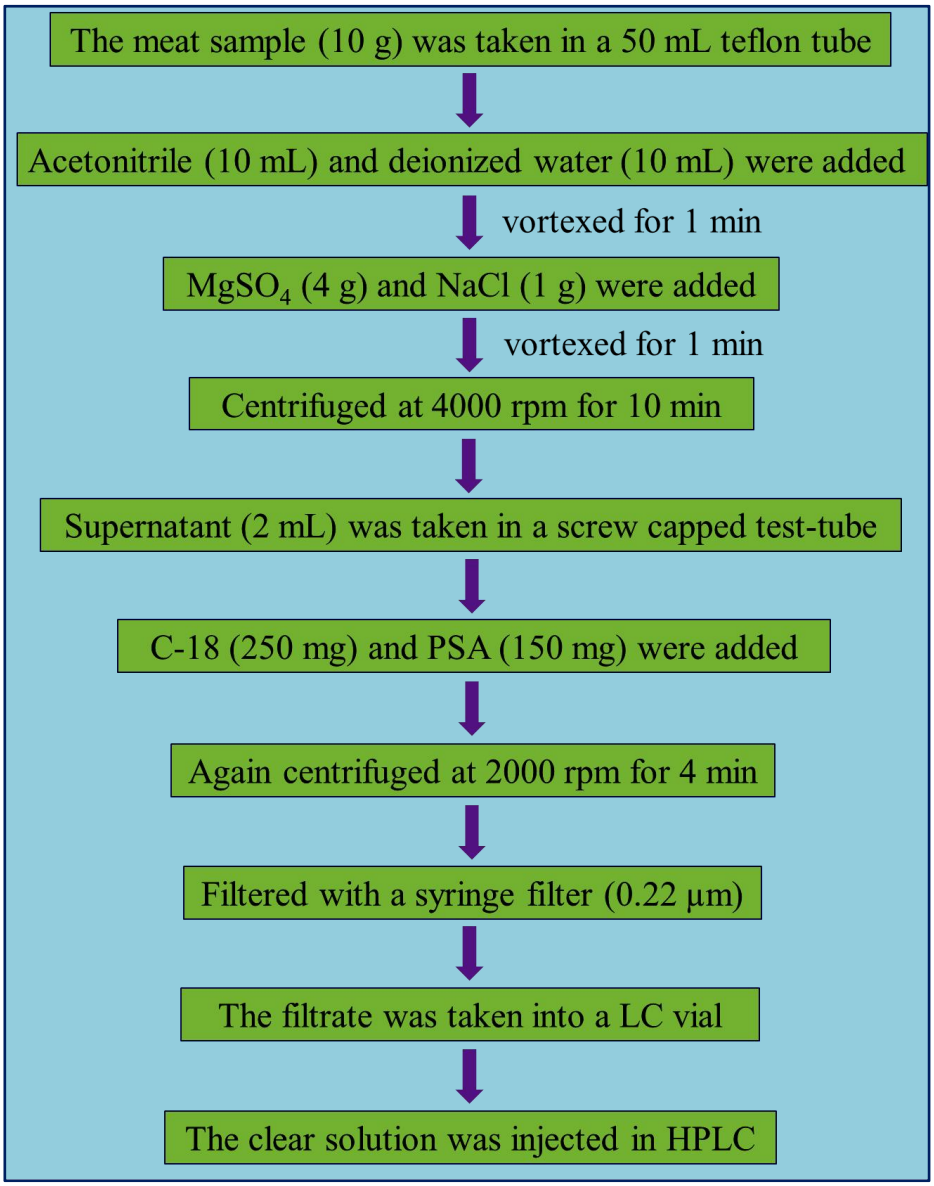
Sample ID	Date of Collection	Location	Number of samples
CM1-CM3	05/01/2019	Maghbazar	3
CM4-CM6	07/01/2019	Jigatola	3
CM7-CM9	10/01/2019	Kazla	3
CM10-CM12	12/01/2019	Tikatuli	3
CM13-CM14	15/01/2019	Nizam Uddin Road	2
CM15-CM18	20/01/2019	Chankharpul	4
CM19-CM22	02/02/2019	Hatirpul	4
CM23-CM26	06/02/2019	Polashi	4
CM27-CM30	10/02/2019	Anandabazar	4

2.4.2 Extraction and Clean-up of Beef and Broiler Chicken Meat Samples

The beef and chicken meat samples were extracted by the modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. A homogenized test sample (10.0 g) was taken into a 50 mL Teflon tube and de-ionized water (10 mL) and acetonitrile (10 mL) were added. The mixture was vortexed for 1 minute in a vortex machine. Then, 4 g magnesium sulfate ($MgSO_4$) and 1 g sodium chloride (NaCl) were added and vortexed for 1 minute. After the vortex, the mixture was centrifuged in a centrifuge machine at 4000 rpm for 5 minutes. The supernatant (2 mL) was transferred into a test tube for clean-up (Scheme1).

Clean up

The clean-up process involved using Primary Secondary Amine (PSA) and octadecyl silica (C-18). To the supernatant (2 mL) obtained after centrifugation, 250 g of C-18 and 150 g of PSA were added, and the mixture was vortexed for one minute with a vortex mixer. Following this, the mixture was subjected to centrifugation at 2000 rpm for four minutes. After centrifugation, the supernatant was filtered using a PTFE syringe filter and subsequently analyzed by RP-HPLC (Scheme 1).



Scheme 1 Extraction procedure for TCs

2.5 Overview of Method for Analysis of TCs

2.5.1 Preparation of primary standard solutions for TCs

Each of the certified Oxytetracycline (OTC), Tetracycline (TC), and Chlortetracycline (CTC) (0.0100 g) was weighed and dissolved separately in a volumetric flask (10.0 mL). The primary standard solutions (1000 mg/L) of OTC, TC, and CTC were prepared by dissolving in methanol. Labeled with permanent black ink, the prepared solution was placed in 10 mL amber bottles with the standard name, concentration, and date of

preparation. The bottles were then placed in a refrigerator at -20°C, away from the sample storage area, until they were needed (Figure 2.22).

Figure 2.22 Standard Solutions (TCs)

2.5.2 Preparation of secondary and working standard solutions

The primary standard solutions were taken from the refrigerator to reach room temperature and checked the meniscus of the layer. Then 1.0 mL of each primary standard solution of tetracycline, chlortetracycline, and oxytetracycline was mixed in a 10 mL volumetric flask and filled to the mark with methanol to prepare 100 mg/L of secondary mixed stock solutions. The standard name, concentration, and preparation date were all listed on the labels of these solutions. The solutions were kept in a refrigerator at -20°C away from the lab and their meniscuses were marked with indelible ink. The middle standard solution of 10 and 1 mg/L was then prepared with the same solvent. Working standard solutions were prepared from 1 mg/L to obtain the standard solutions 1, 2.5, 5 and 10 µg/L daily.

2.5.3 Preparation of Mobile Phase

Oxalic acid (1.26 g) was mixed with de-ionized water (1000 mL) to create the buffer solution.

2.5.4 HPLC Conditions for TCs

A reversed-phase high performance liquid chromatography (HPLC, RF 1200, Prominance, Shimadzu) equipped with a photo-diode array detector (PDA, SPD-M20A Prominance) connected with a Rheodyne injector (20 µL sample loop) was used for this analysis. Separations were performed by keeping the oven temperature at 40 ˚C. A 20 µL sample

loop was used to manually inject the sample extracts. The mobile phase consisted of a combination of methanol, acetonitrile, and oxalic acid buffer solution. The UV wavelengths of 360 nm and 375 nm were used to establish the flow rate at 1.0 mL/min. Oxytetracycline, tetracycline, and chlortetracycline had retention times of 3.8, 4.3 and 7.8 minutes, respectively, under these circumstances. The process of passing samples and external reference standards using HPLC and comparing the respective retention times allowed for the identification of residues (Table 2.2).

Table 2.2 Analytical conditions of HPLC-PDA for analysis of TCs

Parameter	**Type / Value**
Mode	Isocratic
Flow rate	1.0 mL/min
Column Oven Temperature	40° C
Injection Volume	20 µL
The Wavelength of Detection	360 nm and 375 nm
Total Run Time	15 min
Mobile Phase	Oxalic acid buffer solution: acetonitrile: methanol (70: 20: 10%,v/v/v)

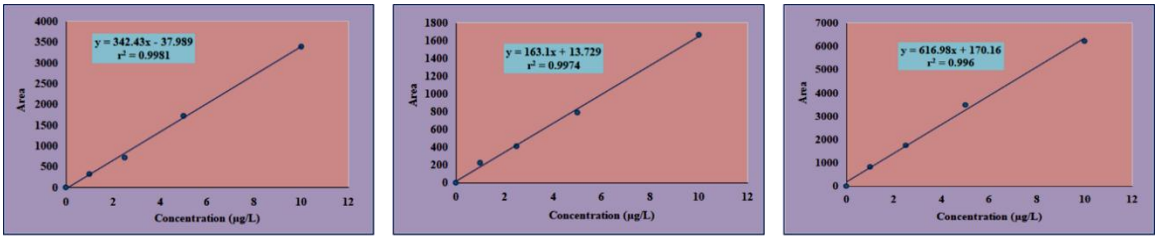
2.5.5 Filtration and Degassing

Oxalic acid buffer, acetonitrile, methanol and HPLC grade water (DI water) were filtered using a solvent filtration apparatus (Restek, Japan) with a Sartorius vacuum pump device (pre-cut membrane with 0.45 µm pore size) and degassed for about half an hour in an ultrasonic before used in HPLC analysis. The sample filtration was done through a polytetrafluoroethylene (PTFE; 0.22 µm) syringe filter.

2.5.6 Calibration Curves for TCs

Solutions containing 1.0, 2.5, 5.0, and 10 µg/L of the corresponding tetracycline antibiotics (OTC, TC, and CTC) were injected into the HPLC to create the calibration curves. The integrated peak areas were then plotted against the standard concentration using Microsoft Excel software. The three antibiotic standards' calibration curves are shown in Figure 2.23. The curves excluded peak areas below the detection limit. Oxytetracycline, tetracycline,

and chlortetracycline had linear correlation coefficients (r^2) of 0.998, 0.997, and 0.991, respectively.



OTC TC CTC

Figure 2.23 Calibration curves of TCs standard

2.5.7 Matrix-matched Calibration Curves for TCs

The matrix-matched calibration was done by spiking a mixture of TCs standard with a control sample to observe the matrix effect. To create matrix-matched calibration curves, solutions containing 1.0, 2.5, 5.0, and 10 µg/L of the corresponding tetracycline antibiotics (OTC, TC, and CTC) were injected into the HPLC. The integrated peak areas were then plotted against the standard concentration using Microsoft Excel software. The curves excluded peak areas below the detection limit. For oxytetracycline, tetracycline, and chlortetracycline, the correlation coefficients (r^2) were found to be linear, with values of 0.999, 0.999, and 0.999, respectively (Figure 2.24).

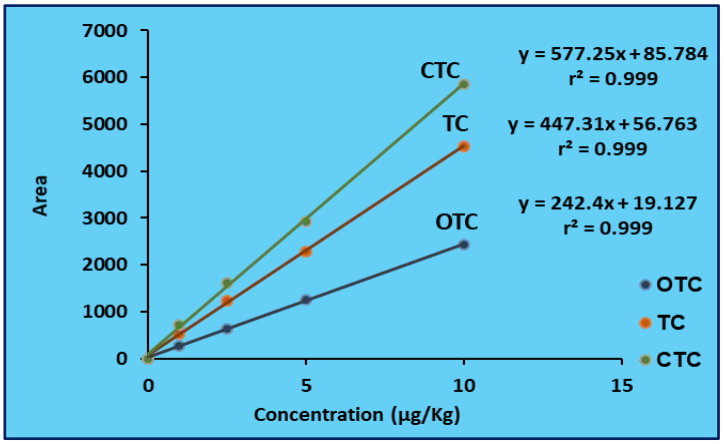


Figure 2.24 Matrix-matched calibration curves of TCs

2.5.8 Recovery Experiment

Samples of broiler chicken meat (10 g) were spiked with a combination of TCs standard at two different spiking concentrations of 2.5 and 5.0 µg/kg. The extraction method and

HPLC analysis parameters were consistent with those used for the standard. The average recoveries were found to be 91, 102, and 106% at the 2.5 µg/kg spiking level, and 100, 100, and 100% at the 5.0 µg/kg spiking level for OTC, TC and CTC, respectively, all falling within a relative standard deviation (RSD%) of 10% (Table 3.2).

2.5.9 Limit of Detection (LOD)

The limit of detection (LOD) was established by injecting progressively diluted mixtures of the standard TC solution using HPLC. For the LOD, the peak area for each standard was evaluated to be three times greater than the baseline noise, meaning the signal-to-noise ratio was set at 3:1. The LOD for the method proposed was 1.05, 1.17, and 1.09 µg/kg for OTC, TC and CTC, respectively (Table 3.2).

2.5.10 Limit of Quantification (LOQ)

Limit of Quantitation (LOQ), the peak area of each standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The corresponding values of LOQ were 3.15, 3.51, and 3.27 µg/kg for OTC, TC and CTC, respectively (Table 3.2).

2.6 Analysis of TCs in Beef, Broiler Chicken Meat and Liver Samples

2.6.1 Samplings

A total of 120 samples of beef, broiler chicken meat, and liver were collected from twelve local markets (Ananda Bazar, Basabo, Chankharpul, Chawk Bazar, Kaptan Bazar, Karwan Bazar, Mohammadpur, Moulovibazar, Noyabazar, Polashi, Tikatuli, and Tongi Bazar) in both the Dhaka North and South City areas of Bangladesh between February and August 2022 (Figure 2.25). Beef and liver samples weighed between 250-500 g, while broiler chicken samples ranged from 900-1000 g. The samples were rinsed with clean water and placed in plastic bags. The uniform samples were then stored in zip-locked plastic bags with appropriate labels. Subsequently, the samples were kept in the refrigerator at -20°C until they were analyzed.

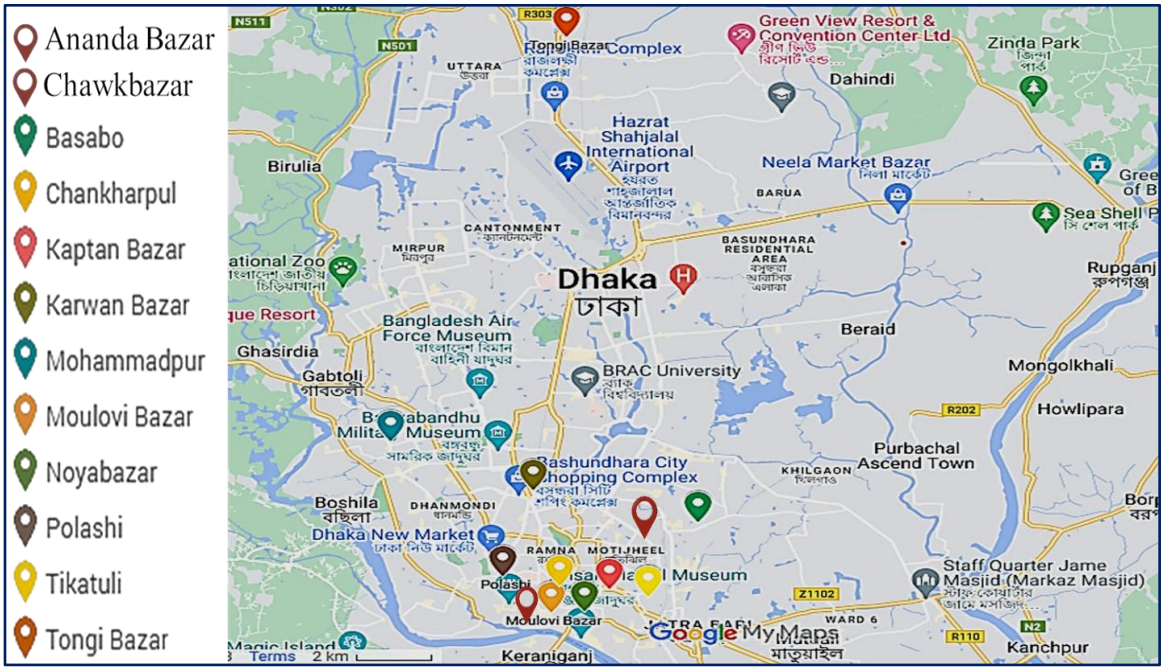


Figure 2.25 Sample Collection Location

2.6.2 Collection of Liver Samples

A total of thirty beef liver (BL) samples were gathered from seven local markets (Tikatuli, Ananda Bazar, Chankharpul, Chawk Bazar, Kaptan Bazar, Noyabazar, Polashi Bazar) in both Dhaka North and South City, Bangladesh, between January and March 2022. The collection dates, locations, and their corresponding ID numbers are listed in Table 2.3.

Table 2.3 List of collected beef liver samples

Sample ID	Collection	Location	Number of samples
BL1, BL4, BL5, BL13, BL14	31/01/2022 08/02/2022	Tikatuli	5
BL2, BL3, BL24, BL30	31/01/2022 08/03/2022	Ananda Bazar	4
BL19, BL25, BL26, BL29	03/02/2022 08/03/2022	Chankharpul	4
BL9, BL10	03/02/2022	Chawk Bazar	2
BL21, BL22, BL23	03/03/2022	Kaptan Bazar	3
BL7, BL8, BL15-BL18, BL27, BL28	03/02/2022 07/03/2022	Noyabazar	8
BL6, BL11, BL12, BL20	02/02/2022 02/03/2022	Polasi Bazar	4

2.6.3 Collection of Beef Meat Samples

Thirty beef meat (BM) samples were collected from six local markets (Basabo, Kaptan Bazar, Karwan Bazar, Mohammadpur, Moulovibazar, and Tongi Bazar) of Dhaka North and South City of Bangladesh from June to August 2022 (Figure 2.26). The collection dates, locations, and their corresponding ID numbers are listed in Table 2.4.

Table 2.4 List of collected beef meat samples

Sample ID	Date of Collection	Location	Quantity (250 -500 g)
BM1-BM5	16/06/2022	Basabo	5
BM6-BM10	18/06/2022	Karwan bazar	5
BM11-BM15	25/06/2022	Tongi bazar	5
BM16-BM20	01/07/2022	Kaptan bazar	5
BM21-BM25	02/07/2022	Moulovibazar	5
BM26-BM30	13/08/2022	Mohammadpur	5

Figure 2.26 Sample Collection (beef meat and liver)

2.6.4 Collection of Broiler Chicken Meat and Liver Samples

Thirty broiler chicken meat (CM) and thirty broiler chicken liver (CL) samples were collected from six local markets (Basabo, Karwan Bazar, Kaptan Bazar, Mohammadpur, Moulovibazar, and Tongi Bazar) of Dhaka North and South City of Bangladesh from July to August 2022 (Figure 2.27). Five samples of each type were collected from each location. The date & area of collections and their ID number are given in Table 2.5.

Table 2.5 List of collected broiler chicken meat and liver samples

Sample ID	Date of Collection	Place	Number of samples
CM1-CM5 CL1-CL5	23/07/2022	Karwan Bazar	5 5
CM6-CM10 CL6-CL10	30/07/2022	Tongi Bazar	5 5
CM11-CM15 CL11-CL15	31/07/2022	Moulovibazar	5 5
CM16-CM20 CL16-CL20	04/08/2022	Kaptan Bazar	5 5
CM21-CM25 CL21-CL25	14/08/2022	Basabo	5 5
CM26-CM30 CL26-CL30	18/08/2022	Mohammadpur	5 5

Figure 2.27 Sample collection (broiler chicken)

2.6.5 Extraction and Clean-up of Beef and Broiler Chicken Meat and Liver Samples

Extraction and clean-up of beef meat and liver; chicken meat and liver samples followed the procedure described in section 2.3.2.

2.6.6 Blank Preparation for TCs

The reagent blank was prepared for removing impurities that might been included in samples during extraction. Five reagent blanks were prepared for TCs analysis.

2.7 Overview of Method for Analysis of TCs

2.7.1 Preparation of primary standard solutions for TCs

Each of the Oxytetracycline (OTC), Tetracycline (TC), and Chlortetracycline (CTC) was weighed 0.0100 g and dissolved separately in a volumetric flask (10.0 mL). The primary standard solutions (1000 mg/L) of Tetracycline, Chlortetracycline, and Oxytetracycline were prepared by dissolving in Methanol. The prepared solutions were placed in amber 10-milliliter bottles, labeled with the standard's name, concentration, and date of preparation. The solutions were then issued labels with permanent black ink and kept in a refrigerated at -20°C, away from the sample storage area, until they were needed.

2.7.2 Preparation of secondary and working standard solutions

Initially, the primary standard solutions were removed from the refrigerator to allow them to reach room temperature, and the meniscus of each solution was inspected. Next, 1 mL of each primary standard solution (OTC, TC and CTC) was combined in a 10 mL volumetric flask and brought up to the mark with methanol to create a secondary mixed stock solution at a concentration of 100 mg/L. Labels detailing the name of the standard, its concentration, and the date of preparation were affixed to these solutions. The menisci of the solutions were carefully marked with permanent ink and stored outside of the lab in a freezer at -20°C. Subsequently, a middle standard solution of 10 and 1 mg/L was prepared using the same solvent. Daily working standard solutions were then prepared starting from 1 mg/L to achieve concentrations of 50, 100, 150, 200, 250, and 300 µg/L daily.

2.7.3 Preparation of Mobile Phase

Oxalic acid (1.26 g) was mixed with de-ionized water (1000 mL) to prepare the buffer solution. The mobile phase was composed of the buffer solution, acetonitrile and methanol.

2.7.4 HPLC Conditions for TCs

The latest version of a reversed-phase High-Performance Liquid Chromatography (HPLC, Prominance-i, LC-2030C 3D Plus, (Shimadzu, Kyoto, Japan), consisting of dual pumps, an auto-injector, an auto-sampler and a photodiode-array detector in the range of 200-800 nm (wavelength). The LC system was fitted with a C-18 column (Shim-pack GIST, Shimadzu, 250 cm × 4.6 mm i.d., particle size: 5 μm) was used for this analysis. Separations were performed by keeping the oven temperature at 40°C. The mobile phase consisted of a combination of methanol, acetonitrile, and oxalic acid buffer solution. The ratio of buffer solution, acetonitrile and methanol was 70: 20: 10 (v/v/v). The UV wavelengths of 360 nm and 375 nm were used to establish the flow rate at 1.0 mL/min. Oxytetracycline, tetracycline, and chlortetracycline had retention times of 4.2, 4.8 and 8.7 minutes, respectively, under these circumstances. The process of passing samples and external reference standards using HPLC and comparing the respective retention times allowed for the identification of residues (Table 2.6).

Table 2.6 Analytical condition of HPLC-PDA for analysis of TCs

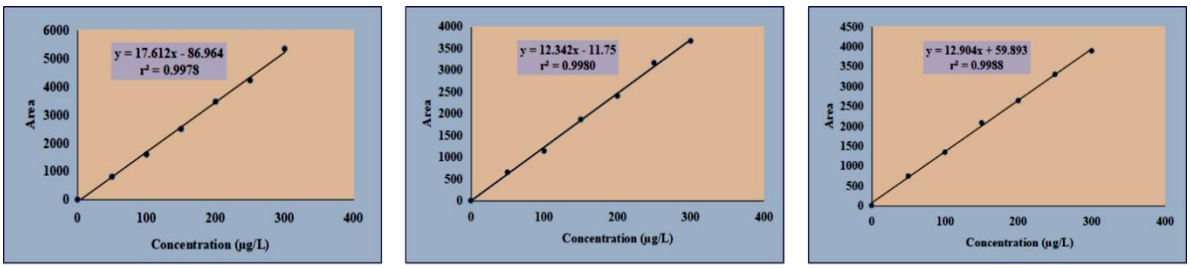
Parameter	**Type / Value**
Mode	Isocratic
Flow rate	1.0 mL/min
Column Oven Tem	40° C
Injection Volume	20 µL
The Wavelength of Detection	360 nm and 375 nm
Total Run Time	15 min
Mobile Phase	Oxalic acid buffer solution: acetonitrile: methanol (70: 20: 10%,v/v/v)

2.7.5 Filtration and Degassing

Oxalic Acid Buffer, Acetonitrile, Methanol and HPLC grade water (DI water) were filtered using a solvent filtration apparatus (Restek, Japan) with a Sartorius vacuum pump device (pre-cut membrane with 0.45 µm pore size) and degassed for about half an hour in an ultrasonic before used in HPLC analysis. The sample filtration was done through a PTFE (0.22 µm) syringe filter.

2.7.6 Calibration Curves for TCs Standard

To create the calibration curves, solutions containing 50, 100, 150, 200, 250, and 300 µg/L of the corresponding tetracycline antibiotics (OTC, TC, and CTC) were injected into the HPLC. The integrated peak areas were then plotted against the reference concentration using Microsoft Excel software. For the curves, peak areas below the detection limit were excluded. The linear correlation coefficients (r^2) for oxytetracycline, tetracycline, and chlortetracycline were determined to be 0.9978, 0.9980, and 0.9988, respectively. (Figure 2.28).

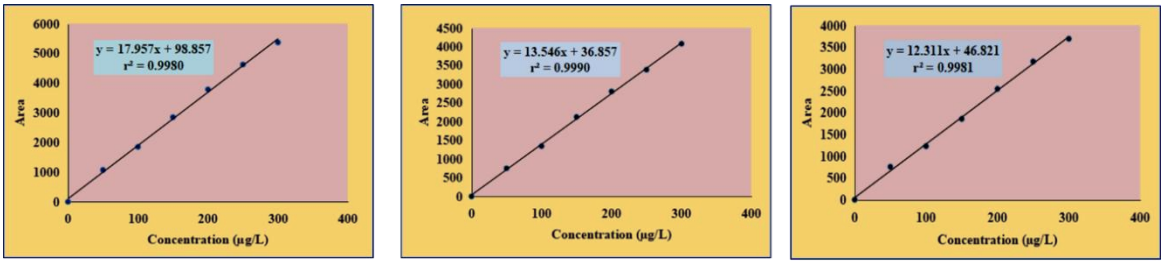


OTC TC CTC

Figure 2.28 Calibration curves of TC Standards

2.7.7 Matrix-matched Calibration Curves for TCs

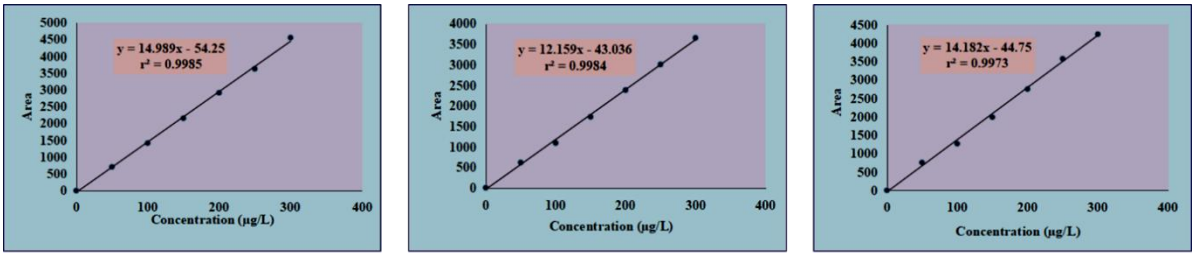
Spiking a combination of TC standards with a control sample allowed for the observation of the matrix effect in the matrix-matched calibration. In order to create the matrix-matched calibration curves, solutions containing 50, 100, 150, 200, 250, and 300 µg/L of the corresponding tetracycline antibiotics (OTC, TC, and CTC) were injected into the HPLC. The integrated peak areas were then plotted against the standard concentration using Microsoft Excel software. The curves excluded peak areas below the detection limit. It was discovered that the correlation coefficients (r2) for oxytetracycline, tetracycline, and chlortetracycline in beef meat were linear, with values of 0.9980, 0.9990, and 0.9981, respectively (Figure 2.29).



OTC TC CTC

Figure 2.29 Matrix-matched calibration curves for OTC, TC and CTC (beef meat)

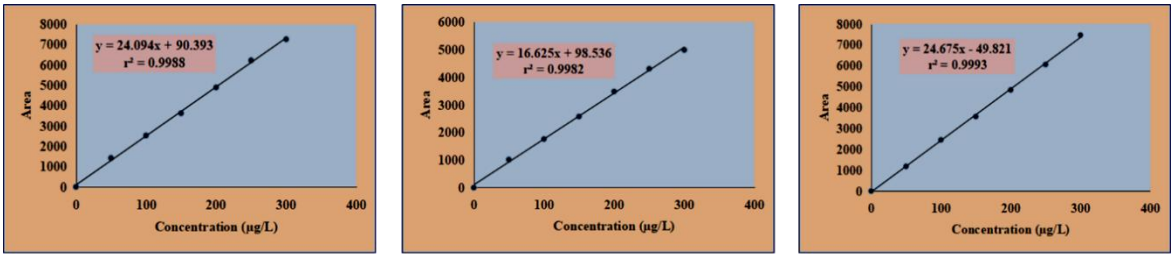
Correlation coefficients (r^2) were found to be linear with 0.9985, 0.9984 and 0.9973 for oxytetracycline, tetracycline, and chlortetracycline, respectively in beef liver (Figure 2.30).



OTC TC CTC

Figure 2.30 Matrix-matched calibration curves for OTC, TC and CTC (beef liver)

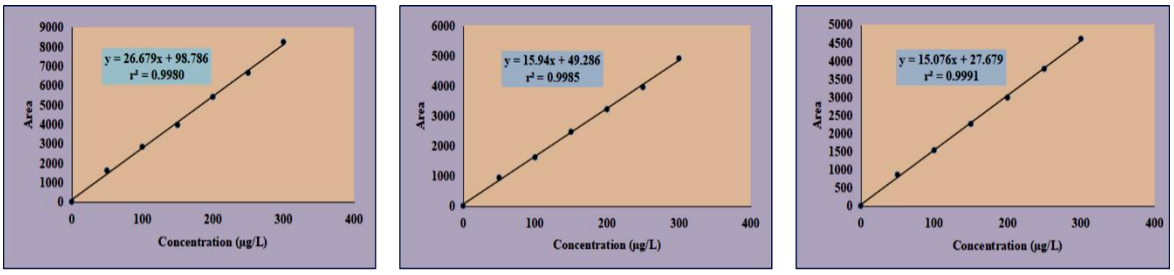
Correlation coefficients (r^2) were found to be linear with 0.9988, 0.9982 and 0.9993 for oxytetracycline, tetracycline, and chlortetracycline, respectively in chicken meat (Figure 2.31).



OTC TC CTC

Figure 2.31 Matrix-matched calibration curves for OTC, TC and CTC (chicken meat)

Correlation coefficients (r^2) were found to be linear with 0.9980, 0.9985 and 0.9991 for oxytetracycline, tetracycline, and chlortetracycline, respectively in chicken liver (Figure 2.32).



OTC TC CTC

Figure 2.32 Matrix-matched calibration curves for OTC, TC and CTC (chicken liver)

2.7.8 Recovery Experiment

The intra-day recovery ranged from 98 to 104 and 99 to 102; 10 to 107, and 81 to 82% for beef meat and liver at spiked level 100 and 150 µg/kg for OTC, respectively. The recovery ranged from 98 to 104 and 94 to 99; 109 to110, and 108 to 109% for beef meat and liver at spiked level 100 and 150 µg/kg for TC, respectively. The recovery ranged from 98 to 102 and 98 to 101; 88 to 106, and 96 to 103% for beef meat and liver at spiked level 100 and 150 µg/kg for CTC, respectively. The recovery ranged from 102 to 103 and 98 to 99 (OTC); 100 to 102 and 100 to 102 (TC); 98 to 102 and 98 to 100% (CTC), respectively at spiked levels 100 and 150 µg/kg for chicken meat and, 99 to 103 and 97 to 101 (OTC); 100 to 102 and 100 to 100 (TC); 99 to 100 and 100 to 100% (CTC), respectively for chicken liver at spiked level 100 and 150 µg/kg. The inter-day (3 days, n = 5x3 replicates) recoveries were also calculated at spiking levels 100 and 150 µg/kg for each matrix and the relative standard deviation was within 10%.

2.7.9 Limit of Detection (LOD)

The limit of detection (LOD) was determined by injecting serially diluted mixtures of TCs standard solution in HPLC. For LOD, the peak area of each standard was considered 3 times higher than the baseline noise *i.e.*, signal to noise ratio was 3:1. The LODs of the proposed method were 1.11, 1.15 and 1.19 for the standard OTC, TC and CTC respectively, and 3.05, 3.78, and 4.33 µg/kg in beef meat; 2.51, 3.08 and 2.83 µg/kg in the beef liver; 1.93, 2.16 µg/kg and 2.03 µg/kg in chicken meat and 1.38, 1.66 and 2.58 µg/kg in the chicken liver for OTC, TC, and CTC, respectively (Table 3.6 and 3.9).

2.7.10 Limit of Quantification (LOQ)

Limit of Quantitation (LOQ), the peak area of each standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The corresponding values of LOQ were 3.71, 3.84 and 3.96 µg/kg for the standard OTC, TC and CTC respectively, and 10.16, 12.59 and 14.45 µg/kg in beef meat; 8.36, 10.28 and 9.43 µg/kg in the beef liver; 6.42, 7.21 and 6.78 µg/kg in chicken meat and 4.59, 5.53 and 8.60 µg/kg in the chicken liver for OTC, TC, and CTC, respectively (Table 3.6 and 3.9).

2.8 Analysis of Amoxicillin in Beef, Broiler Meat and Liver Samples

2.8.1 Samplings

The samplings was the same as section 2.5.

2.8.2 Extraction and Clean-up of Beef and Broiler Chicken Meat and Liver Samples

Extraction and clean-up of beef meat and liver; chicken meat and liver samples followed the procedure described in section 2.3.2.

2.8.3 Blank Preparation for amoxicillin

A reagent blank was prepared for removing impurities that might been included in samples during extraction. Five reagent blanks were prepared for analysis of amoxicillin.

2.9 Overview of Method for Analysis of Amoxicillin

2.9.1 Preparation of primary standard solution for Amoxicillin

Amoxicillin trihydrate was weighed 0.01 g and dissolved separately in a volumetric flask (10 mL). The primary standard solution (1000 mg/L) of amoxicillin was prepared by dissolving in methanol and deionized water (50:50 v/v). Labeled with permanent black ink, the prepared solution was placed in 10 mL volumetric flask with the standard name, concentration, and date of preparation. The bottles were then placed in a refrigerator at -20°C, away from the sample storage area, until they were needed.

2.9.2 Preparation of secondary and working standard solutions

The primary standard solutions were taken from the refrigerator to reach room temperature and checked the meniscus of the layer. Then, 1 mL of primary standard solution of amoxicillin was mixed in a 10 mL volumetric flask and filled to the mark with methanol to prepare 100 mg/L of secondary stock solution. A label with the standard's name, concentration, and preparation date was attached to the solution. Permanent ink was used to indicate the solution's meniscus, which was then kept out of the lab in a refrigerated at -20°C. The appropriate working standard solutions of 10 and 1 mg/L were prepared from the secondary stock solution with the same solvent. Working standard solutions were prepared from 1 mg/L to obtain the standard solutions 1, 5, 10, 15, 20, 25, and 30 µg/L daily.

2.9.3 Preparation of Mobile Phase

Disodium hydrogen phosphate (Na_2HPO_4, 0.500 g) was dissolved in de-ionized water (1000 mL) to make the phosphate buffer solution. The pH was adjusted at 7.0 using phosphoric acid and measured using a pH meter. The mobile phase consisted of phosphate buffer solution and acetonitrile.

2.9.4 HPLC Conditions for Amoxicillin

The latest version of a reversed-phase High-Performance Liquid Chromatograph (HPLC, Prominance-i, LC-2030C 3D Plus, (Shimadzu, Kyoto, Japan), consisting of dual pumps, an auto-injector, an auto-sampler, and a photodiode-array detector in the range of 200-800 nm (wavelength). The LC system was fitted with a C-18 column (Shim-pack GIST, Shimadzu; 250 cm × 4.6 mm i.d., particle size: 5 μm) was used for this analysis. Separations were performed by keeping the oven temperature at 30°C. The mobile phase consisted of a combination of phosphate buffer solution and acetonitrile. The ratio of buffer solution (Na_2HPO_4) and acetonitrile (CH_3CN) was 70: 30 (v/v). The UV wavelength of 230 nm and was used to establish the flow rate at 1.0 mL/min. The retention time of amoxicillin was 3.3 minute under these circumstances. The process of passing samples and external reference standards using HPLC and comparing the respective retention times allowed for the identification of residues. The pH of the buffer solution was adjusted to 7.0 using phosphoric acid (Table 2.7).

Table 2.7 Analytical parameters of HPLC-PDA for the analysis of amoxicillin

Parameter	**Type / Value**
Mode	Low-pressure gradient
Flow rate	1.0 mL/min
Column Oven Tem	30° C
Injection Volume	20 µL
The Wavelength of Detection	230 nm
Total Run Time	10 min
Mobile Phase	Phosphate buffer solution (Na_2HPO_4): acetonitrile (CH_3CN) 70: 30 (v/v)
pH	7.0

2.9.5 Filtration and Degassing

Phosphate Buffer, acetonitrile and HPLC grade water (DI water) were filtered using a solvent filtration apparatus (Restek, Japan) with a sartorius vacuum pump device (pre-cut membrane with 0.45 µm pore size) and degassed for about half an hour in an ultrasonic before used in HPLC analysis. The sample filtration was done through a PTFE (0.22 µm) syringe filter.

2.9.6 Calibration Curve for Amoxicillin Standard

Solutions containing 1, 5, 10, 15, 20, 25, and 30 µg/L of the corresponding amoxicillin antibiotic were injected into the HPLC to create the calibration curve. The integrated peak areas were then plotted against the standard concentration using Microsoft Excel software. The curve excluded peak regions below the detection limit. For amoxicillin, the correlation coefficient (r^2) was determined to be linear at 0.9982 (Figure 2.33).

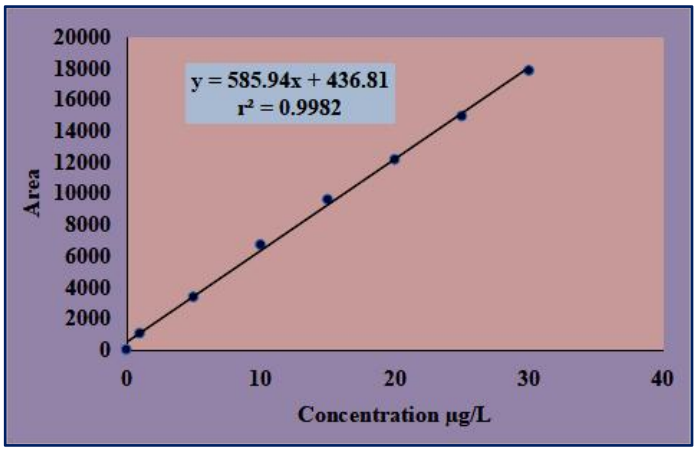
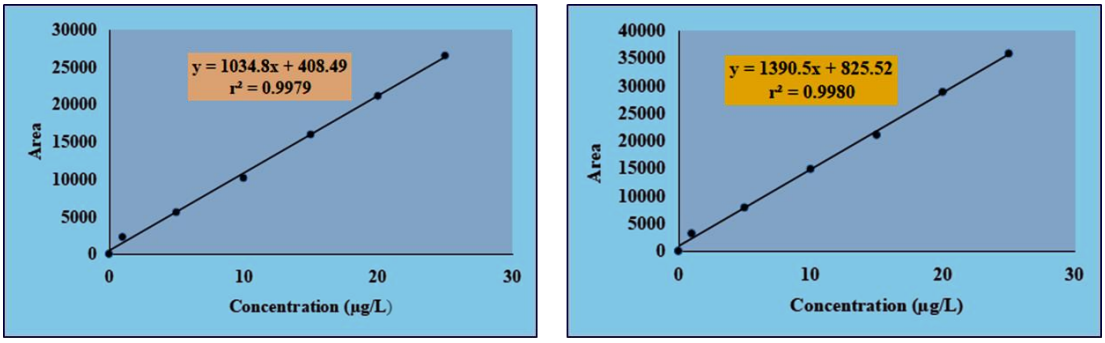


Figure 2.33 Calibration curve of standard amoxicillin

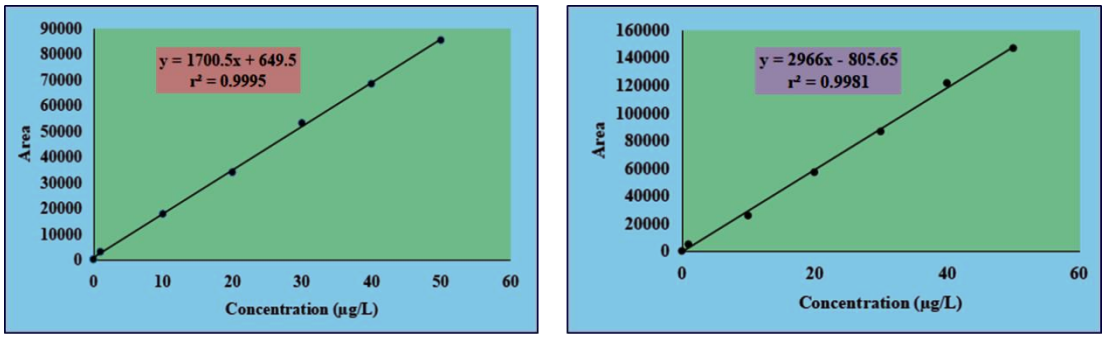
2.9.7 Matrix-matched Calibration Curves for Amoxicillin

The amoxicillin standard was spiked with a control sample in order to detect the matrix effect in the matrix-matched calibration. Using MS Excel software, the integrated peak areas were plotted against the standard concentration after solutions containing 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 µg/L of amoxicillin were injected into the HPLC to create the matrix-matched calibration curves. The curves excluded peak areas below the detection limit. Amoxicillin was found to have linear correlation coefficients (r^2) of 0.9979, 0.9980, 0.9995, and 0.9981 in beef meat, beef liver, and chicken liver, respectively (Figure 2.34 and 2.35).



i ii

Figure 2.34 Calibration curve for amoxicillin in (i) beef meat (ii) beef liver



i ii

Figure 2.35 Calibration curve for amoxicillin in (i) broiler chicken meat (ii) broiler chicken liver

2.9.8 Recovery Experiment

The intra-day recovery ranged from 101 to 103 and 103 to 106% for beef meat, 108 to 112 and 94 to 102% for beef liver at spiked levels of 10 and 15 µg/kg, respectively. The recovery percentages ranged from 95 to 100 and 97 to 101% at spiked levels 10 and 20 µg/kg for chicken meat and, 102 to 106 and 93 to 96% for chicken liver at spiked levels 20 and 30 µg/kg. The inter-day (3 days, n = 5x3 replicates) recoveries were also calculated for each matrix and the relative standard deviation (RSD) was within 10%.

2.9.9 Limit of Detection (LOD)

The limit of detection (LOD) was established by introducing a series of diluted amoxicillin standard solutions into the HPLC system. For determining the LOD, the peak area of each standard was evaluated at a level three times greater than the baseline noise, meaning the signal to noise ratio was set at 3:1. The LOD for the method developed was found to be 0.55 µg/kg for standard amoxicillin. The calculated LOD values for amoxicillin were 0.71, 0.65, 0.51, and 0.57 µg/kg for beef meat, beef liver, chicken meat, and chicken liver, respectively (see Tables 3.12 and 3.16).

2.9.10 Limit of Quantification (LOQ)

Limit of Quantitation (LOQ), the peak area of the standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The LOQ for the amoxicillin standard was 1.84 µg/kg. The corresponding values of LOQ for amoxicillin were 2.35, 2.17, 1.71 and 1.90 in beef meat, beef liver, chicken meat and chicken liver, respectively (Table 3.12 and 3.16).

2.10 Analysis of Patulin in Beef, Broiler Chicken Meat and Liver Samples

2.10.1 Samplings

A total of 120 beef, broiler chicken meat, and liver samples from six local markets (Basabo, Karwan bazar, Kaptan bazar, Mohammadpur, Moulovi bazar, and Tongi bazar) of Dhaka North and South City of Bangladesh in June to August 2022. Five samples of each type (beef meat, beef liver, broiler chicken meat, and liver) were collected from each location. Beef meat and liver samples were 250-500 g and broiler chicken samples were 900-1000 g (Figure 2.36). Samples were then washed with clean water and kept in plastic bags. The homogenous samples were kept in zip-locked plastic bags and properly labeled. Then, the samples were stored at -20°C in the refrigerator until analysis.

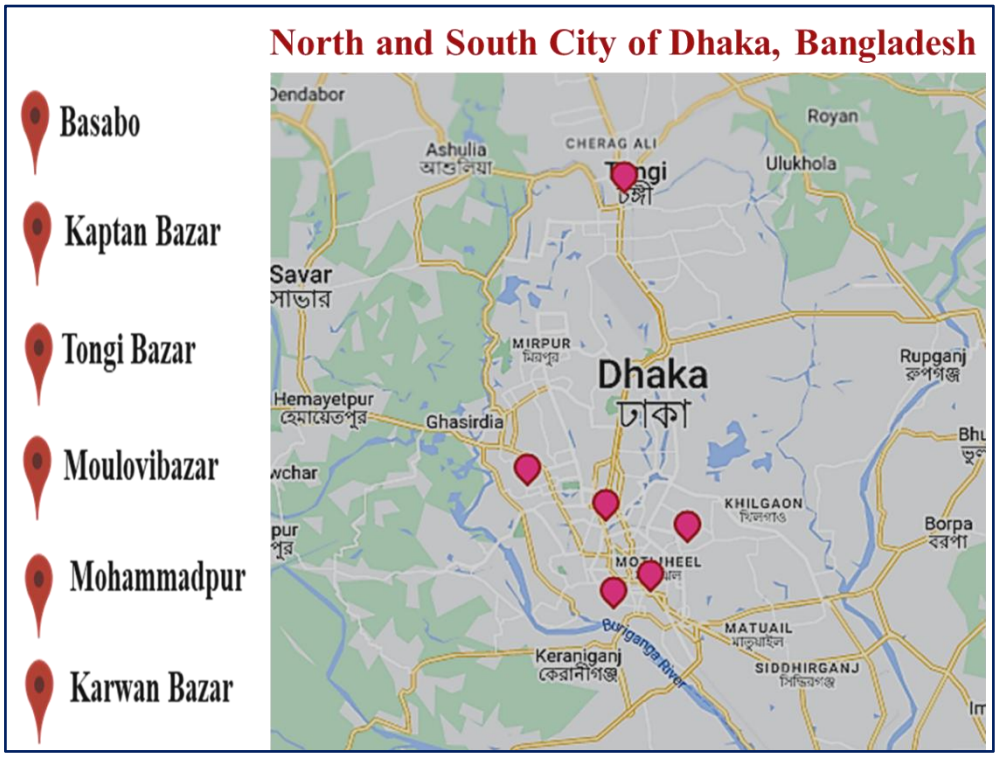


Figure 2.36 Sample Collection Location

2.10.2 Collection of Beef Meat Samples

The Collection of beef meat samples was the same as described in section 2.5.2.

2.10.3 Collection of Beef Liver Samples

Thirty beef liver samples were collected from six local markets (Basabo, Karwan Bazar, Kaptan Bazar, Mohammadpur, Moulovibazar, and Tongi Bazar) of Dhaka North and South City of Bangladesh from April to May 2023. Five samples were collected from each location. The date & area of collections and their ID number are given in Table 2.8.

Table 2.8 List of beef liver samples

Sample ID	Date of Collection	Location	Number of samples
BL1 - BL5	02/04/2023	Basabo	5
BL6 - BL10	08/04/2023	Karwan bazar	5
BL11 - BL15	10/04/2023	Tongi bazar	5
BL16 - BL20	15/04/2023	Kaptan bazar	5
BL21 - BL25	02/05/2023	Moulovibazar	5
BL26 - BL30	04/05/2023	Mohammadpur	5

2.10.4 Collection of Broiler Chicken Meat Samples

The Collection of beef meat samples was the same as described in section 2.5.4.

2.10.5 Collection of Broiler Chicken Liver Samples

Thirty broiler chicken liver (CL) samples were collected from six local markets (Basabo, Karwan Bazar, Kaptan Bazar, Mohammadpur, Moulovibazar, and Tongi Bazar) of Dhaka North and South City of Bangladesh in March 2023. Five samples were collected from each location. The date & area of collections and their ID number are given in Table 2.9.

Table 2.9 List of collected broiler chicken liver samples

Sample ID	Date of Collection	Place	Number of samples
CL1 - CL5	10/03/2023	Karwan Bazar	5
CL6 - CL10	11/03/2023	Tongi Bazar	5
CL11 - CL15	17/03/2023	Moulovibazar	5
CL16 - CL20	18/03/2023	Kaptan Bazar	5
CL21 - CL25	27/03/2023	Basabo	5
CL26 - CL30	29/03/2023	Mohammadpur	5

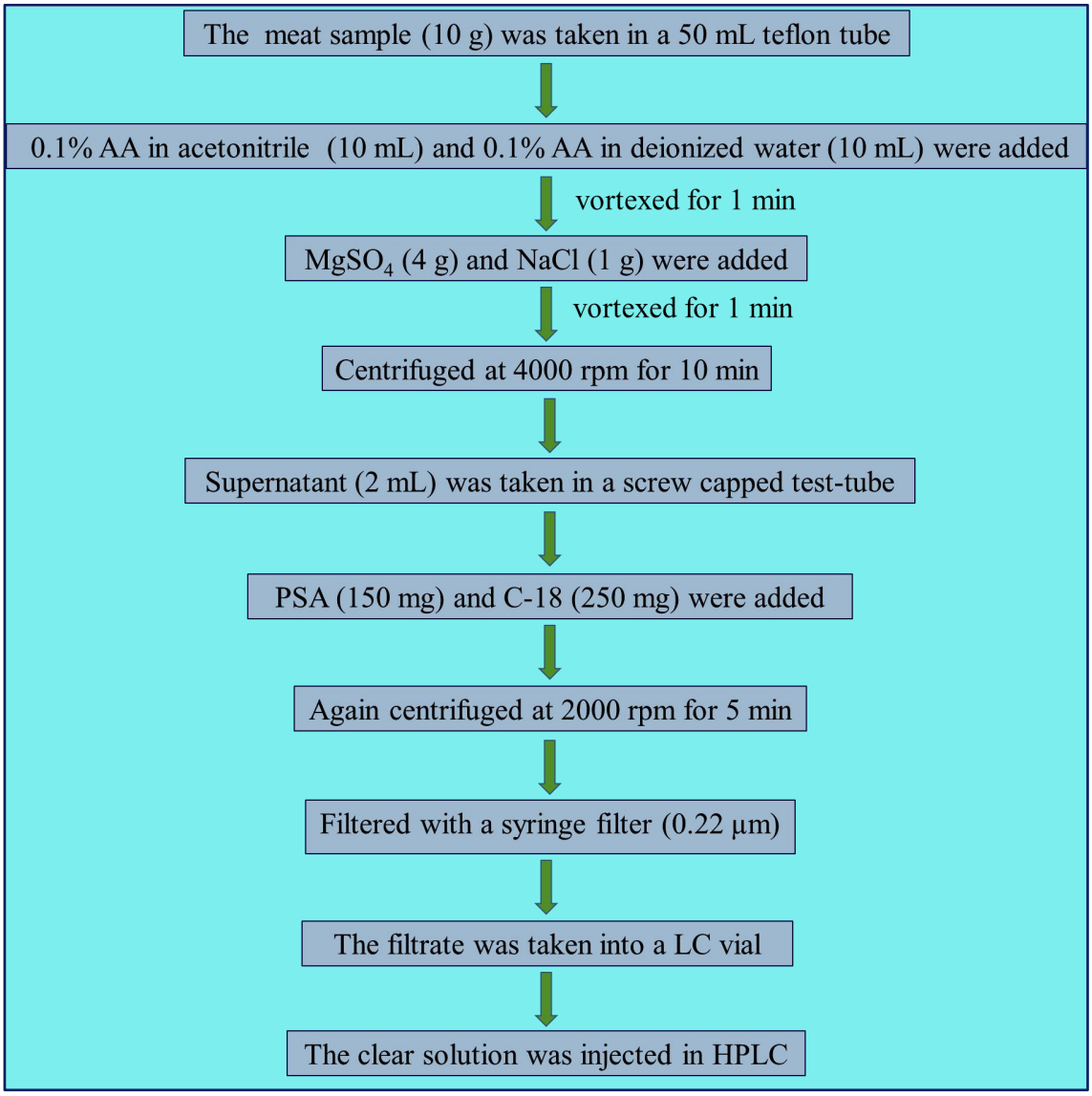
2.10.6 Extraction and Clean-up of Beef and Broiler Chicken Meat Samples

The beef and chicken meat samples were extracted for patulin by the modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method.

A homogenized test sample (10.0 g) was taken into a 50 mL Teflon tube, and 0.1 % acetic acid in de-ionized water (10 mL) and 0.1 % acetic acid in acetonitrile (10 mL) were added. Then the mixture was vortexed for 1 minute in a vortex machine. Then, 4 g magnesium sulfate ($MgSO_4$) and 1 g sodium chloride (NaCl) were added and vortexed for 1 minute. After the vortex, the mixture was centrifuged in a centrifuge machine at 4000 rpm for 5 minutes. The supernatant (2 mL) was transferred into a test tube for clean-up.

Clean up

The Primary Secondary Amine (PSA) and octadecyl silica (C-18) were used for clean-up. C-18 (250 g) and PSA (150 g) were added into the supernatant (2 mL) obtained after centrifugation and vortexed for 1 minute using a vortex mixer. Then, the mixer was centrifuged in a centrifuge machine at 2000 rpm for 4 minutes. After the centrifugation, the supernatant was filtered by using a PTFE syringe filter and analyzed by RP-HPLC. A Scheme is shown below (Scheme 2).



Scheme 2 Extraction procedure for patulin

2.10.7 Blank Preparation for Patulin

A reagent blank was created to eliminate any contaminants that may have been introduced into the samples during the extraction process. Five reagent blanks were generated for the patulin analysis. A pure solvent was also utilized to assess any residues in the analytical column.

2.11 Overview of Method for Analysis of Patulin

2.11.1 Preparation of primary standard solution for Patulin

A patulin standard (5 mg) was dissolved in 50 mL of ethyl acetate to create a stock solution of patulin at a concentration of 100 mg/L. The solution, prepared in a 50 mL volumetric flask, was labeled with the name of the standard, its concentration, and the date it was prepared, all inscribed with a permanent black ink marker. This flask was then stored in the refrigerator at -20°C, separated from the sample storage area, until it is needed for further use.

2.11.2 Preparation of secondary and working standard solution for Patulin

A test tube was filled with 1 mL of the primary standard solution and dried completely using a stream of nitrogen gas at 45ºC. The residue was then re-dissolved in a solvent mixture (0.1% acetic acid in acetonitrile and 0.1% acetic acid in deionized water) within a 100 mL volumetric flask to produce a 1 mg/L secondary standard solution. The solution was labeled to indicate the standard's name, its concentration, and the preparation date. Working standard solutions were created daily from the 1 mg/L to prepare standards at concentrations of 1, 5, 10, 15, 20, 25 and 30 µg/L using the same solvent as that used for the 1 mg/L solution for calibration purposes. All working standard solutions were kept in a refrigerator at 4ºC.

2.11.3 Preparation of Mobile Phase

Glacial acetic acid (0.1%) was mixed with deionized water (H_2O) and shaken thoroughly. The mobile phase consisted of deionized water (H_2O) and acetonitrile (CH_3CN), with a concentration of 0.1% acetic acid.

2.11.4 HPLC Conditions for Patulin

The most recent version of a reversed-phase HPLC, Prominance-i, LC-2030C 3D Plus (Shimadzu, Kyoto, Japan) includes two pumps, an auto-injector, an auto-sampler, and a photodiode-array detector with a wavelength of 200–800 nm. This study was conducted using an LC system equipped with a C-18 column (Shim-pack GIST, Shimadzu; 250 cm × 4.6 mm i.d., particle size: 5 µm). Separations were performed by keeping the oven temperature at 40°C. The mobile phase consisted of a combination of acetonitrile (CH_3CN) and deionized water. The ratio of acetonitrile and deionized water was 10: 90 (v/v). The

UV wavelength of 276 nm and was used to establish the flow rate at 1 mL/min. The retention time of patulin was 4.9 minutes under these circumstances. The process of passing samples and external reference standards using HPLC and comparing the respective retention times allowed for the identification of residues (Table 2.10).

Table 2.10 Analytical parameters of HPLC-PDA for the analysis of patulin

Parameter	**Type/Value**
Mode	Low-pressure gradient
Flow rate	1.0 mL/min
Column Oven Temperature	40° C
Injection volume	20 µL
The wavelength of Detection	276 nm
Total Run Time	10 min
Mobile Phase	Acetonitrile (CH_3CN): 0.1% acetic acid in deionized water (H_2O) 10: 90 (v/v)

2.11.5 Filtration and Degassing

Acetonitrile and HPLC grade water (DI water) were filtered using a solvent filtration apparatus (Restek, Japan) with a Sartorius vacuum pump device (pre-cut membrane with 0.45 µm pore size) and degassed for about half an hour in an ultrasonic before used in HPLC analysis. The sample filtration was done through a PTFE (0.22 µm) syringe filter.

2.11.6 Calibration Curve for Patulin Standard

Solutions containing 1, 5, 10, 15, 20, 25, and 30 µg/L of the corresponding patulin antibiotic were injected into the HPLC to create the calibration curves. The integrated peak areas were then plotted versus the standard concentration using Microsoft Excel software. Figure 2.37 displays the antibiotic calibration curve of the standard. The curve excluded peak regions below the detection limit. For patulin, the correlation coefficient (r^2) was determined to be linear at 0.9991.

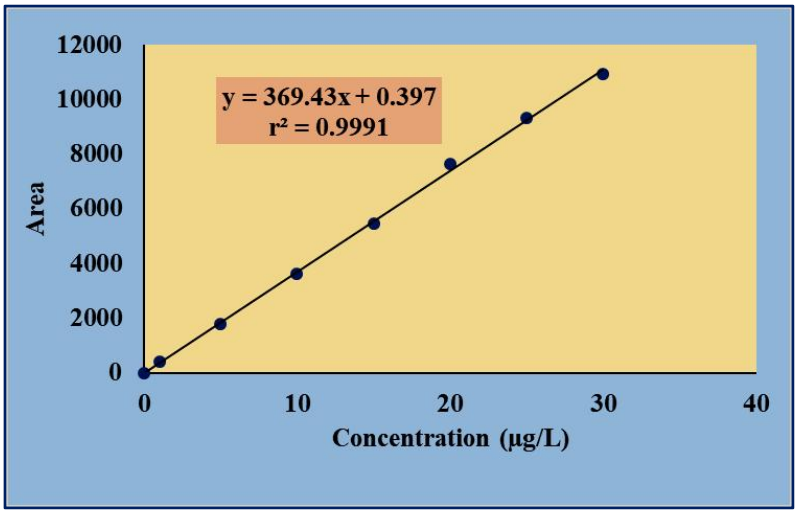
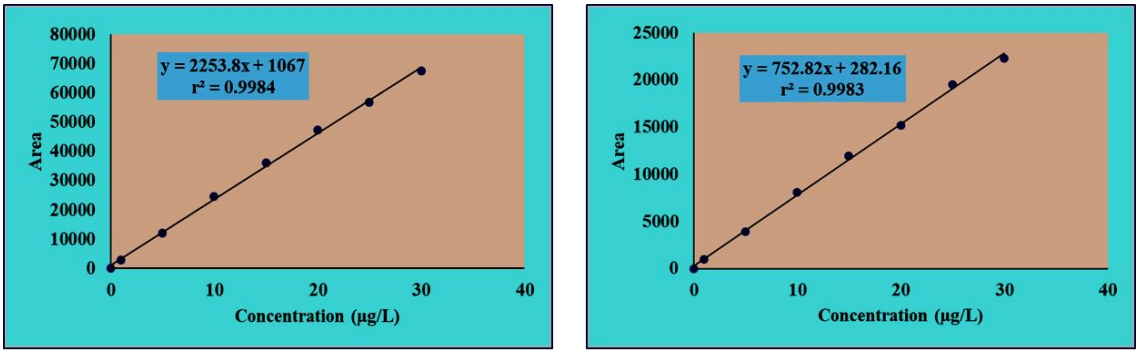


Figure 2.37 Calibration curve for patulin standard

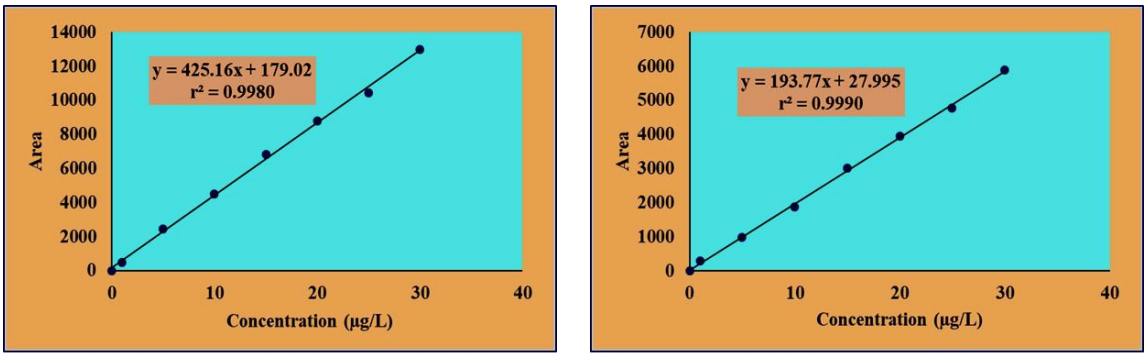
2.11.7 Matrix-matched Calibration Curves for Patulin

The matrix-matched calibration was performed by spiking the patulin standard with a control sample to observe the matrix effect. The integrated peak areas were plotted against the standard concentration after solutions containing 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 µg/L of the patulin were injected into the HPLC to create the matrix-matched calibration curves using MS Excel software. The curves excluded peak areas below the detection limit. Patulin in beef meat, cow liver, and chicken meat had linear correlation values (r^2) of 0.9984, 0.9983, 0.9980, and 0.9990, respectively (Figures 2.38 and 2.39).



i ii

Figure 2.38 Matrix-matched calibration curves for patulin (i) beef meat (ii) beef liver



i ii

Figure 2.39 Matrix-matched calibration curves for patulin (i) broiler chicken meat (ii) broiler chicken liver

2.11.8 Recovery Experiment

The intra-day recovery ranged from 94 to 95, and 96 to 97% for beef meat, 104 to 105, and 102 to 103% for beef liver at spiked levels of 10 and 15 µg/kg, respectively. The recovery ranged from 94 to 95 and 98 to 100% for chicken meat, 100 to 105 and 104 to 105% for chicken liver at spiked levels 10 and 15 µg/kg. The inter-day (3 days, n = 5x3 replicates) recoveries were also calculated for each matrix and the relative standard deviation (RSD%) was within 10%.

2.11.9 Limit of Detection (LOD)

The limit of detection (LOD) was determined by injecting a serially diluted patulin standard solution in HPLC. For LOD, the peak area of each standard was considered 3 times higher than the baseline noise *i.e.*, signal to noise ratio was 3:1. The LOD of the proposed method was 0.18 µg/kg for standard patulin. The corresponding values of LOD for patulin were 0.05, 0.07, 0.21 and 0.30 µg/kg in beef meat, beef liver, chicken meat and chicken liver, respectively.

2.11.10 Limit of Quantification (LOQ)

Limit of Quantitation (LOQ), the peak area of each standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The LOQ of the proposed method was 0.60 µg/kg for standard patulin. The corresponding values

of LOQ for patulin 0.18, 0.25, 0.71 and 0.99 μg/kg in beef meat, beef liver, chicken meat and chicken liver, respectively.

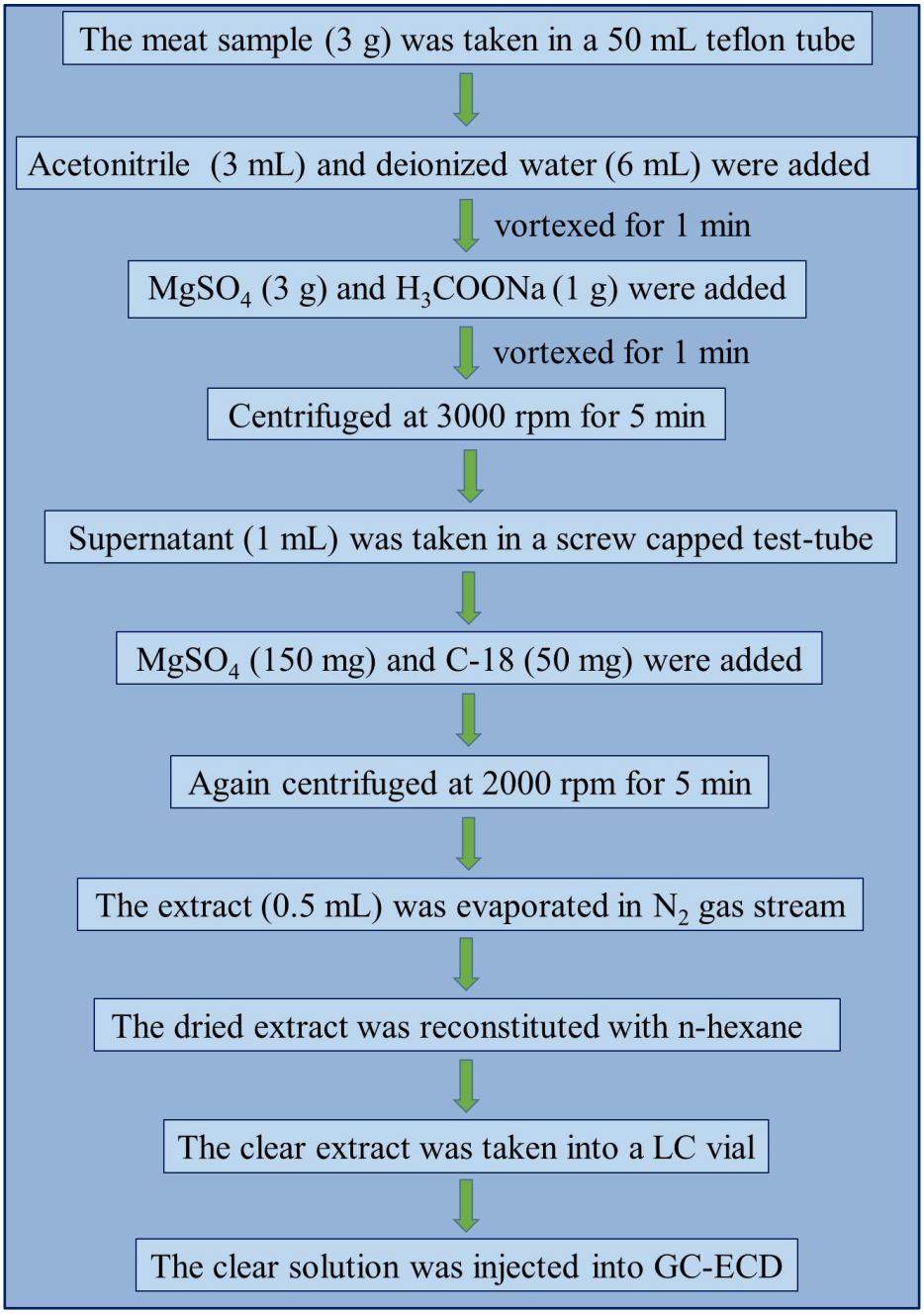
2.12 Analysis of Organochlorine Pesticides in Beef, Broiler Chicken Meat and Liver Samples

2.12.1 Samplings

Samplings were the same as in section 2.9.

2.12.2 Sample Preparation

Sample (3 g) was measured and transferred into a centrifuge tube and 3 mL of water and 3 mL of acetonitrile were added. After intensive stirring on a vortex, 3 g of anhydrous magnesium sulfate and 1 g of anhydrous sodium acetate were added. An exothermic reaction occurred within 1 minute after the intense stirring on the vortex. The sample was then centrifuged for 5 min at 1110 g (approximately 3000 rpm). 1 mL of upper acetonitrile extract was transferred into a 5 mL tube containing 150 mg of anhydrous magnesium sulfate, 100 mg of PSA, and 50 mg of C-18. The tube content was centrifuged for 5 min at 2000 rpm. After centrifuging, purified and clear extract was obtained. After that, 0.5 mL of the extract was evaporated in nitrogen and reconstituted with hexane. The sample prepared in this way was ready for the analysis on GC-ECD. The method of sample preparation was based on the extraction with acetonitrile (ACN) produced by Sigma-Aldrich (St. Louis, MI, USA) in the presence of anhydrous magnesium sulfate and anhydrous sodium acetate (Scheme 3).



Scheme 3 Extraction procedure for OCPs

2.12.3 Blank Preparation for OCPs

A reagent blank was employed to remove any impurities that might have been introduced into the sample during the extraction procedure. In the OCP analysis, five reagent blanks were produced for this goal.

2.13 Overview of Method for Analysis of Organochlorine Pesticides

2.13.1 Preparation of Primary Standard Solution for OCPs

A standard of organochlorine pesticides (OCPs), which includes a blend of 20 components in a 1 mL volume, was obtained from Restek, USA, and dissolved in n-hexane to prepare a stock solution at a concentration of 2000 mg/L. The prepared solution was labeled with the standard name, concentration, and date of preparation. Permanent black ink was utilized to mark the meniscus levels of the solutions, which were subsequently stored in a refrigerator at -20°C, kept separate from the sample storage area until further analysis.

2.13.2 Preparation of Secondary and Working Standard Solution

The primary standard solutions were removed from the refrigerator and allowed to cool down to room temperature to check the layer's meniscus. In order to create a 1000 mg/L secondary standard solution, 5 mL of the primary stock solution was serially diluted up to 1 mg/L in a 10 mL volumetric flask using 5 mL of the matching solvent. After that, working solutions ranging from 1 mg/L to µg/L were made every day. These solutions were labeled with the standard's name, concentration, and preparation date. The meniscuses of the solutions were marked with permanent ink and kept out of the pesticide residue lab in a refrigerator at -20°C.

2.13.3 GC-ECD Conditions for OCPs

The residual organochlorine pesticides (OCPs, consisting of a mix of 20 components) were examined using a Shimadzu GC-2030 Plus gas chromatograph that has a Ni63-electron capture detector (manufactured by Shimadzu, Tokyo, Japan). For the chromatographic analysis of the OCPs, a high-polarity SH-CLP II column (5% Diphenyl – 95% Dimethylpolysiloxane, low bleed, measuring 30 m x 0.32 mm x 0.25 µm, Shimadzu, Japan) was utilized. Nitrogen gas (N_2, with 99% purity) served as the carrier gas. The instrumental parameters were set as follows: the gas chromatography oven temperature started at 150°C for 2 minutes, then increased to 295°C at a rate of 5°C per minute, with a 4-minute hold, resulting in a total runtime of 35 minutes; the injection volume was 1.0 µL, and the nitrogen gas flow rate was 20 mL/min. The quality control analytical procedures were conducted by comparing the results with established standard curves, alongside injector and detector temperatures of 200°C and 300°C. The injector was set to splitless mode, and the overall flow was recorded at 21.5 mL/min.

2.13.4 Calibration Curves for OCP Standard

Solutions containing 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µg/L of the corresponding organochlorine pesticide (OCPs) standard were injected into the GC-ECD to create the calibration curves. The integrated peak areas were then plotted versus the standard concentration using Microsoft Excel software. Peak areas below the detection limit were not included in the curves. Correlation coefficients (r^2) were found to be linear with 0.9999, 0.9994, 0.9990, 0.9988, 0.9980, 0.9997, 0.9991, 0.9994, 0.9993, 0.9996, 0.9983, 0.9978, 0.9995, 0.9985, 0.9990, 0.9997, 0.9981, 0.9991, 0.9994 and 0.9998 for alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan I, 4, 4′-DDE, dieldrin, endrin, 4, 4′-DDD, endosulfan II, endrin aldehyde, 4, 4′-DDT, endosulfan sulfate, methoxychlor and endrin ketone, respectively (Figures 2.40).

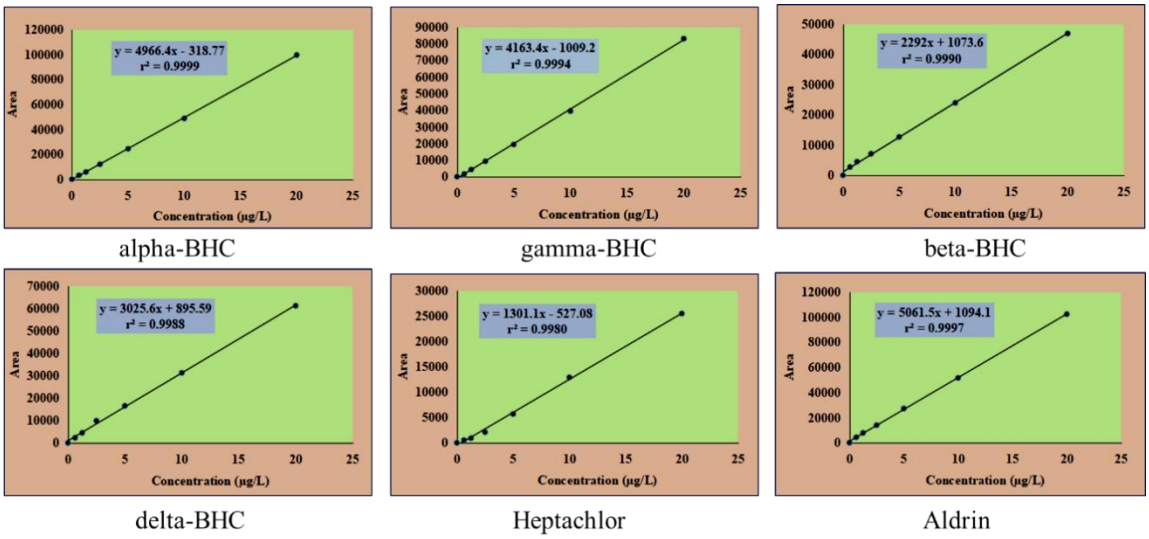


Figure 2.40 Calibration curves for standard 20-mixed OCPs (1)

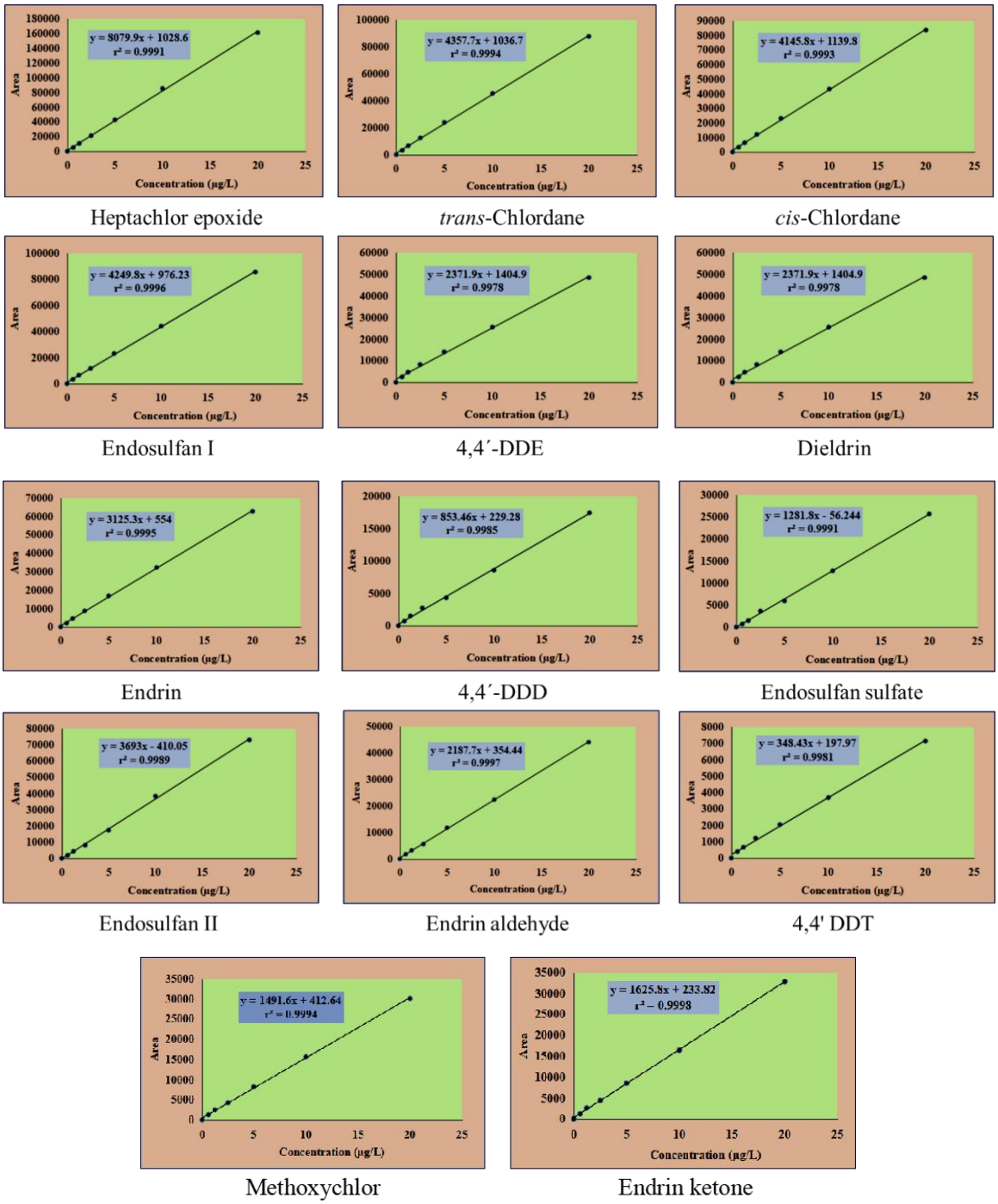


Figure 2.40 Calibration curves for standard 20-mixed OCPs (2)

2.13.5 Matrix-matched Calibration Curves for OCPs

The OCPs standard was spiked with four control samples for four matrices in order to observe the matrix effect in the matrix-matched calibration. To create the matrix-matched calibration curves, solutions containing 0.625, 1.25, 2.5, 5, 10, and 20 µg/L of the OCPs for each matrix in the GC-ECD were injected. The integrated peak areas were then plotted against the standard concentration using Microsoft Excel software. Peak areas below the detection limit were not included in the curves (Figure 2.41 to 2.44).

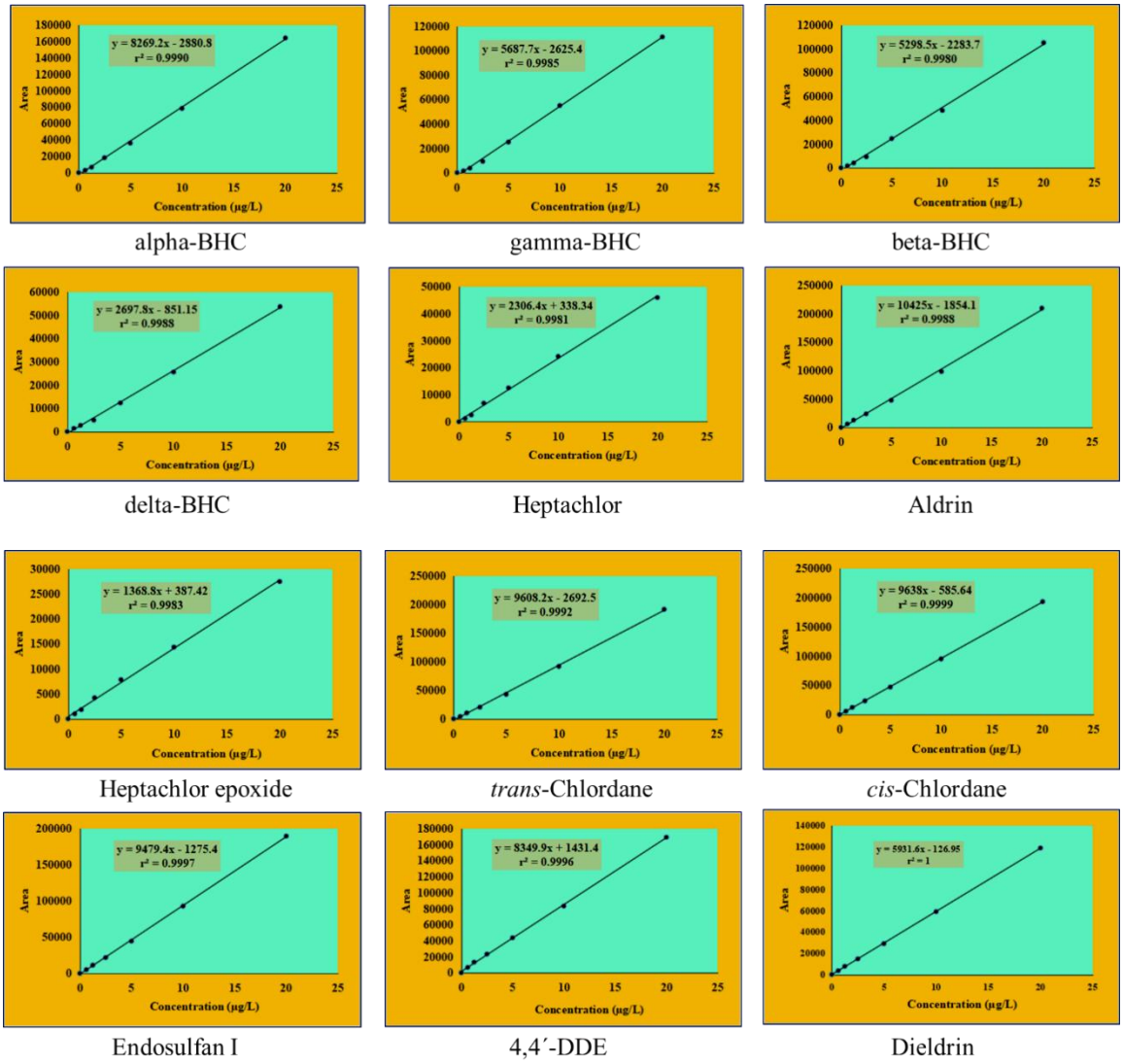


Figure 2.41 Matrix-matched calibration curves for 20-mixed OCPs in beef meat (1)

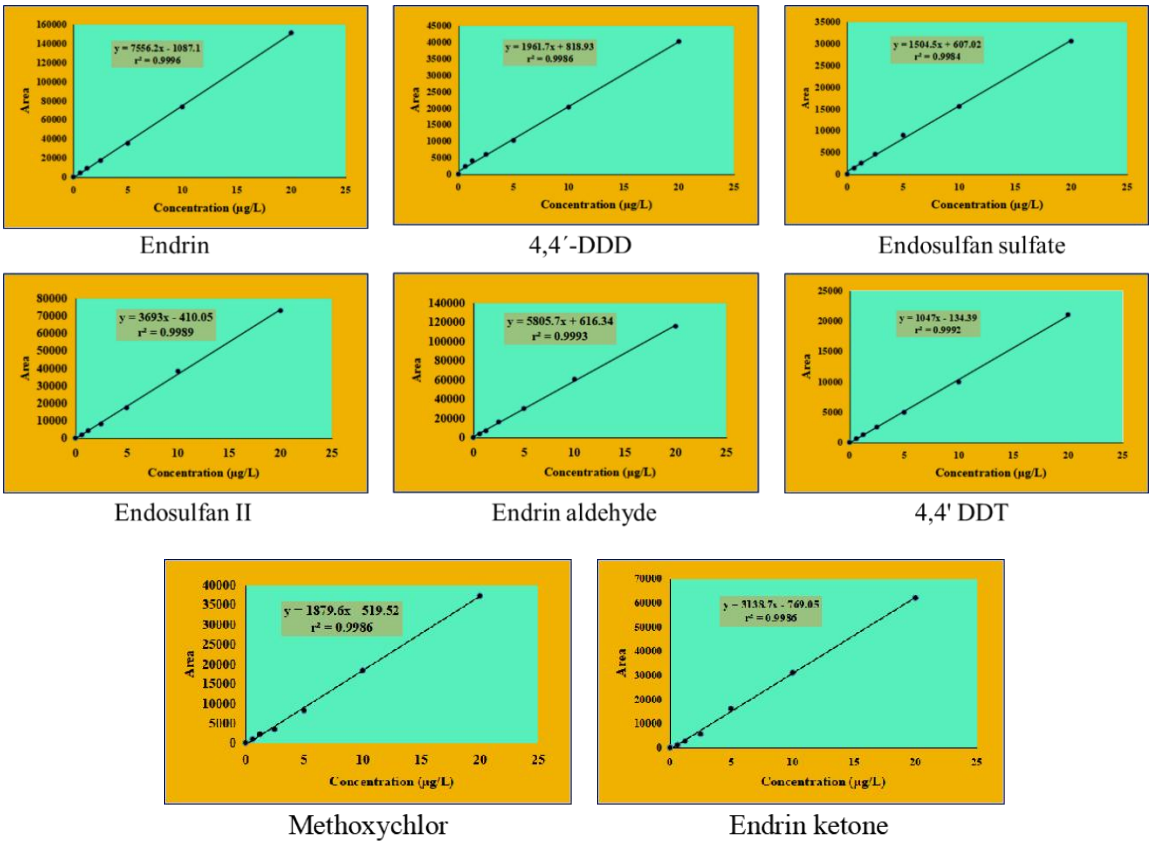


Figure 2.41 Matrix-matched calibration curves for 20-mixed OCPs in beef meat (2)

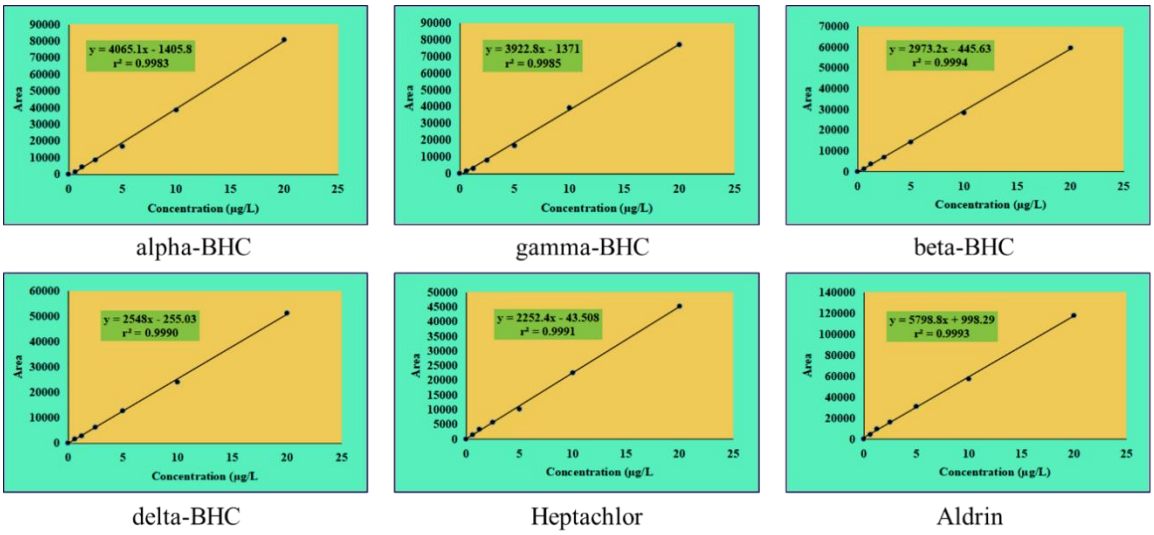


Figure 2.42 Matrix-matched calibration curves for 20-mixed OCPs in beef liver (1)

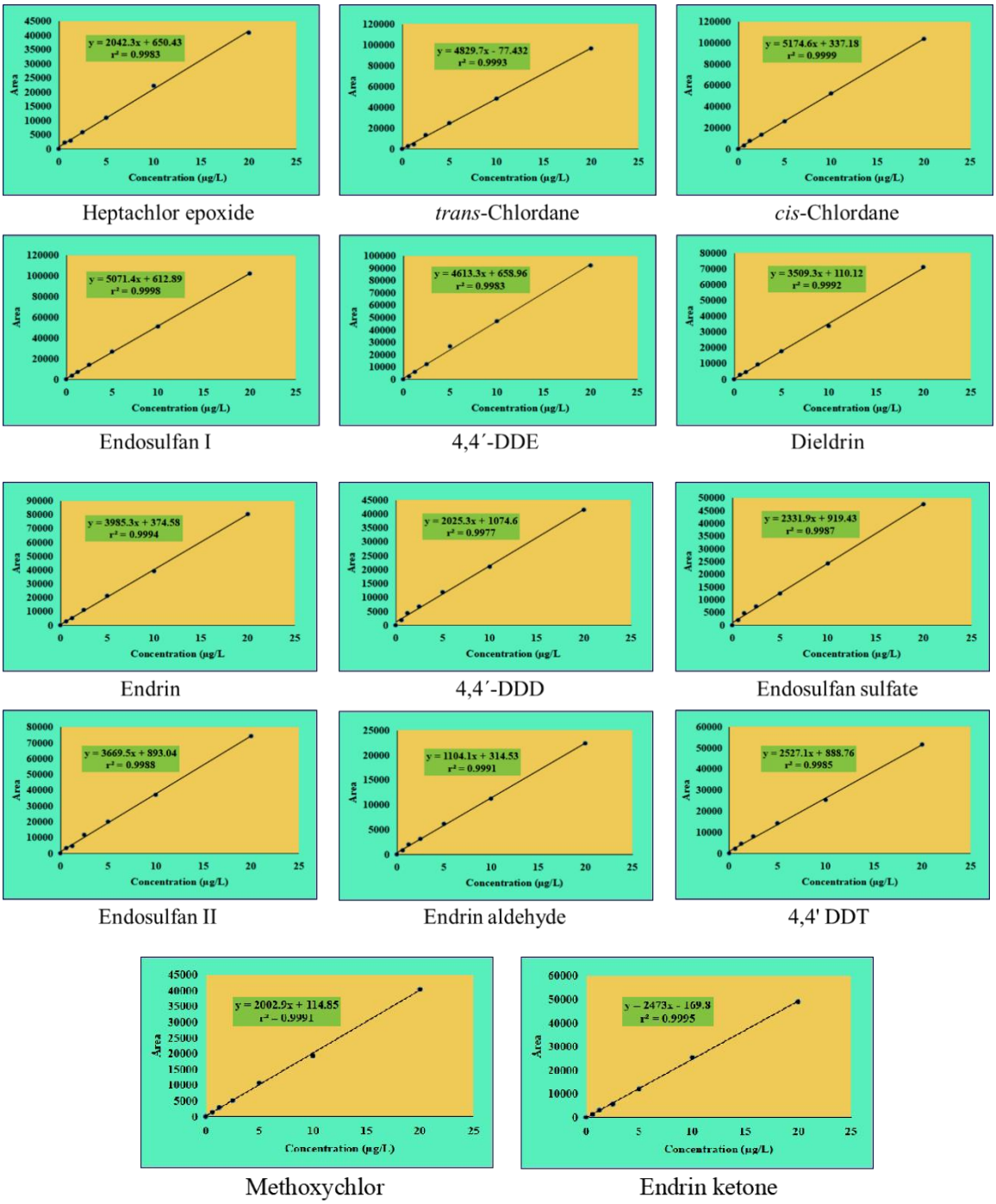


Figure 2.42 Matrix-matched calibration curves for 20-mixed OCPs in beef liver (2)

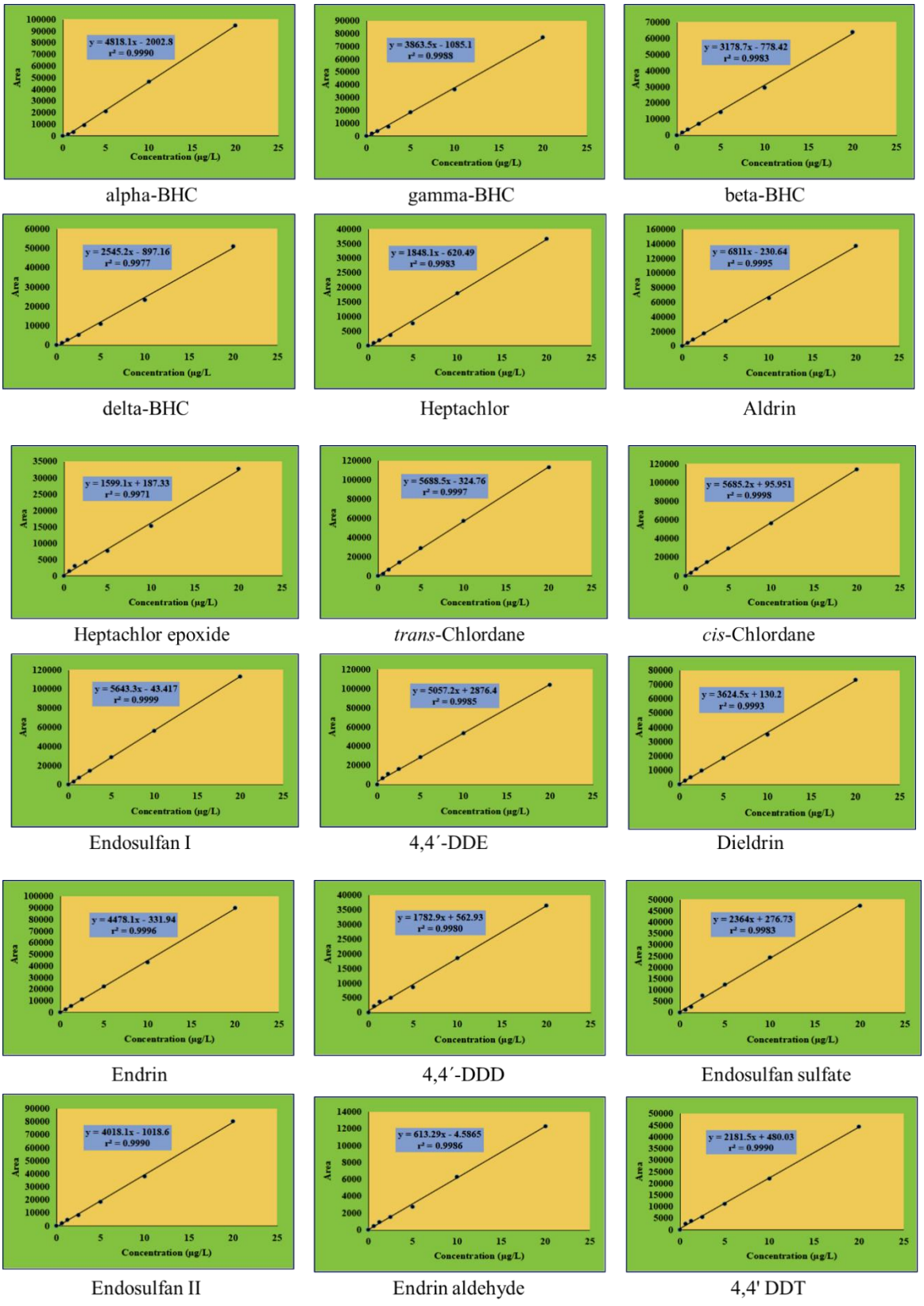
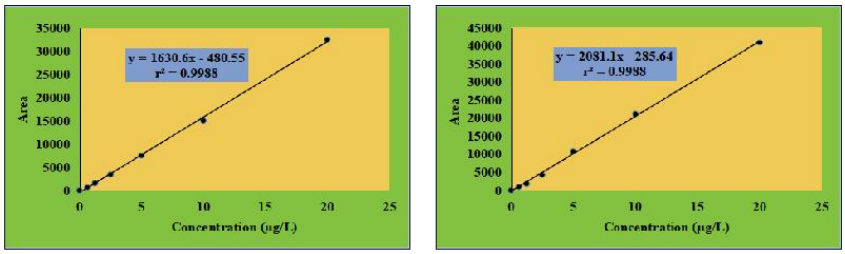


Figure 2.43 Matrix-matched calibration curves for 20-mixed OCPs in chicken meat (1)



Methoxychlor Endrin ketone

Figure 2.43 Matrix-matched calibration curves for 20-mixed OCPs in chicken meat (2)

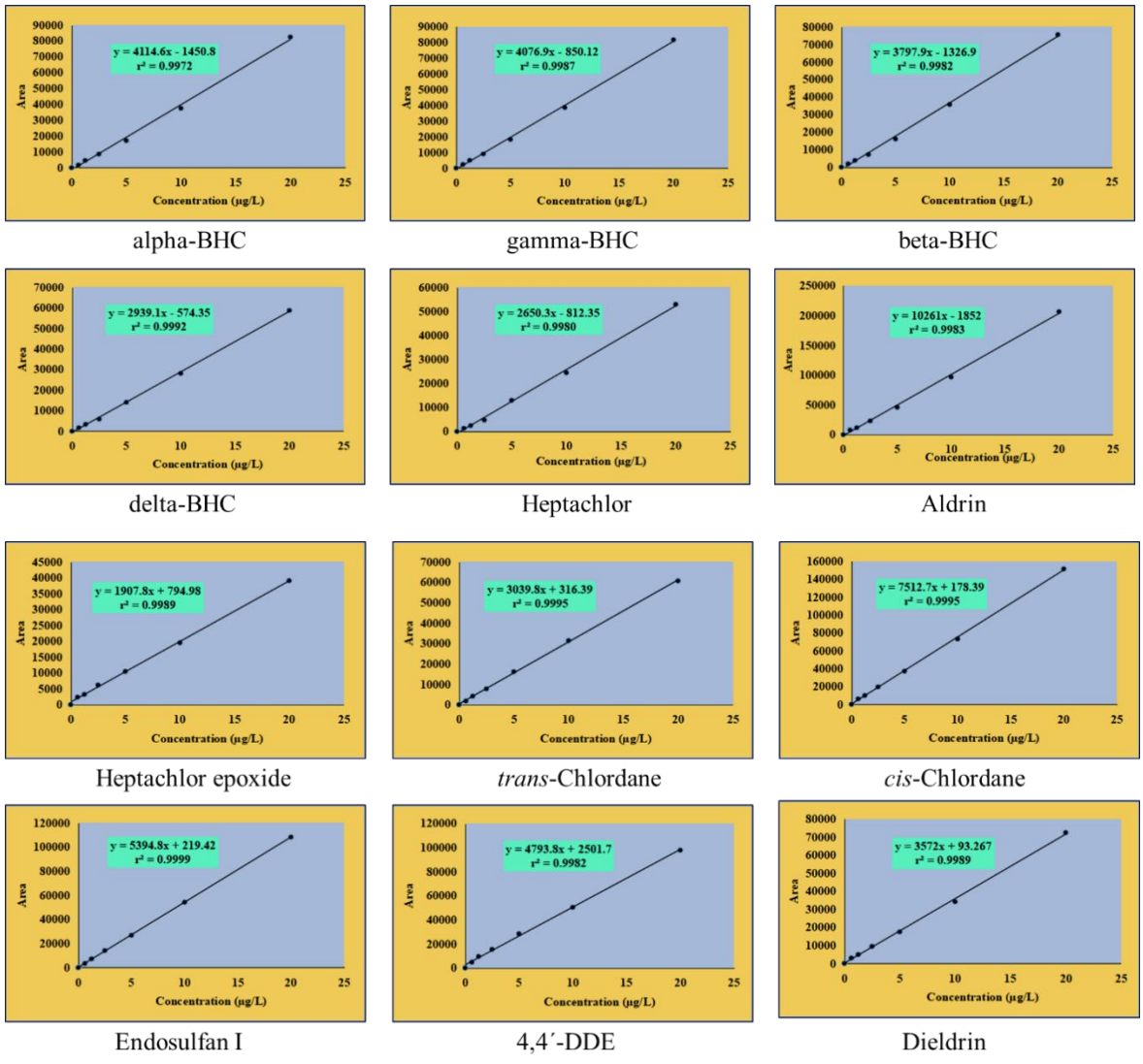


Figure 2.44 Matrix-matched calibration curves for 20-mixed OCPs in chicken liver (1)

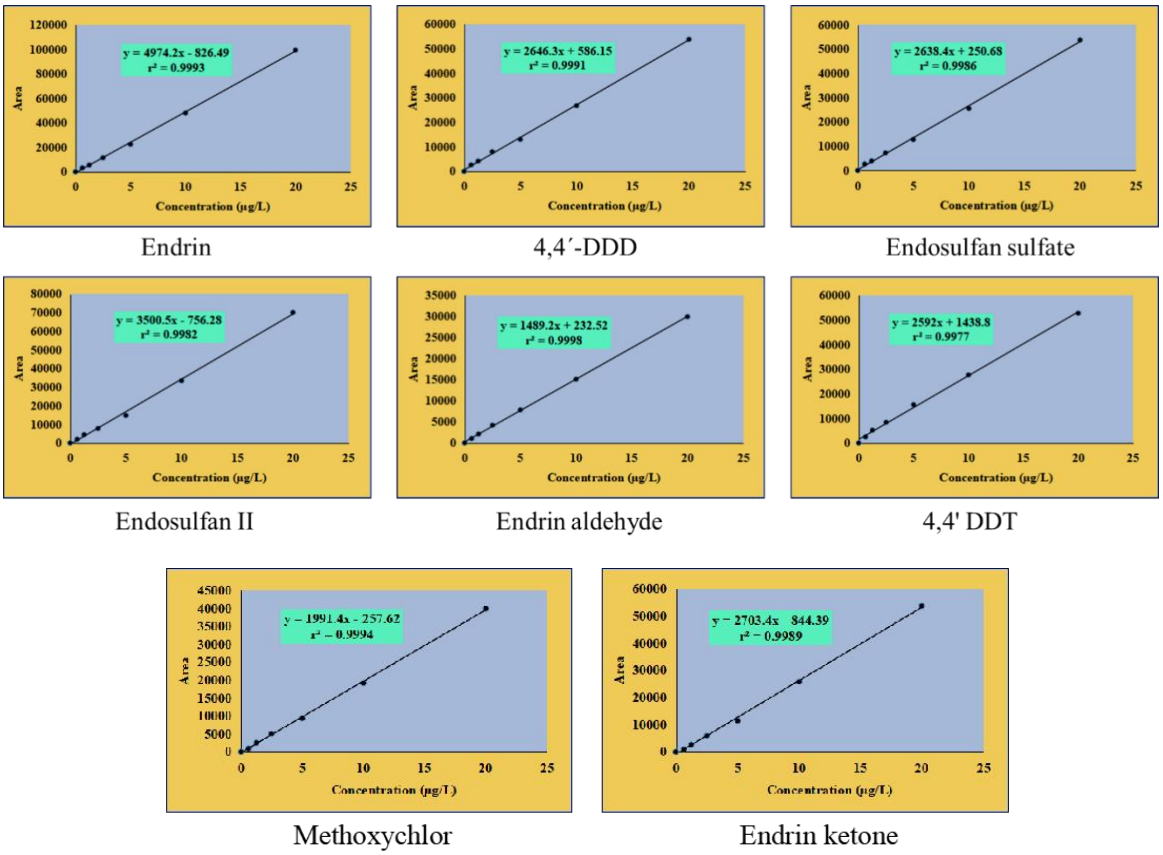


Figure 2.44 Matrix-matched calibration curves for 20-mixed OCPs in chicken liver (2)

2.13.6 Recovery Experiment

The experiment for intra-day recovery was conducted at two spiking concentrations of 2.5 and 5 µg/kg for beef meat, beef liver, chicken meat, and chicken liver, as indicated in Tables 3.30 and 3.33. The relative standard deviation (RSD) remained below 10%.

2.13.7 Limit of Detection (LOD)

The limit of detection (LOD) was established by injecting a solution of serially diluted OCP standard (composed of 20 components) into the GC-ECD. For determining the LOD, the peak area for each standard was assessed to be three times the baseline noise, indicating a signal-to-noise ratio of 3:1. The LOD for the method developed is presented in Table 3.27.

2.13.8 Limit of Quantification (LOQ)

The limit of quantification (LOQ) for each standard was based on a peak area that was deemed to be ten times the level of baseline noise, corresponding to a signal-to-noise ratio of 10:1. The LOQ for the method developed is presented in Table 3.27.

2.13.9 Health Risk Assessment

2.13.9.1 Estimated Daily Intake (EDI) for OCPs

The exposure assessment is outlined as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant" in the Codex Alimentarius Commission Procedural Manual [296, 297]. The lifetime exposure dose (mg/kg/day) for each pesticide exposure can be calculated by multiplying the residual pesticide concentration (mg/kg) in food items by the daily food intake rate (kg/day) of the population and dividing the result by the body weight (kg) [298]. The following formula was utilized to ascertain dietary exposure:

$$\mathbf{EDI = (FCC \times DFC)/BW \ldots \ldots \ldots \ldots \ldots (6)}$$

Here, EDI represents Estimated daily intake (EDI) (mg/(kg/day)); FCC indicated food chemical concentration (mg/kg); DFC is stand for daily food (meat) consumption (kg/day) and BW is used for Body weight (kg). In Bangladesh, the DFC are 14.7 (beef) and 33.1 (chicken) g/person/day for adults, and 8.3 g/person/day children, respectively. The information originated from the "Report of the Household Income and Expenditure Survey, HEIS 2022 and HEIS 2010" [292, 299]. BW represents body weight (60 kg for adults and 27 kg for children) [299] is considered for the dietary exposure calculation.

2.13.9.2 Hazard Risk Index (HI) for OCPs

A Health Risk index (HI) was created to represent the health risks that consumers posed from pesticide-contaminated fruits and vegetables. The estimated daily intake (EDI) was multiplied by the corresponding acceptable daily intake (ADI) values provided by WHO/FAO [300, 301] to determine the HI, which is represented by the following equation:

$$\mathbf{HI} = \frac{\mathbf{EDI}}{\mathbf{ADI}} \ldots\ldots\ldots\ldots\ldots (7)$$

For beef meat, HI_{BM} = Estimated daily intake of beef meat (EDI_{BM}) / ADI_{BM}
Here, EDI_{BM} stands for EDI of beef meat and ADI_{BM} stands for acceptable daily intake of pesticides.

For broiler chicken meat, HI_{CM} = Estimated daily intake of beef meat (EDI_{CM}) / ADI_{CM}
Here, EDI_{CM} stands for EDI of beef meat and ADI_{CM} stands for acceptable daily intake of pesticides.

Food is deemed acceptable when HI is less than 1. Food is deemed hazardous to consumers if the HI is higher than 1 [301, 302].

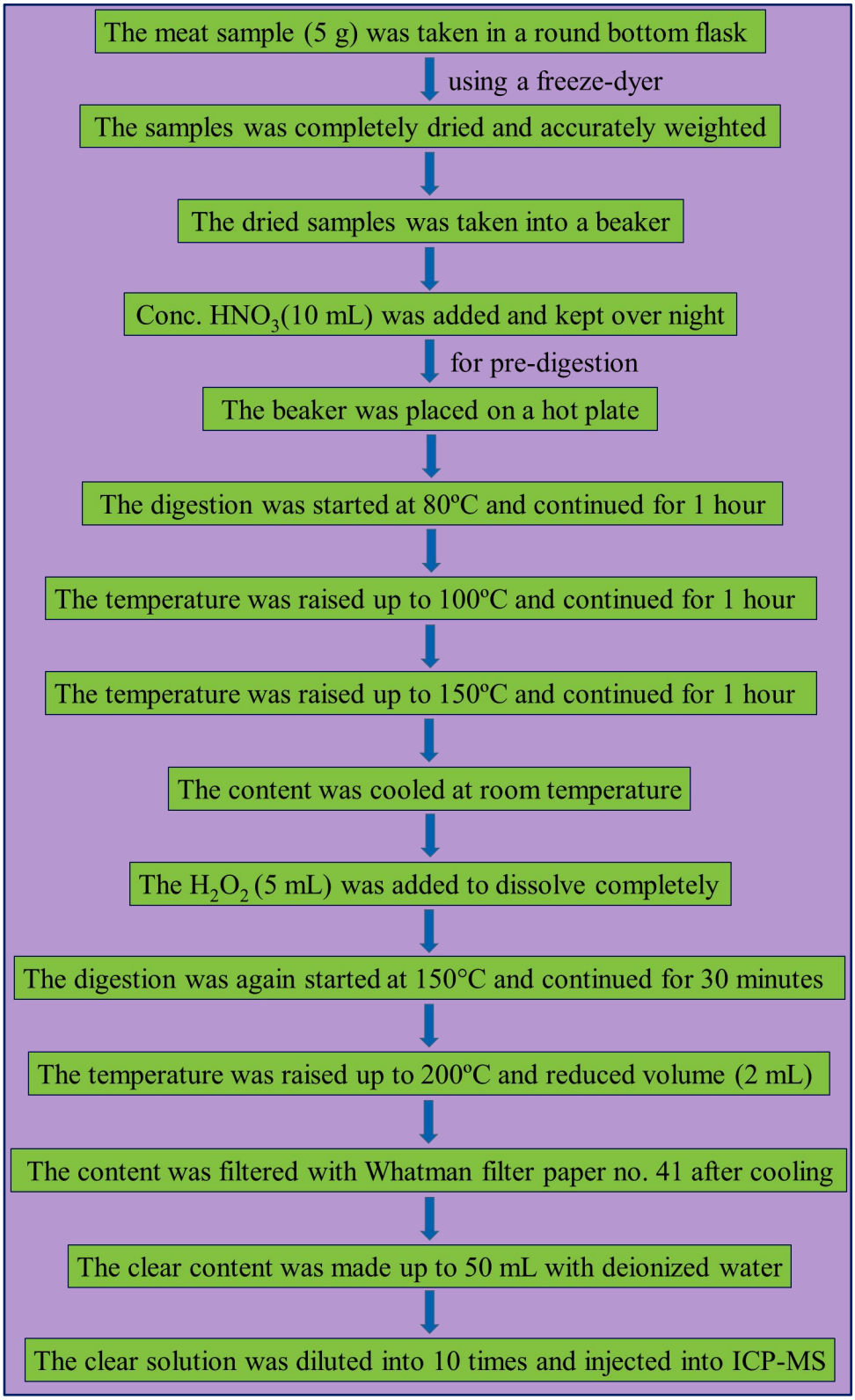
2.14 Analysis of Heavy Metals in Beef, Broiler Meat and Liver Samples

2.14.1 Samplings

Samplings were the same as in section 2.5.

2.14.2 Sample Preparation

All chemicals utilized were of analytical reagent quality, and deionized water was consistently applied during the analysis. HNO_3 (69% from VWR Chemicals, USA) and hydrogen peroxide (30% H_2O_2 from EMSURE, Darmstadt, Germany) were employed for the digestion of the sample matrix. Homogenized minced meat samples (5 g) were thoroughly dried using a freeze-dryer and precisely weighed. One gram of each dried sample was placed in a beaker, and 10 mL of concentrated HNO_3 (69% from Sigma-Aldrich) was added for overnight digestion. The samples were then transferred to a hot plate, starting digestion at 80°C, increasing to 100°C after one hour, and finally reaching 150°C after another hour for further digestion. Once the sample solutions had cooled to room temperature, 5 mL of hydrogen peroxide (H_2O_2) was added to ensure complete dissolution of the samples. Digestion resumed at 150°C, subsequently increasing to 200°C after 30 minutes, which reduced the volume by approximately 2 mL. After cooling the sample solutions, the mixtures were filtered through Whatman filter paper no. 41. Ultimately, each sample solution was diluted to a final volume of 50 mL with deionized water (Scheme 4).



Scheme 4 Sample preparation for heavy metals

2.14.3 Blank Preparation

To prevent contamination of reagents, a reagent blank was prepared, consisting of a solution for each sample group made with all the reagents except for the samples. The purpose of the reagent blank was to eliminate any impurities that may have been introduced during the dissolution of the sample in acid for each batch. Four reagent blank solutions were generated following the same procedure, and a nitric acid solution was utilized to prevent both chemical and sample contamination.

2.15 Overview of Method for Analysis of Heavy Metals (HMs)

2.15.1 Preparation of Primary Standard Solution

All chemicals utilized were of analytical reagent quality, and deionized water was consistently employed throughout the analysis. HNO_3 (69% from VWR Chemicals, USA) was used to prepare the standard stock solution. A standard stock solution containing 1000 µg/L of the relevant metals was created in a 2% (w/w) HNO_3 solution. The glassware was soaked in a 10% (w/w) HNO_3 solution for 24 hours, then rinsed with distilled water and dried in a dust-free environment prior to use.

2.15.2 Preparation of Secondary and Working Standard Solution

A secondary standard solution of 500 µg/L was created from the primary stock solution. Working solutions of 4, 8, 12, and 20 µg/L for the specific heavy metals (Cr, Ni, Pb, As, Mn, Co, Cu, and Zn) and 1, 2, 3, and 5 µg/L for Cd and Se were prepared by diluting the secondary solution with nitric acid.

2.15.3 ICP-MS Conditions for Heavy Metals

The manufacturer's instructions regarding instrument configuration and operating conditions were adhered to. The ICP-MS operational requirements are given in Table 2.11.

Table 2.11 NexiON ICP-MS Instrument Components and Operating Conditions

Instrument Component	**Type / Value**
Nebulizer	ST-PFA Microflow
Spray Chamber	Quartz cyclonic
Torch	One-piece quartz torch, 2 mm i.d. injector
Cones	Nickel sampler and skimmer Aluminium hyper-skimmer
Peristaltic Pump Tubing	Carrier/internal standard: orange/green (0.38 mm i.d)
	Waste: gray/gray Santoprene (1.30 mm i.d.)
Sample Uptake Rate	0.2 mL/min
Operating Conditions	**Type/Value**
RF Power	1600 w
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	1.2 L/min
Nebulizer Gas Flow	Optimized for CeO^+/Ce^+ <2.5 %; Ce^{++}/Ce <2.5 %
Cell Gas	Oxygen, Helium

Using equation 8, the following trace metal concentrations (in mg/kg, fresh weight) were found in this investigation,

$$\textbf{Element}, (\mu g/kg) = (R_{sample} - R_{blank}) \times DF \times (V_{extract}/W_{sample}) \times 10^{-3} \quad \ldots\ldots\ldots\ldots (8)$$

2.15.4 Calibration Curves for Heavy Metals

An ICP-multi-element standard solution XIII (Merck, Germany) was used to prepare standard calibration curves at parts per billion (ppb) levels. The calibration curves were made by injecting solutions at concentrations 4, 8, 12, and 20 µg/L of the respective heavy metals (Cr, Ni, Pb, As, Mn, Co, Cu and Zn) and 1, 2, 3 and 5 µg/L for Cd and Se in the ICP-MS. MS Excel software was used to plot the integrated peak regions versus the standard concentrations for each metal. The calibration curves of the standard of heavy

metals are presented in Figures 2.45 and 2.46. Peak areas below the detection limit were not included in the curve. The correlation coefficient (r^2) was found to be linear with 0.9988, 0.9989, 0.9990, 0.9991, 0.9991, 0.9994, 0.9991, 0.9994, 0.9998 and 0.9993 for chromium (Cr), nickel (Ni), lead (Pb), cadmium (Cd), arsenic (As), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn) and selenium (Se), respectively.

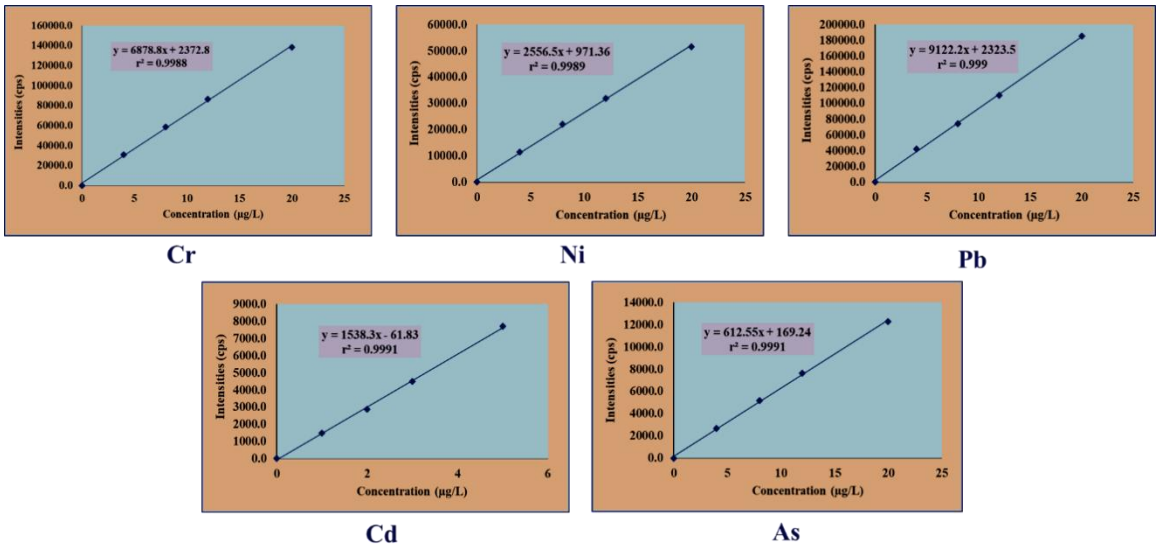


Figure 2.45 Calibration curves for heavy metals (Cr, Ni, Pb, Cd and As)

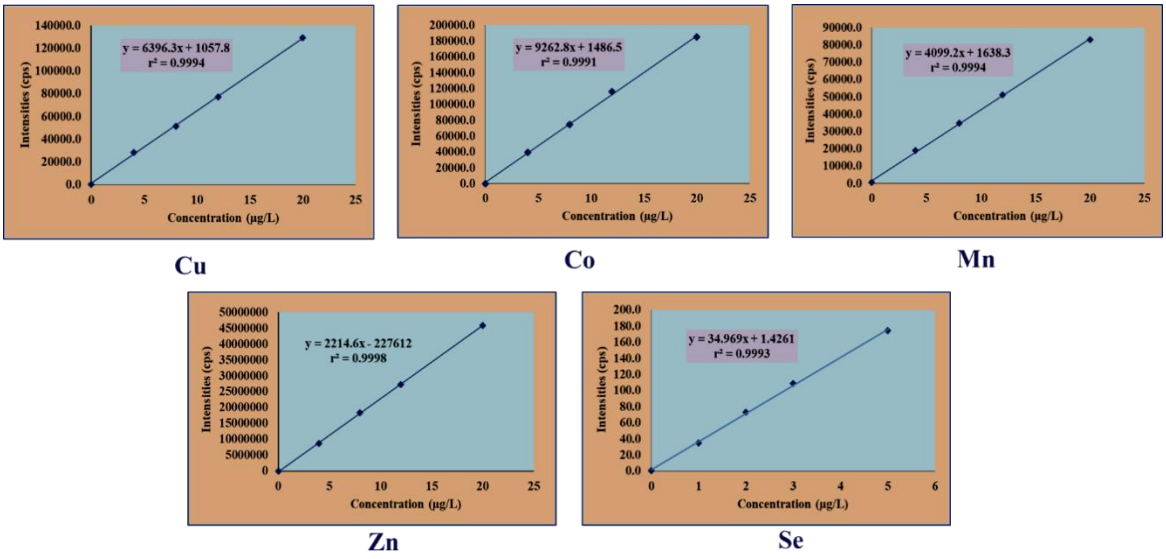


Figure 2.46 Calibration curves for heavy metals (Mn, Co, Cu, Zn and Se)

2.15.5 LOD and LOQ for Heavy Metals

LOD was determined by injecting a serially diluted heavy metal standard (mix-10 components) solution in ICP-MS. For LOD, the peak area of each standard was considered 3 times higher than the baseline noise *i.e.*, signal to noise ratio was 3:1. LOQ the peak area of each standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The LOD and LOQ for ten heavy metals is presented in Table 3.36.

2.15.6 Sample Analysis

The levels of heavy metals were assessed using inductively coupled plasma-mass spectrometry (ICP-MS; PerkinElmer, Model: NexION 2000; USA). The sample, which is nebulized, can enter the center of the argon (Ar) plasma at a rate of approximately 0.35 ml/min in the ICP-MS system. A quadrupole mass detector analyzed the heavy metal ions produced in the high-temperature plasma by measuring their mass to charge ratio (m/z).

2.15.7 Quality Assurance and Quality Control

All glassware used in this experiment was cleaned and rinsed with ultra-pure acid and water to reduce the possibility of contamination during sample preparation and analytical operations. The performance of ICP-MS equipment was checked by NexION Setup solution (PerkinElmer, USA) before calibration with standards. A five-point calibration variance, $r^2 > 0.9995$, was achieved over a dynamic range of 1.0 to 25 ng/mL. A minimum of three replicates were conducted to confirm instrument stability. The maximum relative standard deviation (RSD) of 5–8% was considered. The instrument's detection (DL) and quantification (QL) limits were defined when the response for specific metal was three and ten times greater than the background noise, respectively. A standard reference material SRM 1643f (Trace Elements in Water) from the National Institute of Standards (NIST) was used to check the recovery percentage of elements in the sample and showed 96–103.7% recovery for significant elements. The procedure blanks, SRM recoveries, and spike recoveries were performed as part of the quality control process to minimize error. The concentrations of trace metals were corrected from recovery and blank sample analysis.

2.15.8 Health Risk Assessment

Human health risk assessment estimates the probability that exposure to particular pollutants for a specified time will harm human health. There are many ways that pollutants might come into contact with people. These pathways could be gastrointestinal,

respiratory, or cutaneous. This research assessed the dangers of consuming heavy metals while eating edible beef, chicken meat, and liver [303].

2.15.8.1 Estimated Daily Intake (EDI) of Heavy Metals

The mean metal concentration of samples, a daily meat intake rate, and the body weight of a individual were used to determine the EDI of specific heavy metals. Equation (9) was utilized to compute in accordance with [304]

$$\mathbf{EDI = (FIR \times MC)/BW \ldots \ldots \ldots \ldots \ldots (9)}$$

FIR is the daily food consumption rate (g/person/day). In Bangladesh, the FIR are 14.7 (beef) and 33.1 (chicken) g/person/day for adults, and 8.3 g/person/day children, respectively. The information originated from the "Report of the Household Income and Expenditure Survey, HEIS 2022 and HEIS 2010" [292, 299]. The symbol MC represents the mean metal content (mg/kg fresh weight) in chicken samples. BW represents Body weight (60 kg for adults and 27 kg for children) [305].

2.15.8.2 Target Hazard Quotient (THQ)

The non-carcinogenic health hazards associated with meat consumption were examined in this study using target hazard quotients (THQs), and calculations were carried out utilizing the assumption of meat consumption for an integrated USEPA risk analysis, as shown by Equation 10 taken from [306, 307].

$$\mathbf{THQ = \frac{EFr \times ED \times FIR \times MC}{Rf D \times BW \times ATn} \times 10^{-3} \ldots \ldots \ldots \ldots \ldots (10)}$$

Where, EFr is the exposure frequency (365 days a year), ED is the exposure duration (70 years for adults and 14 years for children), and THQ is the dimensionless target hazard quotient, all of which are comparable to the average human lifetime [305]. The acronyms FIR (grams per person per day), MC (milligrams per kilogram fresh weight), BW (average body weight; adults weigh 60 kg, children weigh 27 kg), AT (averaging time for non-carcinogens; 365 days/year number of exposure years) [305] and RfD (oral reference dose; milligrams per kilogram day) are used in scientific research. The RfDs for Cr, Ni, Pb, Cd, As, Mn, Co, Cu, Zn and Se are 1.5, 0.02, 0.0035, 0.0005, 0.003, 0.024, 0.0003, 0.04, 0.3 and 0.005 based on mg/kg/day, respectively [308]. The RfDs estimate the daily exposure to which an individual may be subjected continuously for the duration of their lives

without really running the danger of negative effects. A potential health issue exists if the THQ is equal to or more than one, in which case relevant interventions and preventative measures ought to be put in place [309]. Addiction and interaction effects have been related to exposure to two or more harmful components [310]. The total THQ (TTHQ, individual meat item) of heavy metals for each type of meat was determined by adding the THQ values for each metal [311].

$$\mathbf{TTHQ = TTHQ\ metal1 + TTHQ\ metal2 + \ldots + TTHQ\ metaln} \ldots\ldots\ldots\ldots (\mathbf{11})$$

The Hazard Index (HI) method originated by the USEPA to assess the potential risk of non-carcinogenic effects offered by a variety of metals [306, 312]. For a specific receptor/pathway/combination (e.g., food), HI was obtained as follows (Eq. 12).

$$\mathbf{HI = TTHQ1 + TTHQ2 + TTHQ3 + \ldots + TTHQn} \ldots\ldots\ldots\ldots\ldots (\mathbf{12})$$

There is a risk of health issues when HI is greater than one [313].

2.15.8.3 Target Carcinogenic Risk (TCR)

Carcinogen risks were determined as the total probability (i.e., incremental or excess individual lifetime cancer risk) that an individual will develop cancer during their lifetime if exposed to that possible carcinogen. The appropriate risk ranges for carcinogens are 10^{-4} to 10^{-6}. To determine the target cancer risk (lifetime cancer risk), apply the following formula [306].

$$\mathbf{TCR} = \frac{\mathbf{EFr \times ED \times FIR \times MC \times CSFo}}{\mathbf{BW}} \times \mathbf{10^{-3}} \ldots\ldots\ldots\ldots\ldots (\mathbf{13})$$

Where TCR is the target cancer risk and CSF_o is the oral carcinogenic slope factor. The CSF_o for Cr, Ni, Pb, Cd and As was 0.5, 1.7, 0.0085, 0.38 and 1.5 (mg/kg/day)-1 [307, 314].

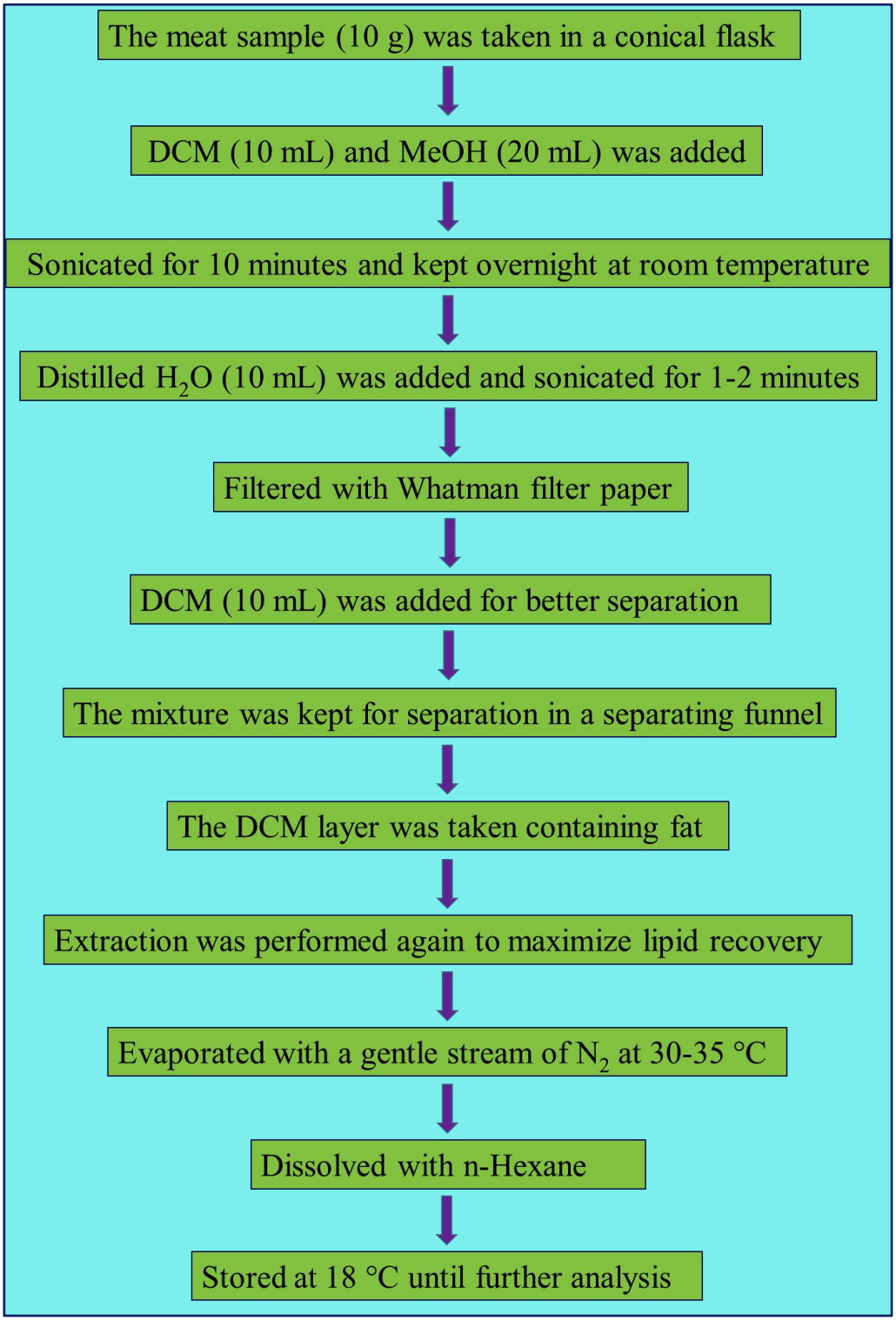
2.16 Analysis of Fatty Acids in Beef, Broiler Chicken Meat and Liver Samples

2.16.1 Samplings

Samplings were the same as in section 2.9.

2.16.2 Sample Preparation

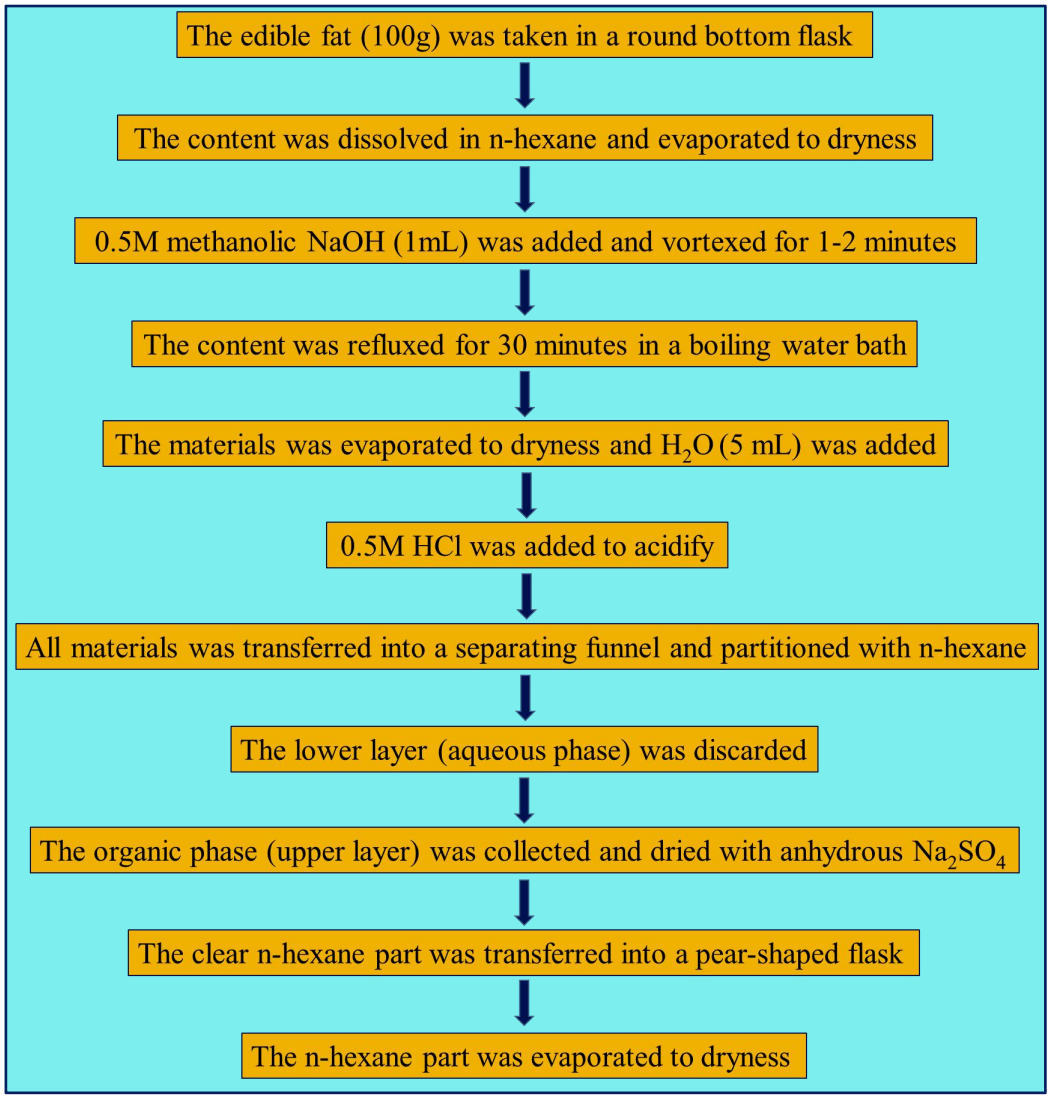
A 10 g meat sample was weighed and placed into a conical flask. Subsequently, 10 mL of dichloromethane (DCM) and 20 mL of methanol were introduced. The sample underwent sonication for 10 minutes, after which it was left at room temperature overnight. Following this, 10 mL of distilled water was added and the mixture was sonicated for an additional 1-2 minutes. The mixture was then filtered using Whatman filter paper. Another 10 mL of dichloromethane was added to the filtrate to enhance separation. The filtrate was then transferred to a separating funnel and allowed to settle for a period to facilitate separation. The dichloromethane layer, which contained the fat, was collected. To ensure maximum lipid recovery, the extraction was repeated. The combined filtrate was evaporated completely under a gentle stream of N_2 gas at a temperature of 30-35°C or utilizing a rotary evaporator. The resulting dry material was then reconstituted in n-hexane and stored at -18°C in a refrigerator for subsequent analysis (Scheme 5).



Scheme 5 Extraction of fatty acids

2.16.3 Saponification of Fatty Acids

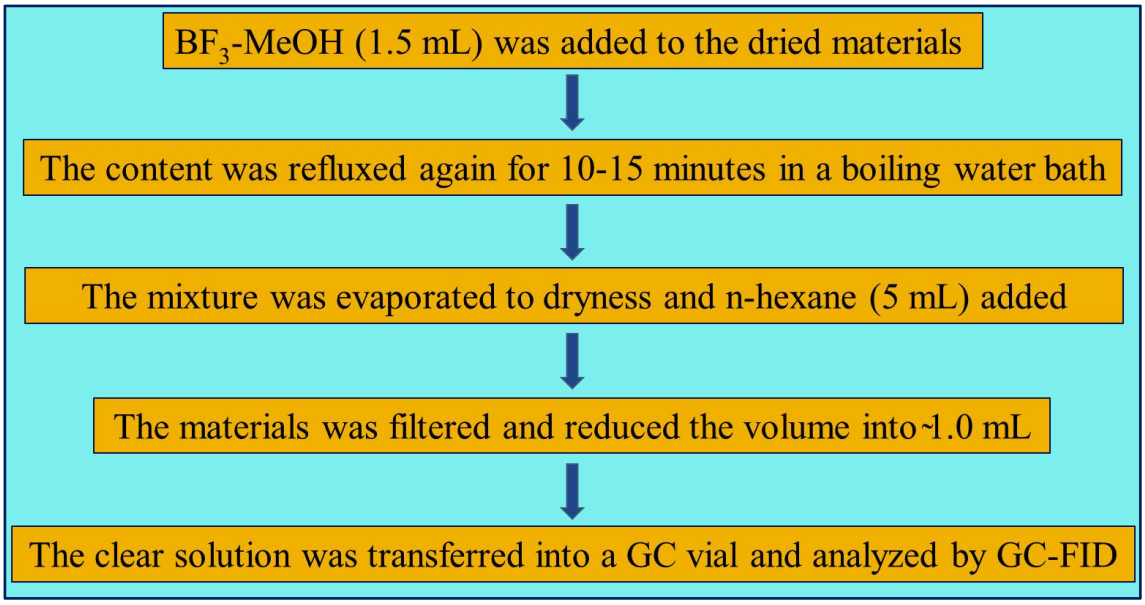
A round bottom flask was used to take in 100 mg of edible fat. The fat was dissolved in n-hexane and then dried using a rotary evaporator. Following this, a methanolic sodium hydroxide (NaOH) solution of 0.5 M was introduced, and the mixture was vortexed for 1-2 minutes. The mixture was refluxed for a duration of 30 minutes in a boiling water bath. After evaporation to dryness, 5 mL of distilled water was added. Hydrochloric acid (HCl) at a concentration of 0.5 M was used to acidify the solution. The entire mixture was then transferred to a separating funnel and extracted with n-hexane. The aqueous layer was discarded. The organic layer (upper phase) was collected and dried using anhydrous sodium sulfate (Na_2SO_4) to eliminate water. The clear organic phase in n-hexane was placed in a pear-shaped flask and evaporated to dryness (Scheme 6).



Scheme 6 Saponification of fatty acids

2.16.4 Conversion of Fatty Acids into Methyl Ester

Following saponification, 1.5 mL of the boron trifluoride-methanol (BF_3-MeOH) complex was added to the dried substances. The mixture was then refluxed for an additional 10-15 minutes in a boiling water bath. Subsequently, the solution was filtered through Whatman filter paper and concentrated to approximately 1 mL. The resulting clear solution was then placed into a GC vial for analysis using GC-FID (Scheme 7).



Scheme 7 Conversion of fatty acids into methyl ester

2.17 Overview of Method for Analysis of Fatty Acids (FAs)

2.17.1 Preparation of Primary Standard Solution for Methyl Ester of Fatty Acids

Certified Standard (FAME, 100 mg, mix-20 components) was acquired from Restek, USA and dissolved with HPLC grade dichloromethane (DCM) in a 100 mL volumetric flask to create a concentrated primary stock standard solution of 1000 mg/L. The prepared solution's label included the standard's name, concentration, and preparation date. Prior to reuse, the meniscuses of the solutions were marked using permanent black ink and stored in a refrigerator at -20°C, separated from the sample storage area.

2.17.2 Preparation of Secondary Standard Solution for Methyl Ester of Fatty Acids

A secondary stock solution at a concentration of 500 mg/L was created using dichloromethane (DCM) from the primary stock solution. This 500 mg/L solution was subsequently serially diluted with the same solvent to produce daily working solutions of 250.0, 125, 62.5, 31.25, 15.615, and 7.8125 mg/L.

2.17.3 GC-FID Conditions for FAME Analysis

The FAMEs were examined using a Shimadzu GC-2030 Plus gas chromatograph that included a flame ionization detector unit (Shimadzu, Tokyo, Japan). For the chromatographic assessment of the FAMEs, a high-polarity SH-FAME (Crossbond Carbowax Polyethylene Glycol, 30 m x 0.32 mm x 0.25 µm, Shimadzu, USA) fused silica column was utilized. Nitrogen (99% purity) was used as the carrier gas. The hydrogen flow rate was set to 32 mL/min, the air flow rate to 200 mL/min, and the remaining flow to 24 mL/min. The gas chromatography parameters were established as follows: injector temperature at 250°C, detector temperature at 250°C, split less mode set to 20:0, and total flow at 30 mL/min. The initial column temperature program commenced at 150°C, increased at a rate of 5°C/min to 200°C, held for 10 minutes, and then increased again at 7°C/min to 240°C and held for another 10 minutes. An injection volume of 1 µL was applied. The entire analysis duration was 36 minutes.

2.17.4 Calibration Curves for FAMEs Standard

Solutions containing 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µg/L of the corresponding methyl ester of fatty acids (FAMEs) standard were injected into the GC-FID to create the calibration curves. The integrated peak areas were then plotted versus the standard concentration using Microsoft Excel software. Peak areas below the detection limit were not included in the curves. Correlation coefficients (r^2) were found to be linear with 0.9998, 0.9994, 0.9999, 0.9999, 0.9999, 0.9999, 0.9999, 1.0, 0.9999, 0.9998, 0.9999, 0.9994, 1.0, 0.9999, 0.9999, 0.9995, 0.9999, 0.9990, 0.9994 and 0.9997 for Methyl myristate, Methyl myristoleate, Methyl palmitate, Methyl palmitoleate, Methyl stearate, Methyl oleate, Methyl vaccenate, Methyl linoleate, Methyl linolenate, Methyl arachidate, Methyl 11-ecosenate, Methyl 11-14 eicosadienoate, Methyl arachidonate, Methyl eicosatrienoate, Methyl eicosapentanoate, Methyl behenate, Methyl erucate, Methyl lignocerate, Methyl docosahexaenoate and Methyl nervonate, respectively (Figure 2.47).

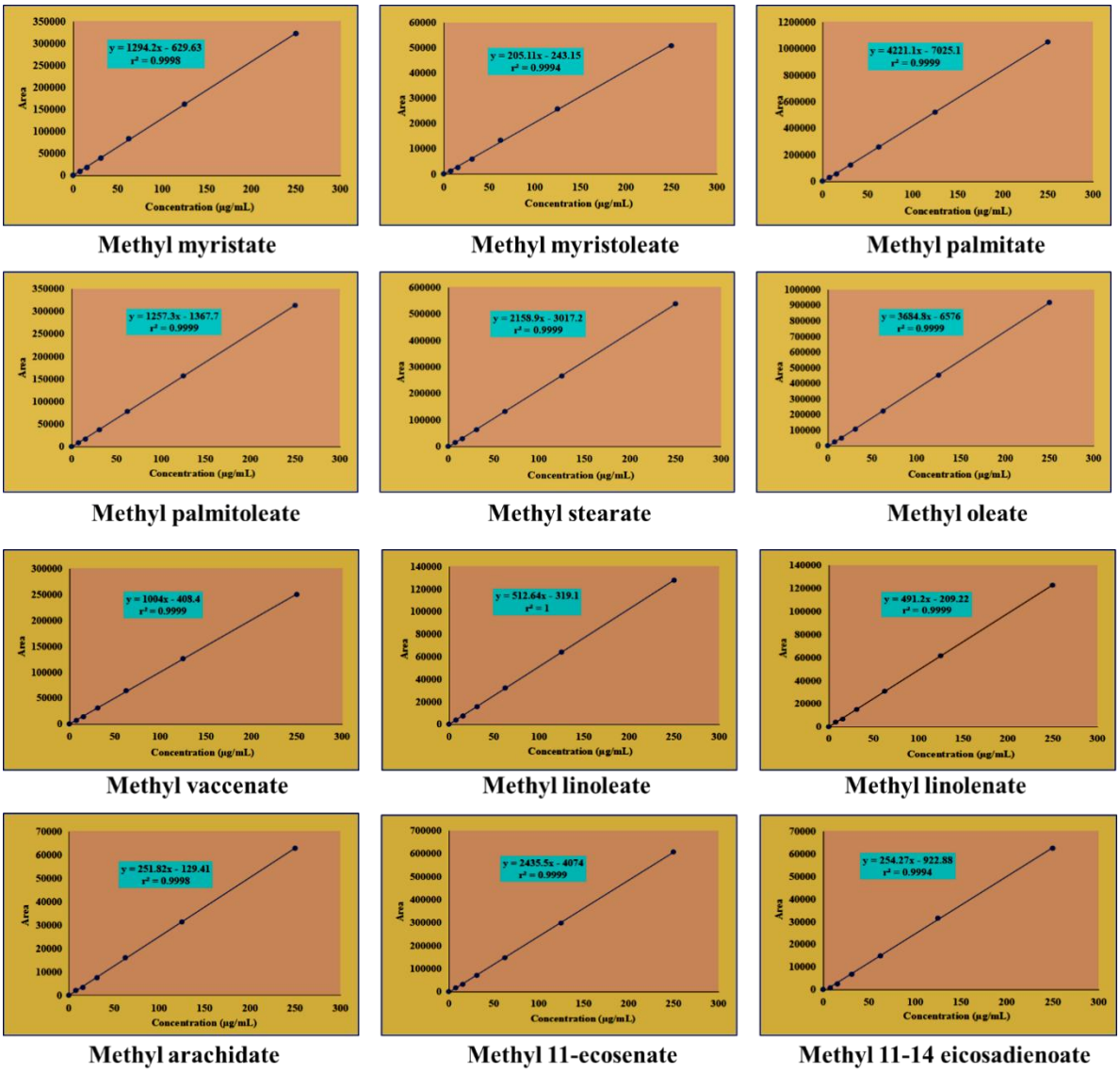


Figure 2.47 Calibration curves of methyl esters of fatty acids (1)

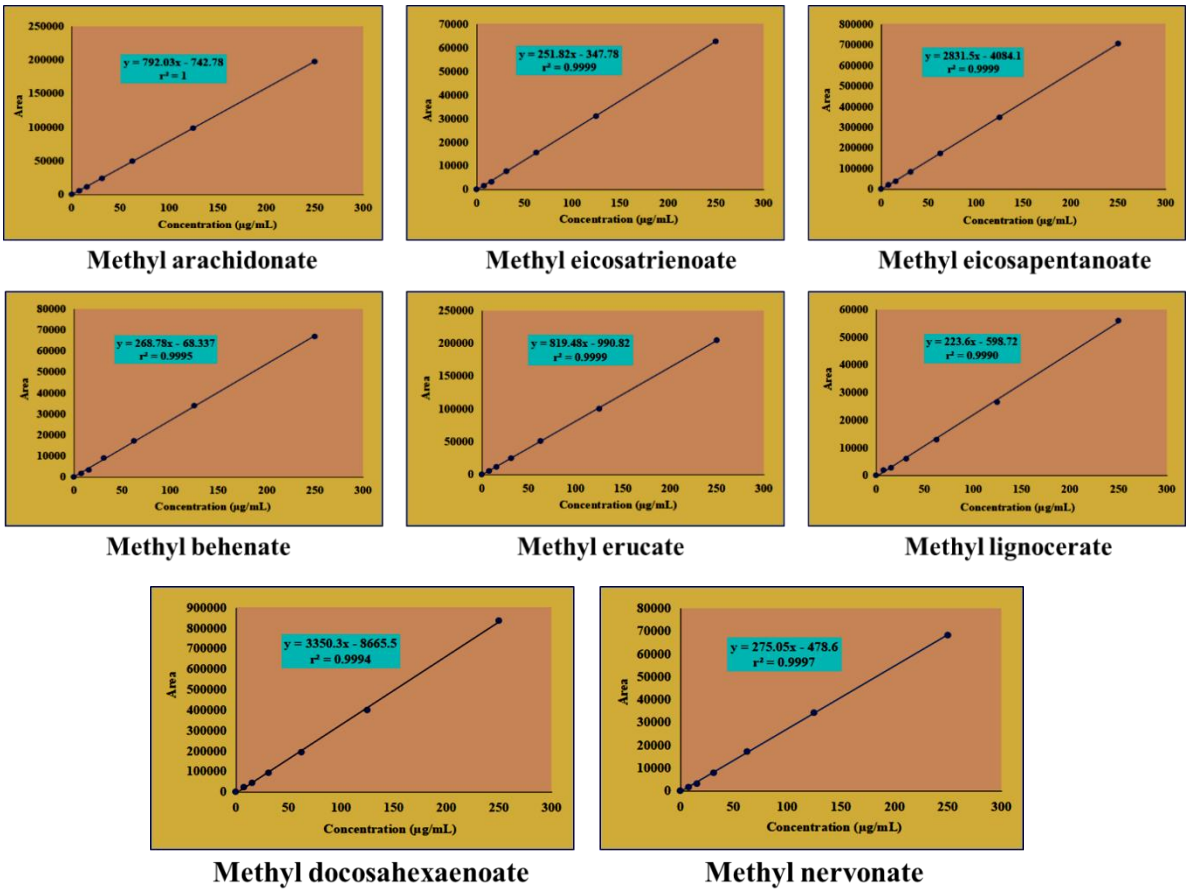


Figure 2.47 Calibration curves of methyl esters of fatty acids (2)

2.17.5 Fat Content

The percentage of fat was calculated using the following formula (14).

$$\textbf{Fat}(\%) = \frac{\textbf{W2} - \textbf{W1}}{\textbf{Sample Weight}} \times \textbf{100} \ldots\ldots\ldots\ldots\ldots\ldots (\textbf{14})$$

Where, W_1 is the weight of a round bottom flask without a sample,
W_2 is the weight of a round bottom flask with a sample.

2.17.6 Composition of Fatty Acids

The following formula (15) calculated the composition of fatty acids in beef meat and liver, chicken meat and liver.

$$\textbf{Relative Abundance of FA}\ (\%) = \frac{\textbf{Area of Indivisual FA}}{\textbf{Total Area}} \times \textbf{100} \ldots\ldots\ldots\ldots (\textbf{15})$$

Here, FA= Fatty Acid

2.17.7 Limit of Detection (LOD)

The limit of detection (LOD) was determined by injecting a serially diluted FAMEs standard (mix-20 components) solution in GC-ECD. For LOD, the peak area of each standard was considered 3 times higher than the baseline noise *i.e*., signal to noise ratio was 3:1. The LOD of the developed method is shown in Table 3.49.

2.17.8 Limit of Quantification (LOQ)

Limit of Quantitation (LOQ), the peak area of each standard was considered 10 times higher than the baseline noise i.e., signal to noise ratio was 10:1. The LOQ of the developed method is shown in Table 3.49.

Chapter 3

RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSIONS

3.1 Analysis of Tetracycline Antibiotics in Broiler Chicken Meat

3.1.1 Determination of Residual TCs in Broiler Chicken Meat

A total of thirty samples of chicken meat (n=30) were acquired from various superstores and local markets in Dhaka, and a control sample free from antibiotics was obtained from a rural area. Three samples from each of the ten different locations were collected. Each of these samples had a weight ranging from 900 to 1000 grams. The poultry meat samples, which consisted of uniform muscle parts (thigh and breast), were stored at -20°C until they were analyzed.

Analysis of poultry meat samples for residual tetracycline (OTC, TC, and CTC) was conducted using reversed-phase HPLC (HPLC; RF 1200, Prominence, Shimadzu) equipped with a photodiode array detector (PDA; SPD-M20A Prominence). On the C18 column, the TCs standard solution was separated at two wavelengths: 360 and 375 nm. The average retention times of OTC, TC, and CTC were 3.8, 4.3, and 7.8 minutes, respectively. For every standard OTC, TC, and CTC, linear calibration curves were generated in the 0 to 10 µg/L range. The correlation coefficient (r^2) for each standard was between 0.99 and 1.00 (Figure 3.1). This investigation was aimed at evaluating the validity of the proposed method. The calibration curve in the range of 0 to 10 µg/kg (0, 1, 2.5, 5, and 10 µg/kg) for the OTC, TC, and CTC standard solutions at five points with triplicate analysis (Table 3.1).

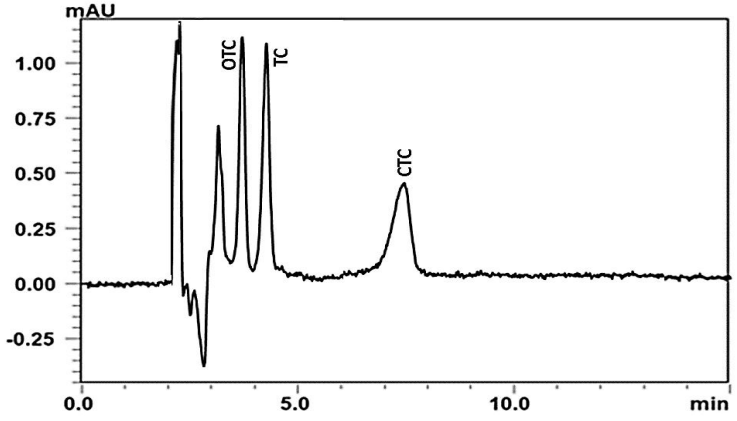


Figure 3.1 Chromatogram of TCs standard

Matrix-matched calibration curves were also obtained, and a similar correlation coefficient was found for poultry meat samples (Figure 3.2). The correlation coefficient obtained for each calibration curve showed that the correlation between peak area and concentration was excellent.

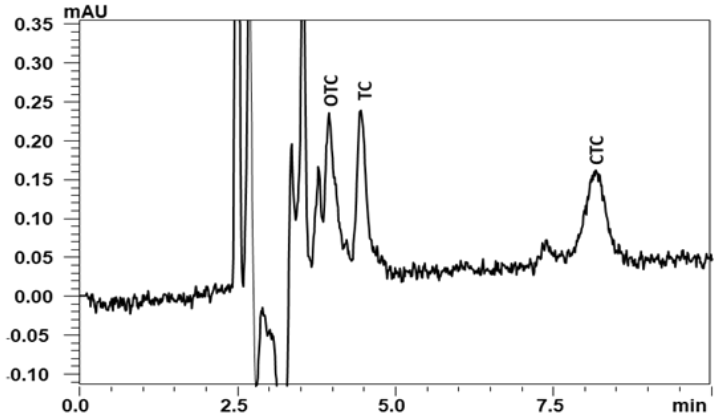


Figure 3.2 Chromatogram of TC standards at spiking level 5 µg/kg

Table 3.1 Slope, intercept and correlation coefficient (r^2) of TCs standard and matrix-match TCs for broiler chicken meat

Antibiotic	Linear Regression Equation of standard	Correlation coefficient (r^2) (standard)	Linear Regression Equation of matrix-matched	Correlation coefficient (r^2) (matrix-matched)
OTC	Y = 342.43x -37.99	1.000	Y=242.40x + 19.13	0.999
TC	Y = 161.10x +13.73	0.997	Y = 447.31 + 56.76	0.999
CTC	Y = 616.98x + 170.16	0.996	Y = 577.25 + 85.78	0.999

The experiment aimed at assessing recovery was conducted at two spiking concentrations (2.5 and 5 µg/kg) for meat from broiler chickens. The average recoveries were found to be 91, 102, and 106% at 2.5 µg/kg for OTC, TC, and CTC, respectively; all TCs showed a recovery rate of 100% at 5 µg/kg. The limits of detection (LOD) were measured at 1.05 ± 0.06, 1.17 ± 0.11, and 1.09 ± 0.03 for OTC, TC, and CTC, respectively. The limits of quantification (LOQ) were determined to be 3.15, 3.51, and 3.27 µg/kg for OTC, TC, and CTC, respectively. The relative standard deviations (RSD%) for OTC, TC, and CTC in broiler chicken meat were 5.5, 9.14, and 2.35, respectively, all remaining below 10% (Table 3.2).

Table 3.2 Spiking level, mean recovery, average, RSD, LOD, and LOQ of TCs

Antibiotics	Spiking Level (µg/kg)	Mean Recovery (%)	RSD (%)	LOD (µg/kg) (3:1)	LOQ (µg/kg) (10:1)
OTC	2.5 and 5	91 and100	5.5	1.05	3.15
TC		102 and 100	9.14	1.17	3.51
CTC		106 and 100	2.35	1.09	3.27
SD = Standard Deviation; RSD =Relative Standard Deviation; LOD = Limit of Detection; LOQ = Limit of Quantitation					

3.1.2 Discussion

Validation of the method for TCs

The deviation of the results from the actual values indicates the measurement's accuracy. Precision refers to the variation in the results, quantified by the relative standard deviation (RSD%) from a series of replicate measurements, and it is represented as the percentage of recovery. To evaluate the linearity of the high-performance liquid chromatograph (HPLC) combined with a photo diode array (PDA) detector, calibration curves were employed. A calibration curve was utilized to ascertain the remaining tetracycline levels in the samples, including spiked samples and a control group (Figure 3.3). This calibration curve plotted peak area against concentration (µg/kg) and exhibited an excellent linear correlation coefficient (r^2 = 0.997, 0.996, and 1.000 for OTC, TC, and CTC, respectively).

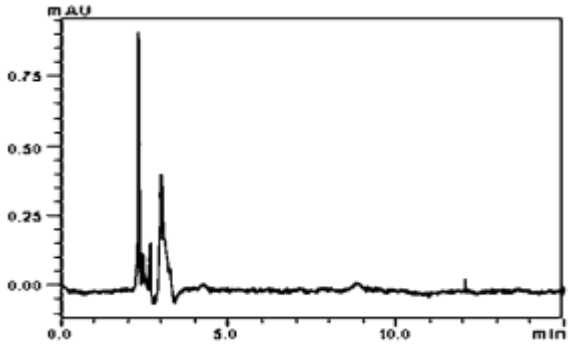
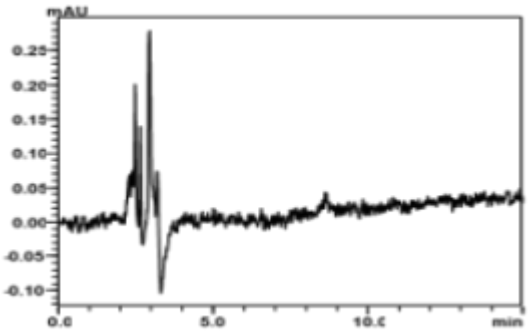


Figure 3.3 Reagent Blank



Broiler chicken meat

Blank matrices, blank matrices treated with TCs mixtures concurrently, and retention periods were used to assess selectivity. The chromatograms of the standard, blank, and spiked samples were compared to identify any undesired elements influencing the analytes. Under comparable LC conditions, there was no interference peak at the TCs retention time.

LOD and LOQ were determined to assess the sensitivity of the LC system. Regarding the background noise recorded for the blank sample, the LOD was calculated using a signal-to-noise ratio of 3, whereas the LOQ was calculated using a signal-to-noise ratio of 10.

For five replicate evaluations, recovery and precision (RSD %) were assessed for both intraday (n = 3) and interday (n = 9, 3 days) experiments. The response of samples spiked into the sample matrix before sample preparation and the response of spiked samples following all sample preparation processes were compared to evaluate the recovery. Thus, the recovery computation did not include any surface recovery loss owing to matrix suppression. To measure recoveries, poultry meat samples were spiked in three duplicates at two different concentrations (2.5 and 5 μg/kg). Table 3.2 shows that the total precision for poultry meat (muscle from the breast and thigh) varied from 2.35 to 9.14 (RSD%). The obtained recovery values of all analytes in various samples ranged between 60 and 120 percent of the values of OTC, TC, and CTC of poultry meat samples, which is the permitted range for trace analysis as defined by the Association of Official Analytical Chemists. Less than 10% of the relative standard deviation (RSD%) surpassed the Codex Alimentarius Commission for compliance. The RSD% for the intra-day and inter-day studies was less than 10%. When the RSD values are ideal, the method is verified.

Food safety is a major concern for consumers worldwide and should be taken very seriously. Antibiotics are now commonly used in poultry and livestock for three purposes: first, they are administered to animals in relatively short amounts for therapeutic purposes; second, they are used for prophylactic purposes, which involves giving animals medium

doses of antibiotics for a duration; and third, they are used for sub-therapeutic antibiotic doses to enhance growth. For instance, it has been reported that animals receive dosages that are 10 or 100 times lower than therapeutic levels for extended periods or their entire lives. Tetracycline analysis of poultry meat samples can be performed effectively, sensitively, and reliably using the HPLC-PDA method. In this study, no residual TCs were found in broiler chicken meat samples, as the TC residues might be below the detection levels (Figure 3.3).

3.2 Analysis of Tetracycline Antibiotics in Beef Meat and Liver

3.2.1 Assessment of Residual TCs in Beef Meat and Liver

Beef meat (n=30) and beef liver (n=30) samples were collected from six different local marketplaces of Dhaka North and South City, Bangladesh. Each of these samples had a weight ranging from 250 to 500 g. The homogenized meat samples were stored at -20°C until analysis, and a specific and rapid reversed-phase high-performance liquid chromatography (HPLC) method was validated for the determination and quantification of tetracycline in beef meat and liver, chicken meat, and liver. Effective chromatographic separation was achieved using High-Performance Liquid Chromatography (HPLC; LC-2030C 3D Plus, Prominence i, Shimadzu) with a photodiode array detector (PDA; SPD-M20A Prominence). On a C-18 column (4.6 × 250 mm, 5µm particle size), the TCs standard solution was separated at two different wavelengths: 360 and 375 nm. The average retention times for OTC, TC, and CTC were 4.2, 4.8, and 8.7 minutes, respectively, and the correlation coefficients (r^2) were 0.998, 0.998, and 0.999 for oxytetracycline, tetracycline, and chlortetracycline standards, respectively at concentrations of 50, 100, 150, 200, 250, and 300 µg/L, respectively (Figure 3.4).

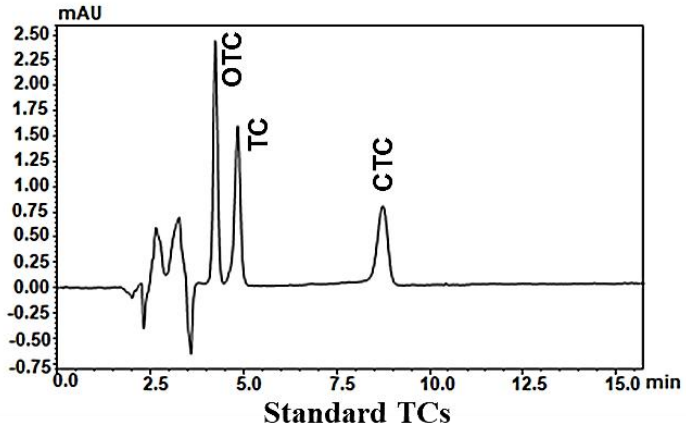


Figure 3.4 Chromatogram of standard TCs

The matrix-matched calibration for OTC, TC and CTC at seven concentrations ranged 50-300 µg/kg and the value of correlation coefficient (r^2) are 0.998, 0.999 and 0.998 for beef meat, respectively.The recovery percentages were ranged from 98.37 to 103.64 and 99.77 to 102.35; 98.39 to 104.29 and 94.13 to 99.13; 97.65 to 101.68 and 98.01 to 100.77for oxytetracycline, tetracycline and chlortetracycline in beef meat at spiking level 100 and 150 µg/kg, respectively. The inter-day recoveries and relative standard deviations (RSD%) for beef are presented in Table 3.3. The chromatograms of the matrix-matched TCs in beef are shown in Figure 3.5.

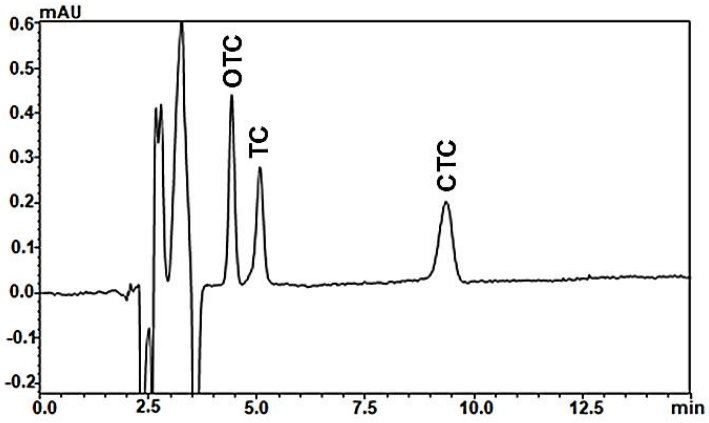


Figure 3.5 Chromatogram of matrix-matched TCs in beef meat (150 µg/kg)

Table 3.3 Recovery percentage of OTC, TC and CTC for beef meat

Antibiotic (BM)	**Spiked Level (µg/kg)**	**Intra-Day Recovery (%) Day-1 (n= 5)**	**Intra-Day Recovery (%) Day-2 (n= 5)**	**Intra-Day Recovery (%) Day-3 (n= 5)**	**Inter-day Recovery (%) 3 days (n= 15)**	**RSD (%) Intra-day (n= 5)**	**RSD (%) Inter-day 3 days (n= 15)**
OTC	100	98.37	101.82	103.64	101.28	1.07 2.73 0.80	1.53
	150	98.77	102.37	102.35	101.16	0.69 0.83 0.46	0.66
TC	100	98.39	104.07	104.29	102.25	1.44 1.23 0.72	1.13
	150	94.13	97.40	99.13	96.89	1.14 1.83 0.51	1.16
CTC	100	97.65	99.55	101.68	99.63	1.11 0.54 2.08	1.23
	150	98.01	98.49	100.77	99.09	0.49 1.50 1.83	1.27

The matrix-matched calibration for OTC, TC, and CTC at seven concentrations ranged from to 50-300 µg/kg and the values of the correlation coefficient (r^2) were 0.9985, 0.9984, and 0.9973 for beef liver, respectively; the recovery percentages ranged from 106.29 to 106.50 and 81.59 to 81.47; 109.59 to 108.94 and 108.72 to 108.30; 87.65 to 106.24 and 96.02 to 102.81for oxytetracycline, tetracycline, and chlortetracycline in beef liver at spiking levels of 100 and 150 µg/kg, respectively. The inter-day recoveries and relative standard deviations (RSD%) for the beef liver are presented in Table 3.4. The chromatogram of the matrix-matched TCs in beef liver is shown in Figure 3.6.

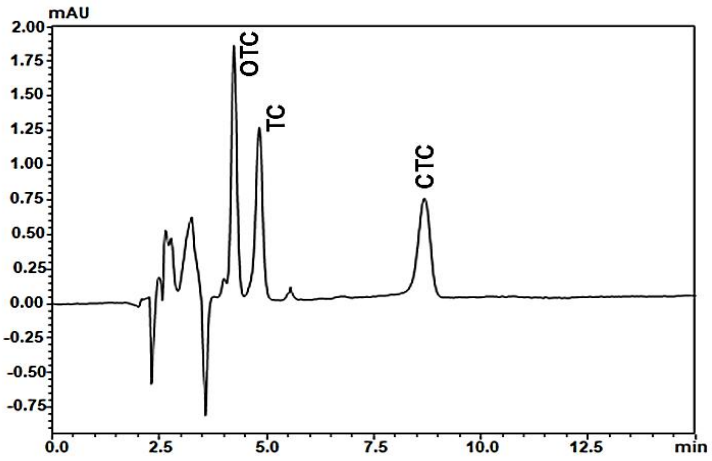


Figure 3.6 Chromatogram of matrix-matched TCs in beef liver (150 µg/kg)

Table 3.4 Recovery percentage of OTC, TC and CTC for beef liver

Antibiotic (BL)	Spiking Level (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
OTC	100	106.29	107.56	106.50	106.65	2.28 1.96 1.78	2.01
	150	81.59	80.75	81.47	81.27	0.73 0.57 0.70	0.67
TC	100	109.59	108.48	108.94	109.00	2.78 2.51 2.42	2.57
	150	108.72	108.54	108.30	108.52	1.83 3.45 1.58	2.29
CTC	100	87.56	105.48	106.24	99.76	6.10 2.05 2.85	3.67
	150	96.02	100.75	102.81	99.86	4.59 2.27 1.51	2.79

The limit of Detection (LOD) (S/N ratio) was 1.11, 1.15 and 1.19 µg/kg for standard oxytetracycline, tetracycline and chlortetracycline respectively. The LOQ (S/N ratio) were 3.71, 3.84 and 3.96 µg/kg for standard oxytetracycline, tetracycline and chlortetracycline respectively. The LOD of OTC, TC and CTC in beef meat were 3.05, 3.78 and 4.33 µg/kg, and the LOQ were 10.16, 12.59 and 14.45 µg/kg, respectively. The LOD of OTC, TC and CTC in the beef liver were 2.51, 3.08 and 2.83 µg/kg, and the LOQ were 8.36, 10.28 and 9.43 µg/kg, respectively (Table 3.5).

Table 3.5 Correlation coefficient (r^2), standard deviation, LOD and LOQ of standard and matrix-matched TCs for beef meat and liver

Antibiotic	Correlation Coefficient (r^2)	Standard Deviation for LOD	LOD (µg/kg)	LOQ (µg/kg)
OTC (STD)	0.9978	0.37	1.11	3.71
TC (STD)	0.9980	0.38	1.15	3.84
CTC (STD)	0.9988	0.40	1.19	3.96
BM (OTC)	0.9980	1.02	3.05	10.16
BM (TC)	0.9990	1.26	3.78	12.59
BM (CTC)	0.9981	1.44	4.33	14.45
BL (OTC)	0.9985	0.84	2.51	8.36
BL (TC)	0.9984	1.03	3.08	10.28
BL (CTC)	0.9973	0.94	2.83	9.43
STD = Standard; BM = Beef Meat; BL = Beef Liver				

3.2.2 Discussion for Residual TC Analysis in Beef Meat and Liver

The maximum residue limits (MRLs) for TCs have been recommended by several agencies. The US Food and Drug Administration (FDA) and the Codex Alimentarius Commission have established a maximum residue level (MRL) of 200 µg/kg in muscle, 600 µg/kg in the liver, and 1200 µg/kg in fat and kidney beef and chicken for the presence of total TC residues in animal products to protect human health (FAO/WHO Expert Committee on Food Additives, 2004). The European Union (EU) established 100, 300, and 600 µg/kg as comparable values (Commission Regulation (EU) No. 37/2010, 2009; Council Regulation (EEC) No. 2377/90/EC, 1990). Sixty beef meat and liver samples were analyzed using reverse-phase HPLC-PDA for the detection and quantitation of residual tetracycline antibiotics (OTC, TC, and CTC). The residual tetracycline antibiotics in the beef liver samples are presented in Table 3.6. Among the beef liver samples, residual oxytetracycline (OTC) was found in eight samples, and one sample (BL18) was above MRL value set by the EU that was 368.97 µg/kg. No residual tetracycline (OTC, TC, and CTC) was found in any beef sample, which might be below the detection limit.

Table 3.6 Amount of residual TCs in beef liver samples

Sample ID	Amount of TCs (µg/kg)		
	Oxytetracycline	Tetracycline	Chlortetracycline
BL1	BDL	BDL	BDL
BL2	BDL	BDL	BDL
BL3	BDL	BDL	BDL
BL4	BDL	BDL	BDL
BL5	BDL	BDL	BDL
BL6	BDL	BDL	BDL
BL7	BDL	BDL	BDL
BL8	BDL	BDL	BDL
BL9	BDL	BDL	BDL
BL10	BDL	BDL	BDL
BL11	BDL	BDL	BDL
BL12	BDL	BDL	BDL
BL13	BDL	BDL	BDL
BL14	86.76	BDL	BDL
BL15	229.67	BDL	BDL
BL16	244.88	BDL	BDL
BL17	200.05	BDL	BDL
BL18	368.97	BDL	BDL
BL19	162.95	BDL	BDL
BL20	BDL	BDL	BDL
BL21	BDL	BDL	BDL
BL22	BDL	BDL	BDL
BL23	BDL	BDL	BDL
BL24	BDL	BDL	BDL
BL25	BDL	BDL	BDL
BL26	95.57	BDL	BDL
BL27	99.04	BDL	BDL
BL28	BDL	BDL	BDL
BL29	BDL	BDL	BDL
BL30	BDL	BDL	BDL

Selectivity was evaluated by analyzing blank matrices, blank matrices concurrently treated with the TCs mixture, and the retention times. Chromatograms of the standard, blank, and spiked samples were compared to identify any unwanted components affecting the analytes. There was no interference peak at the TCs retention time under similar LC conditions. Reagent blanks detected background interference and contamination from chemicals and analytical equipment. While not going through the entire analysis scheme, each analytical reagent was placed into the blank matrix in the exact amount indicated by the analytical procedure. The chromatograms of the solvent blank used as the mobile phase in HPLC and the reagent blank are shown in Figure 3.7. The chromatograms of blank beef meat samples and OTC found in beef liver samples are shown in Figure 3.8 and 3.9, respectively.

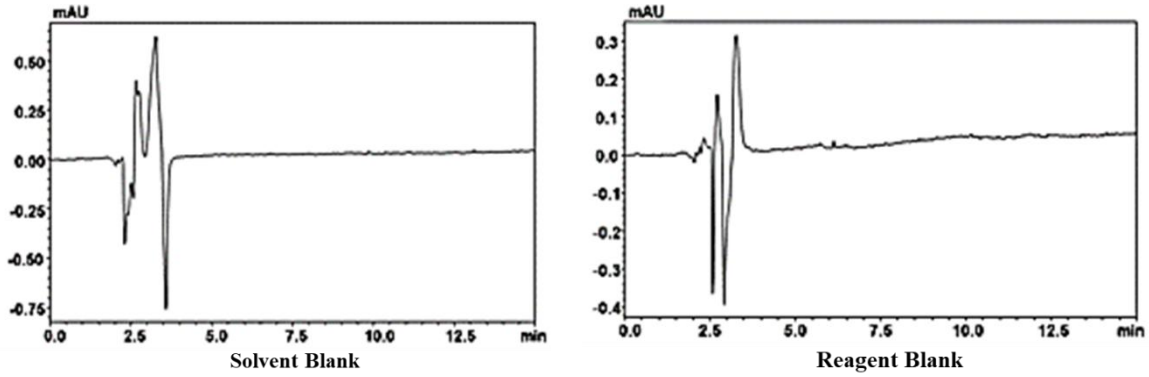


Figure 3.7 Chromatogram of (a) Solvent blank and (b) Reagent blank for TCs

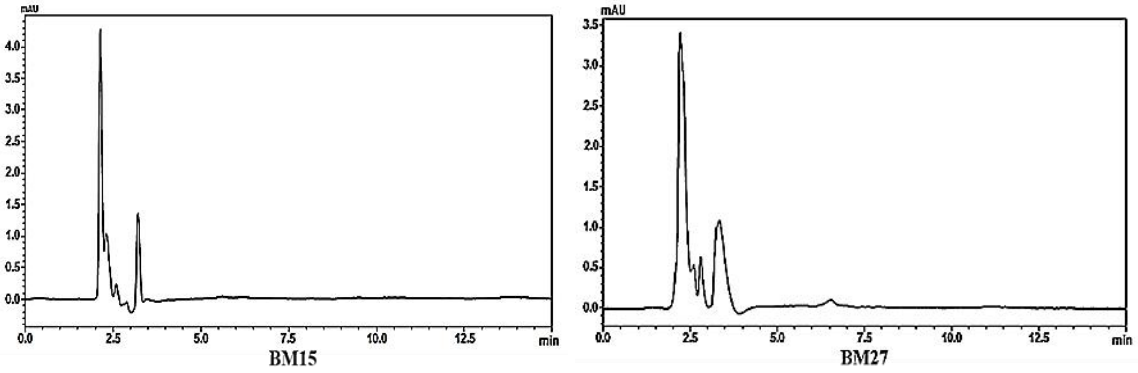


Figure 3.8 Chromatograms of beef meat containing no residual TCs

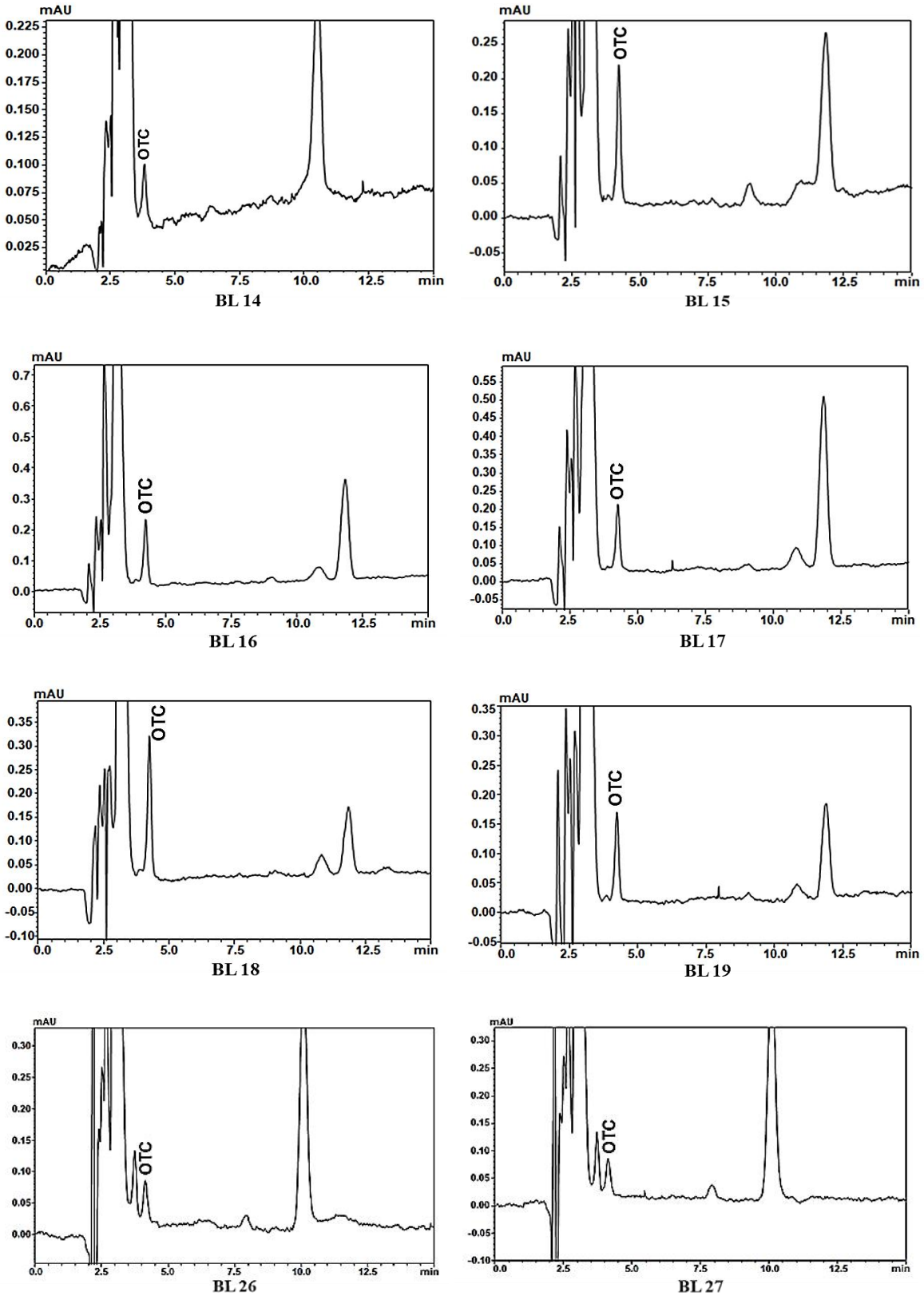


Figure 3.9 Chromatograms of residual OTC in beef liver

A total of 26.67% samples of beef liver were found contaminated with oxytetracycline residues. Among thirty beef liver samples, oxytetracycline was detected and quantified in eight samples (Figure 3.10, 3.11).

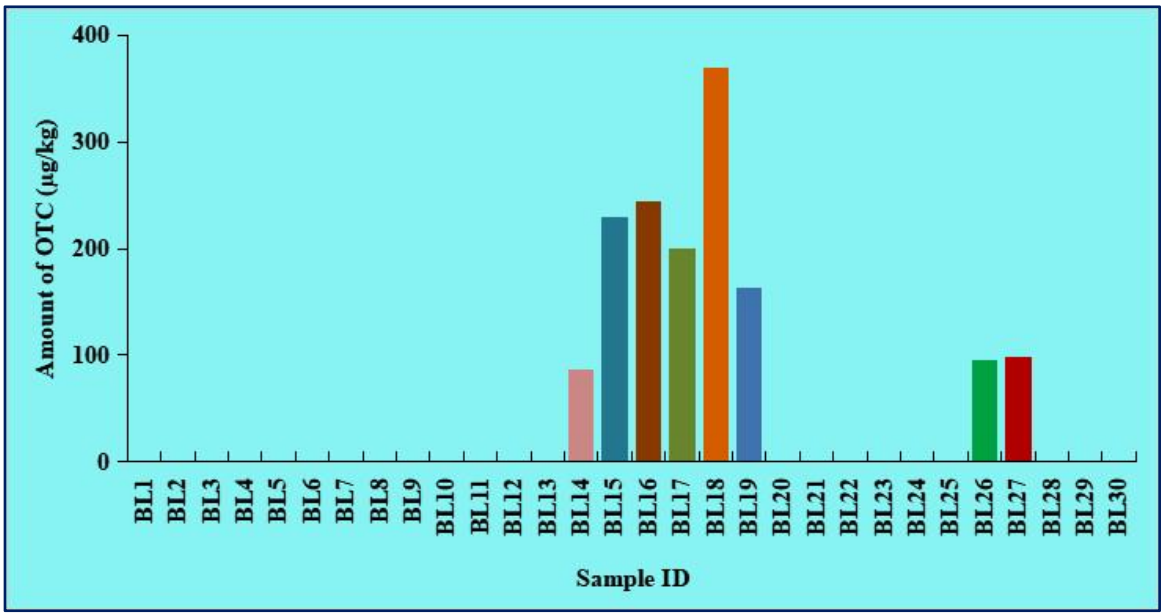


Figure 3.10 Residual amount of OTC in beef liver (µg/kg)

3.3 Analysis of Tetracycline Antibiotics in Broiler Chicken Meat and Liver

3.3.1 Assessment of Tetracycline Antibiotics in Broiler Chicken Meat and Liver

Broiler chicken meat (n=30) and broiler chicken (n=30) samples were purchased from six different local markets in Dhaka North and South City, Bangladesh. Each chicken sample weighed 900 to1000 g. Homogenized meat and liver samples were kept at -20°C until analysis.

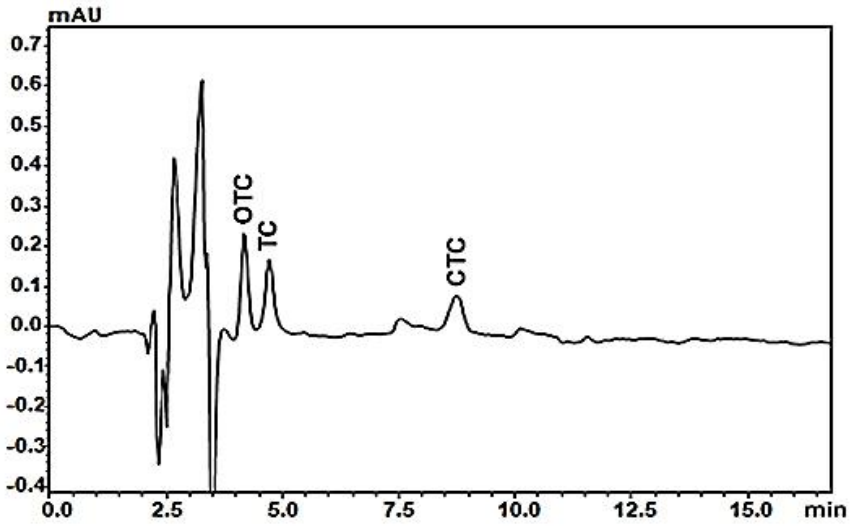


Figure 3.11 Chromatogram of matrix-matched TCs in chicken meat (100 µg/kg)

The matrix-matched calibration for OTC, TC, and CTC at seven concentrations ranged from 50 to 300 µg/kg and the correlation coefficients (r^2) were 0.9988, 0.9982, and 0.9993 for broiler chicken meat, respectively. The chromatograms of matrix-matched TCs in broiler chicken meat are shown in Figure 3.11. The recoveries were ranged from 101.70 to 103.27 and 98.32 to 99.36; 100.14 to 101.99 and 99.56 to 101.62; 98.01 to 101.72 and 97.99 to 99.52% for oxytetracycline, tetracycline and chlortetracycline in broiler chicken meat at spiking level 100 and 150 µg/kg, respectively. The inter-day recoveries and relative standard deviations (RSD%) for broiler chicken meat are presented in Table 3.7.

Table 3.7 Recovery percentage of OTC, TC and CTC for broiler chicken meat

Antibiotic (CM)	**Spiking Level (µg/kg)**	**Intra-Day Recovery (%) Day-1 (n= 5)**	**Intra-Day Recovery (%) Day-2 (n= 5)**	**Intra-Day Recovery (%) Day-3 (n= 5)**	**Inter-day Recovery (%) 3 days (n= 15)**	**RSD (%) Intra-day (n= 5)**	**RSD (%) Inter-day 3 days (n= 15)**
OTC	100	101.70	102.32	103.27	103.43	0.48 0.35 0.37	0.40
	150	98.32	99.23	99.36	98.96	0.48 0.33 0.35	0.39
TC	100	100.14	100.52	101.99	100.88	0.54 0.65 0.90	1.18
	150	99.56	99.32	101.62	100.17	0.40 0.69 0.95	0.68
CTC	100	98.01	100.45	101.72	100.06	0.54 1.11 0.28	0.65
	150	97.99	98.76	99.52	98.75	0.28 0.35 0.42	0.35

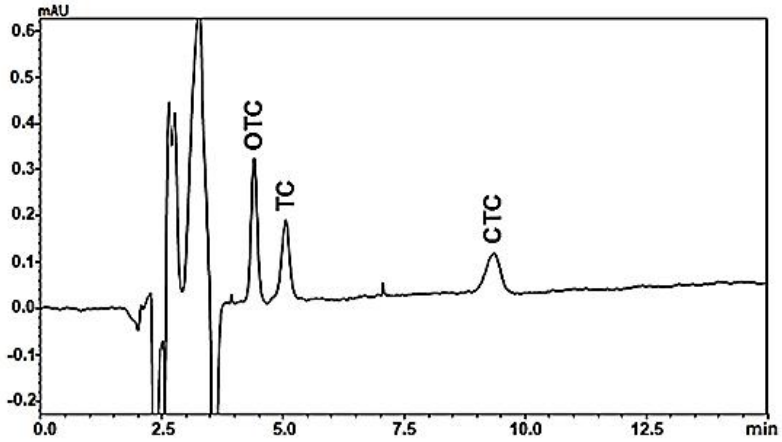


Figure 3.12 Chromatogram of matrix-matched TCs in chicken liver (100 µg/kg)

The matrix-matched calibration for OTC, TC, and CTC at seven concentrations ranged from to 50-300 µg/kg and the values of the correlation coefficients (r^2) were 0.9980, 0.9985, and 0.9991 for broiler chicken liver, respectively. The chromatogram of matrix-matched TCs in broiler chicken liver is shown in Figure 3.12. The recovery percentages were ranged from 99.47 to 103.39 and 96.52 to 101.00; 99.57 to 101.63 and 99.63 to 99.75; 99.49 to 99.94 and 99.94 to 99.88for oxytetracycline, tetracycline and chlortetracycline in chicken meat at spiking level 100 and 150 µg/kg, respectively. The inter-day recoveries and relative standard deviations (RSD%) for broiler chicken liver are presented in Table 3.8.

Table 3.8 Recovery percentage and RSD (%) of OTC, TC and CTC for broiler chicken liver

Antibiotic In CL	Spiking Level (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
OTC	100	99.47	102.21	103.39	101.69	0.95 1.51 0.72	1.06
	150	96.52	98.38	101.00	99.63	0.45 1.28 0.87	0.87
TC	100	99.57	100.71	101.63	100.63	1.00 1.44 10.26	1.18
	150	99.63	99.96	99.75	99.78	0.78 0.28 1.09	0.72
CTC	100	99.49	99.92	99.94	99.78	1.12 0.39 1.43	0.98
	150	99.94	99.17	99.88	99.67	0.39 0.48 0.58	0.48

The limit of Detection (LOD) (S/N ratio) were 1.11, 1.15 and 1.19 µg/kg for standard oxytetracycline, tetracycline and chlortetracycline respectively. The LOQ (S/N ratio) were 3.71, 3.84 and 3.96 µg/kg for standard oxytetracycline, tetracycline and chlortetracycline respectively. The LOD of OTC, TC and CTC in chicken meat were 1.19, 2.16 and 2.03 µg/kg, and the LOQ were 6.42, 7.21 and 6.78 µg/kg, respectively. The LOD of OTC, TC

and CTC in the chicken liver were 1.38, 1.66 and 2.58 µg/kg, and the LOQ were 4.59, 5.33 and 8.60 µg/kg, respectively (Table 3.9).

Table 3.9 Correlation coefficient (r^2), standard deviation, LOD and LOQ of standard and matrix-matched TCs for broiler chicken meat and liver

Antibiotic	Correlation Coefficient (r^2)	Standard Deviation for LOD	LOD (µg/kg)	LOQ (µg/kg)
OTC (STD)	0.9978	0.37	1.11	3.71
TC (STD)	0.9980	0.38	1.15	3.84
CTC (STD)	0.9988	0.40	1.19	3.96
CM (OTC)	0.9988	0.64	1.93	6.42
CM (TC)	0.9982	0.72	2.16	7.21
CM (CTC)	0.9993	0.68	2.03	6.78
CL (OTC)	0.9980	0.46	1.38	4.59
CL (TC)	0.9985	0.55	1.66	5.53
CL (CTC)	0.9991	0.86	2.58	8.60
STD = Standard; CM = Chicken Meat; CL= Chicken Liver				

3.3.2 Discussion of Residual TC Analysis in Broiler Chicken Meat and Liver

Broiler chicken meat (n=30) and liver (n=30) samples were analyzed using reversed-phase HPLC-PDA for the detection and quantitation of residual tetracycline antibiotics (OTC, TC, and CTC). The chromatograms of the solvent and reagent blanks are shown in Figure 3.13. Residual oxytetracycline, tetracycline, and chlortetracycline were found in three (CM18, CM21, and CM25), two (CM17 and CM20), and one (CM23) broiler chicken meat samples, respectively (Figure 3.14). Four positive samples exceeded the MRL value recommended by the Codex. However, according to the EU, six positive samples exceeded the MRL value set by EU (Table 3.10). No residual tetracycline was found in the broiler chicken liver samples, which might be below the detection limit (Figure 3.15).

Table 3.10 Amount of TCs in broiler chicken meat samples

Sample ID	Amount of TCs (µg/kg)		
	Oxytetracycline	Tetracycline	Chlortetracycline
CM1	BDL	BDL	BDL
CM2	BDL	BDL	BDL
CM3	BDL	BDL	BDL
CM4	BDL	BDL	BDL
CM5	BDL	BDL	BDL
CM6	BDL	BDL	BDL
CM7	BDL	BDL	BDL
CM8	BDL	BDL	BDL
CM9	BDL	BDL	BDL
CM10	BDL	BDL	BDL
CM11	BDL	BDL	BDL
CM12	BDL	BDL	BDL
CM13	BDL	BDL	BDL
CM14	BDL	BDL	BDL
CM15	BDL	BDL	BDL
CM16	BDL	BDL	BDL
CM17	BDL	715.00	BDL
CM18	220.94	BDL	BDL
CM19	BDL	BDL	BDL
CM20	BDL	698.88	BDL
CM21	153.45	BDL	BDL
CM22	BDL	BDL	BDL
CM23	BDL	BDL	677.35
CM24	BDL	BDL	BDL
CM25	101.32	BDL	BDL
CM26	BDL	BDL	BDL
CM27	BDL	BDL	BDL
CM28	BDL	BDL	BDL
CM29	BDL	BDL	BDL
CM30	BDL	BDL	BDL

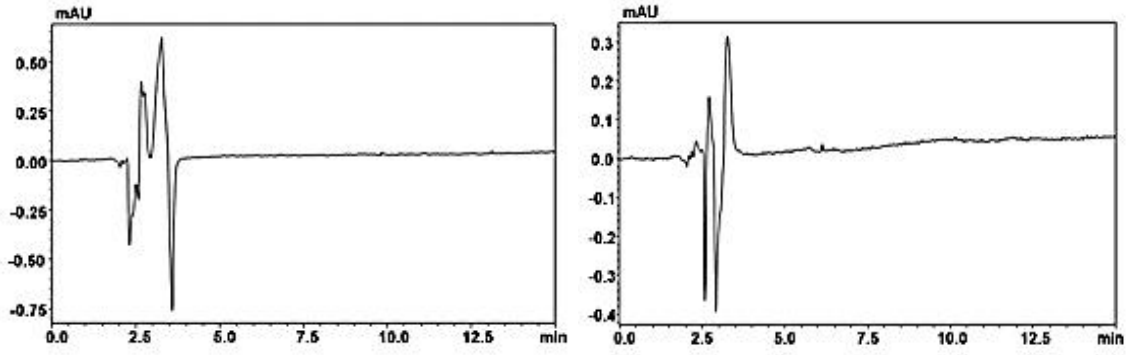


Figure 3.13 Chromatogram of (a) Solvent Blank and (b) Reagent Blank

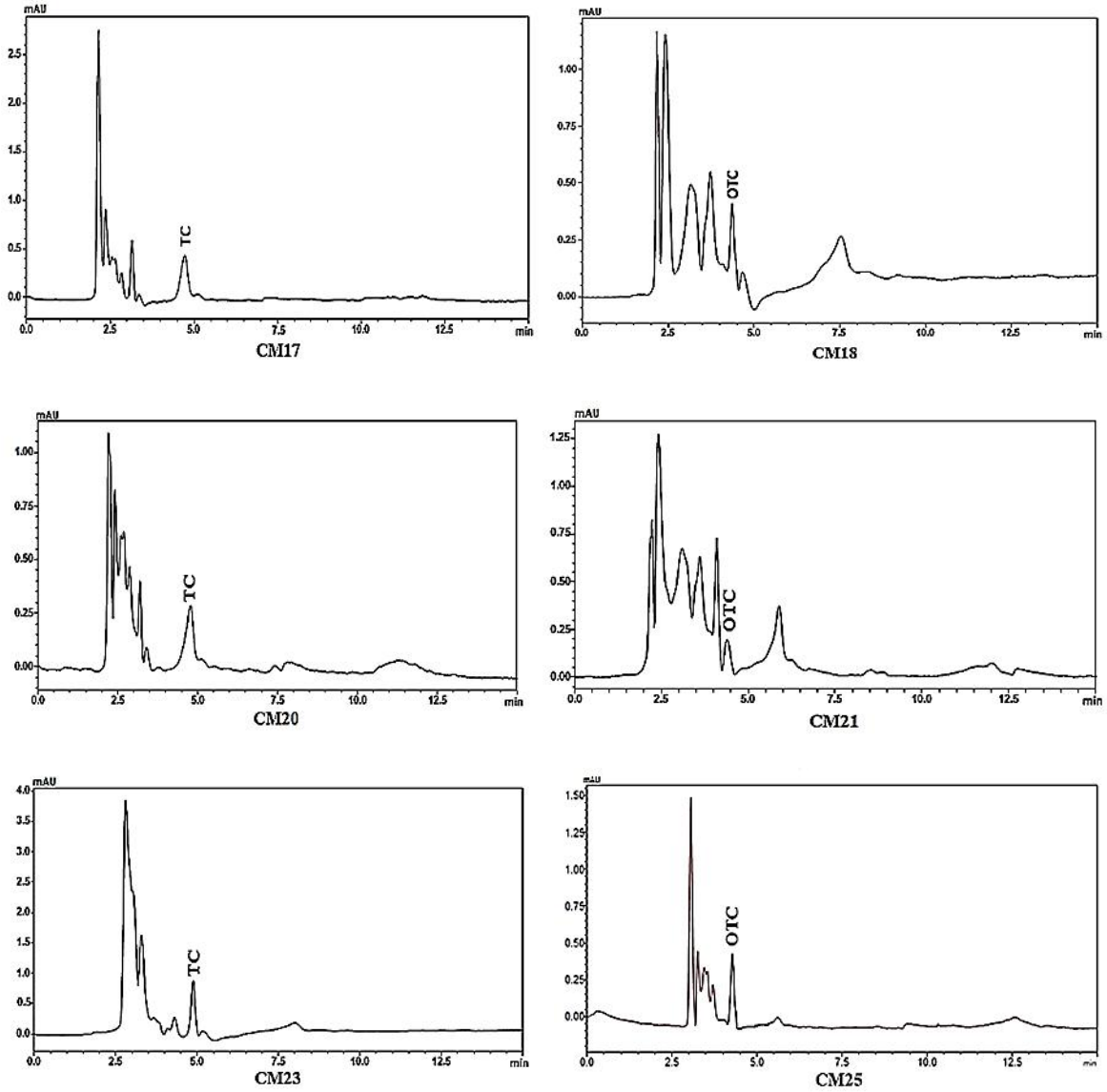


Figure 3.14 Chromatograms of chicken meat containing residual TCs

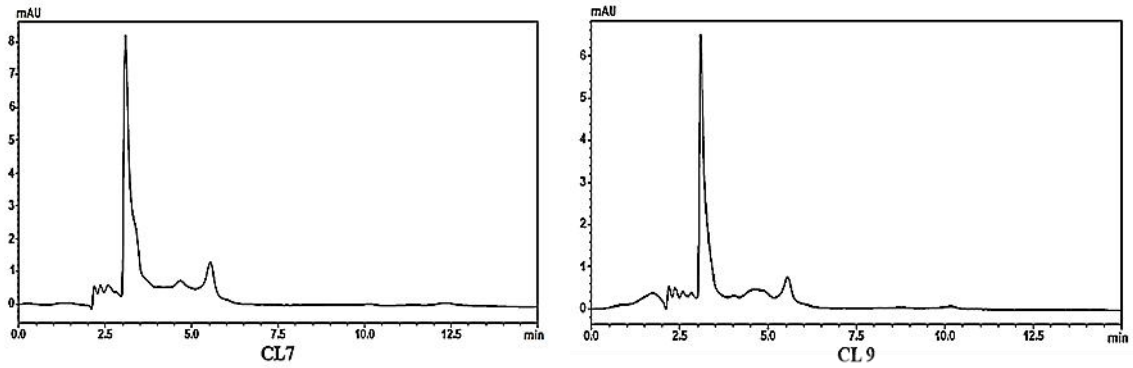


Figure 3.15 Chromatograms of chicken liver containing no residual TCs

OTC, TC and CTC were found 10, 6.67 and 3.33 % among thirty broiler chicken meat samples (Figure 3.16).

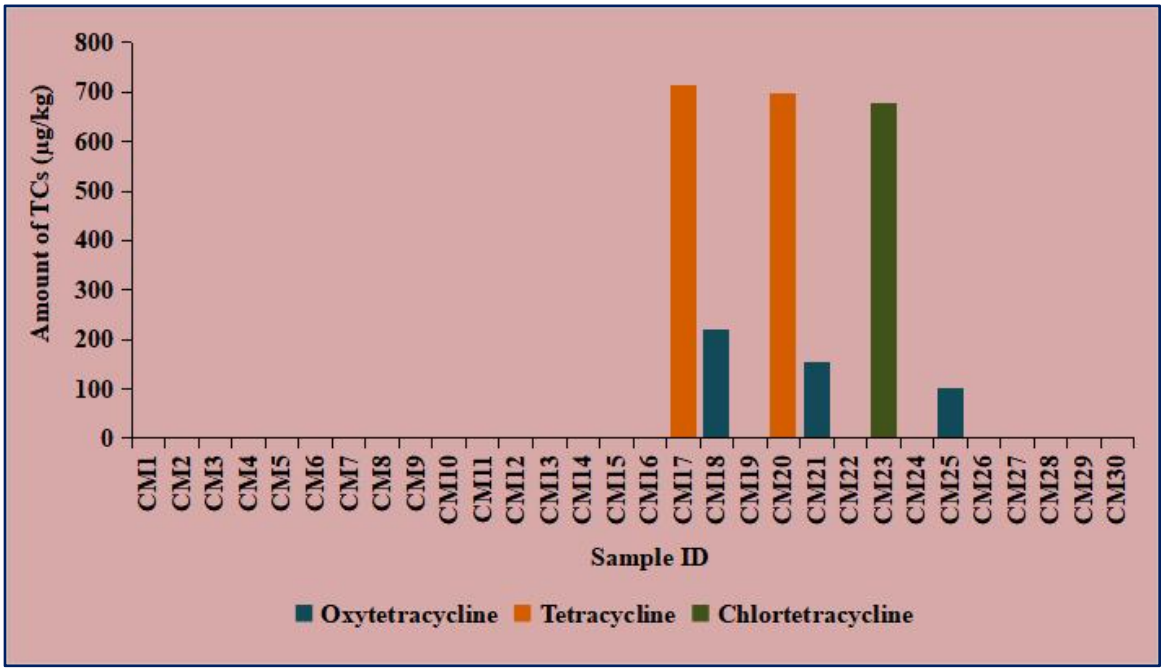


Figure 3.16 Amount of Residual TCs in chicken meat samples (µg/kg)

3.4 Analysis of Amoxicillin in Beef Meat and Liver

3.4.1 Evaluation of Residual Amoxicillin in Beef Meat and Liver

Beef meat (n=30) and beef liver (n=30) samples were purchased from six different local marketplaces of Dhaka North and South City, Bangladesh, for the determination of residual amoxicillin. Each sample weighed between 250 and 500 g. Homogenized meat samples were kept at -20°C until analysis.

The detection and quantification of amoxicillin in beef liver and meat have been confirmed using precise and quick reversed-phase HPLC-PDA. Effective chromatographic separation was achieved using High-Performance Liquid Chromatography (HPLC; LC-2030C 3D Plus, Prominence i, Shimadzu) with a photodiode array detector (PDA). On a C-18 column(4.6 × 250 mm, 5µm particle size), the amoxicillin standard solution was separated at a wavelength 230 nm. The correlation coefficient (r^2) was 0.9982 for the amoxicillin standard at concentrations of 1, 5, 10, 15, 20, 25, and 30 µg/L. The retention time of amoxicillin was 3.3 minutes (Figure 3.17).

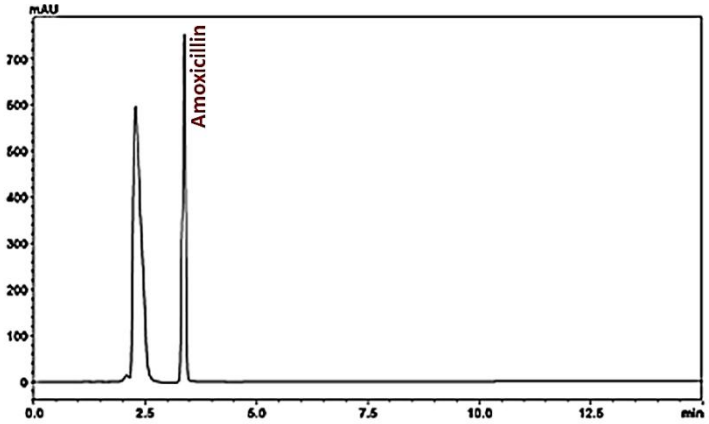


Figure 3.17 Chromatogram of standard amoxicillin

The matrix-matched calibration for amoxicillin at seven concentrations ranged from 1 to 30 µg/kg and the correlation coefficient (r^2) value was 0.9973 for beef meat. The recovery percentages ranged from 103.06 to 100.79 and 102.96 to 106.23 for amoxicillin in beef meat at spiking levels of 10 and 15 µg/kg, respectively (Figure 3.18). The inter-day recoveries were 101.09 and 104.20% and, RSD% were 3.47 and 2.47% for beef meat at spiking levels of 10 and 15 µg/kg, respectively as presented in Table 3.11.

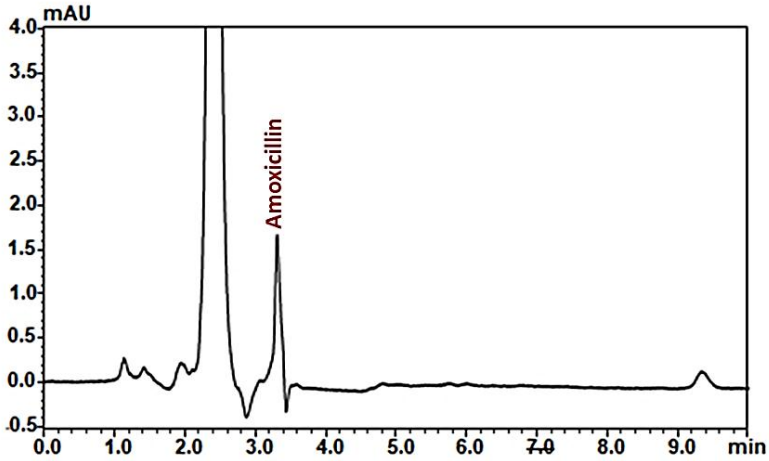


Figure 3.18 Chromatogram of matrix-matched amoxicillin for beef meat (10 µg/kg)

Table 3.11 Recovery percentage and RSD (%) of amoxicillin for beef meat

Amox (BM) (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
10	103.06	99.42	100.79	101.09	4.28 3.65 2.50	3.47
15	102.96	103.42	106.23	104.20	1.82 2.26 3.33	2.47

The matrix-matched calibration for amoxicillin at seven concentrations ranged from 1 to 30 µg/kg, the correlation coefficient (r^2) value was 0.9973 for beef meat, and the recovery percentages ranged from 108.43 to 111.51 and 94.34 to 101.66 for amoxicillin in beef liver at spiking levels of 10 and 15 µg/kg, respectively (Figure 3.19). The inter-day recoveries were 109.68 and 98.61% and, the relative standard deviations (RSD%) were 1.94 and 3.39% for beef meat at spiking levels of 10 and 15 µg/kg, respectively, as presented in Table 3.12.

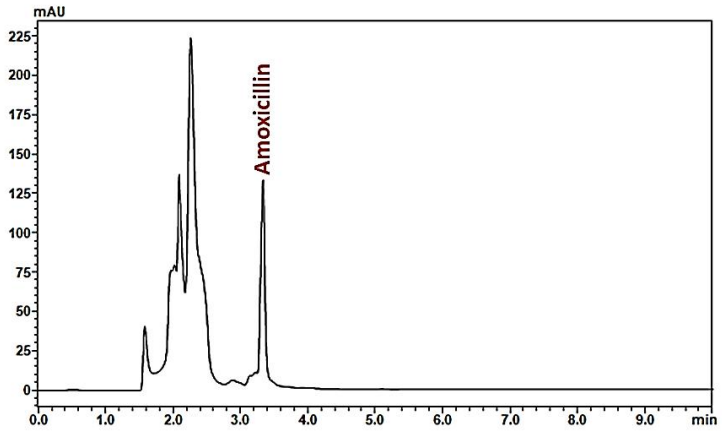


Figure 3.19 Chromatogram of matrix-matched amoxicillin for beef liver (15 µg/kg)

Table 3.12 Recovery percentage and RSD (%) of amoxicillin for beef liver

Amox (BL) (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
10	111.51	108.43	109.10	109.68	1.51 1.21 3.09	1.94
15	94.34	99.84	101.66	98.61	3.95 2.67 3.56	3.39

3.4.2 Discussion for Residual Amoxicillin in Beef Meat and Liver

The correlation coefficient (r^2) was 0.9980 for the amoxicillin standard at concentrations of 1, 5, 10, 15, 20, 25, and 30 µg/kg. The LOD and LOQ (S/N ratio) were 0.55 and 1.85 µg/kg. For matrix-matched amoxicillin for beef meat and beef liver at concentrations of 1, 5, 10, 15, 20, 25, and 30 µg/kg, the correlation coefficient (r^2) values were 0.9979 and 0.9980, respectively. The LOD and LOQ for amoxicillin in beef meat and liver were 0.71 and 0.65 µg/kg and, 2.35 and 2.17 µg/kg (Table 3.13).

Table 3.13 Correlation Coefficient (r^2), standard Deviation, LOD and LOQ of standard amoxicillin and matrix-matched for chicken meat and liver

Antibiotic	**Correlation Coefficient (r^2)**	**Standard Deviation for LOD**	**LOD (µg/kg)**	**LOQ (µg/kg)**
Amox (STD)	0.9980	0.18	0.55	1.84
BM (Amox)	0.9979	0.24	0.71	2.35
BL (Amox)	0.9980	0.22	0.65	2.17
STD = Standard; Amox = Amoxicillin; BM = Beef Meat; BL = Beef Liver				

The Codex Alimentarius Commission and European Union (EU) have set a maximum residue level (MRL) of 50 µg/kg for amoxicillin in beef, chicken meat, and the liver. Residual amoxicillin was detected in 7 beef meat samples ranged 4.89 to 9.36 µg/kg (Figure 3.22) and 15 beef liver samples ranged 10.39 to 89.47 µg/kg. Two beef liver samples exceeded the MRL value that are 53.46 µg/kg ((BL10) and 89.47 µg/kg (BL14) (Table 3.14). The chromatograms of solvent blank, reagent blank, beef meat and liver samples for the analysis of residual amoxicillin in Figure 3.20, 3.21 and 3.22.

Table 3.14 Amount of residual amoxicillin in beef meat and liver

Sample ID	Amount of Amoxicillin (µg/kg)	Sample ID	Amount of Amoxicillin (µg/kg)
BM1	BDL	BL1	BDL
BM2	BDL	BL2	BDL
BM3	BDL	BL3	11.50
BM4	BDL	BL4	33.43
BM5	BDL	BL5	30.95
BM6	BDL	BL6	BDL
BM7	BDL	BL7	BDL
BM8	BDL	BL8	BDL
BM9	BDL	BL9	BDL
BM10	7.89	BL10	53.46
BM11	BDL	BL11	BDL
BM12	9.36	BL12	BDL
BM13	BDL	BL13	24.21
BM14	8.54	BL14	89.47
BM15	BDL	BL15	BDL
BM16	BDL	BL16	16.42
BM17	6.56	BL17	BDL
BM18	4.89	BL18	40.70
BM19	BDL	BL19	21.12
BM20	BDL	BL20	BDL
BM21	BDL	BL21	17.41
BM22	6.10	BL22	17.61
BM23	7.79	BL23	11.55
BM24	BDL	BL24	BDL
BM25	BDL	BL25	17.05
BM26	BDL	BL26	10.39
BM27	BDL	BL27	BDL
BM28	BDL	BL28	20.73
BM29	BDL	BL29	BDL
BM30	BDL	BL30	BDL

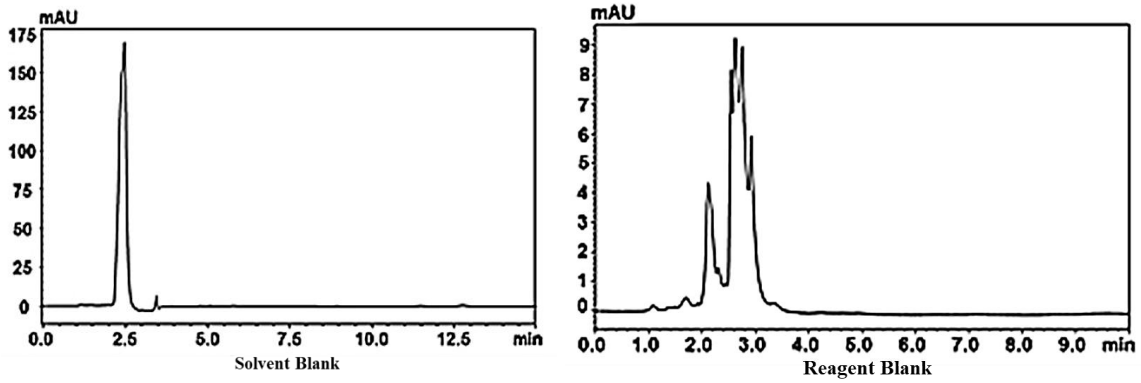


Figure 3.20 Chromatogram of (a) Solvent blank and (b) Reagent blank

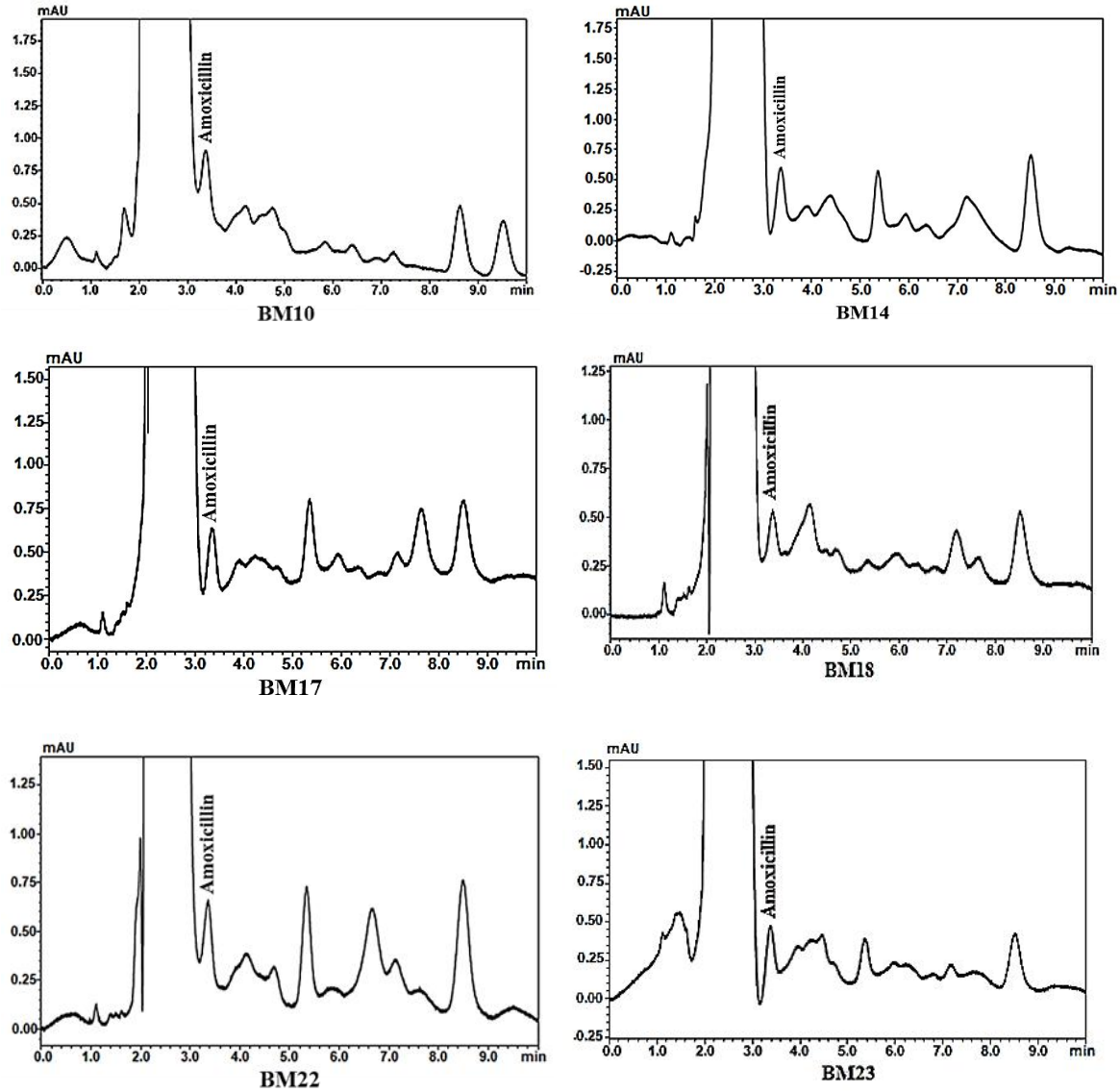


Figure 3.21 Chromatogram of amoxicillin in beef meat

Amoxicillin was identified and quantified in 7 beef meat samples. 23.33% was the positive samples containing residual amoxicillin among the total beef meat samples (Figure 3.22).

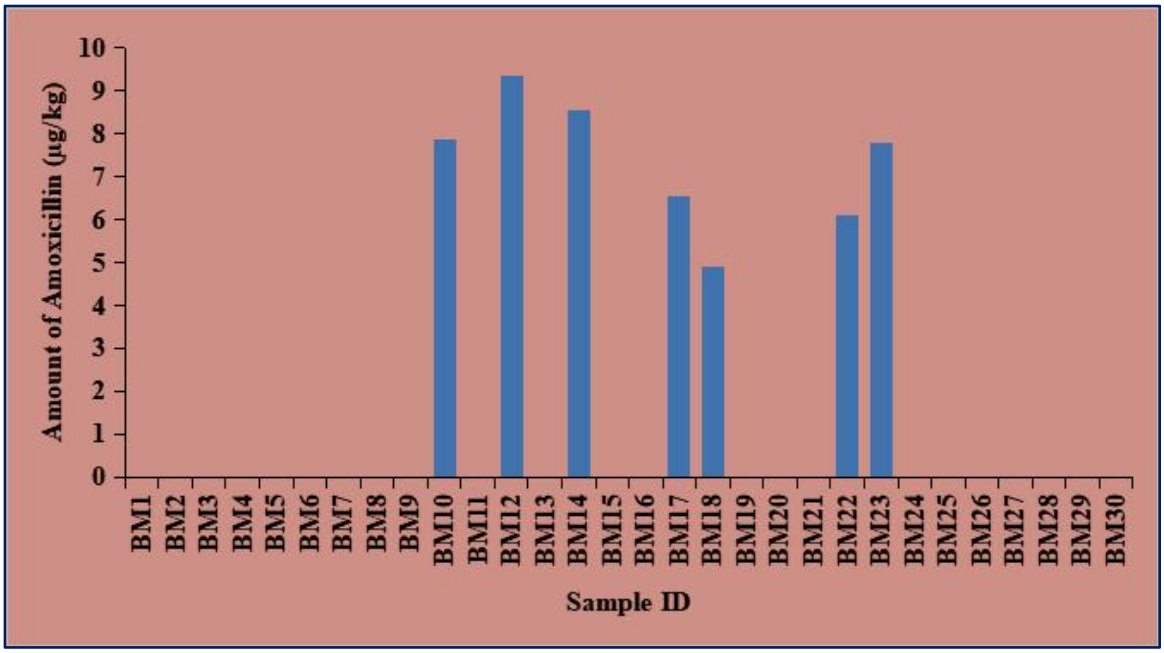
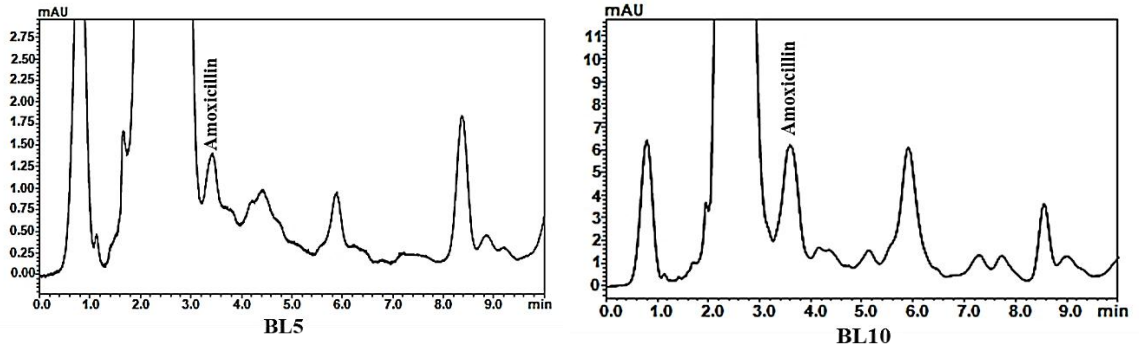


Figure 3.22 Residual amount of amoxicillin in beef meat samples (µg/kg)

The chromatograms of some beef liver samples of residual amoxicillin are presented in Figure 3.23.



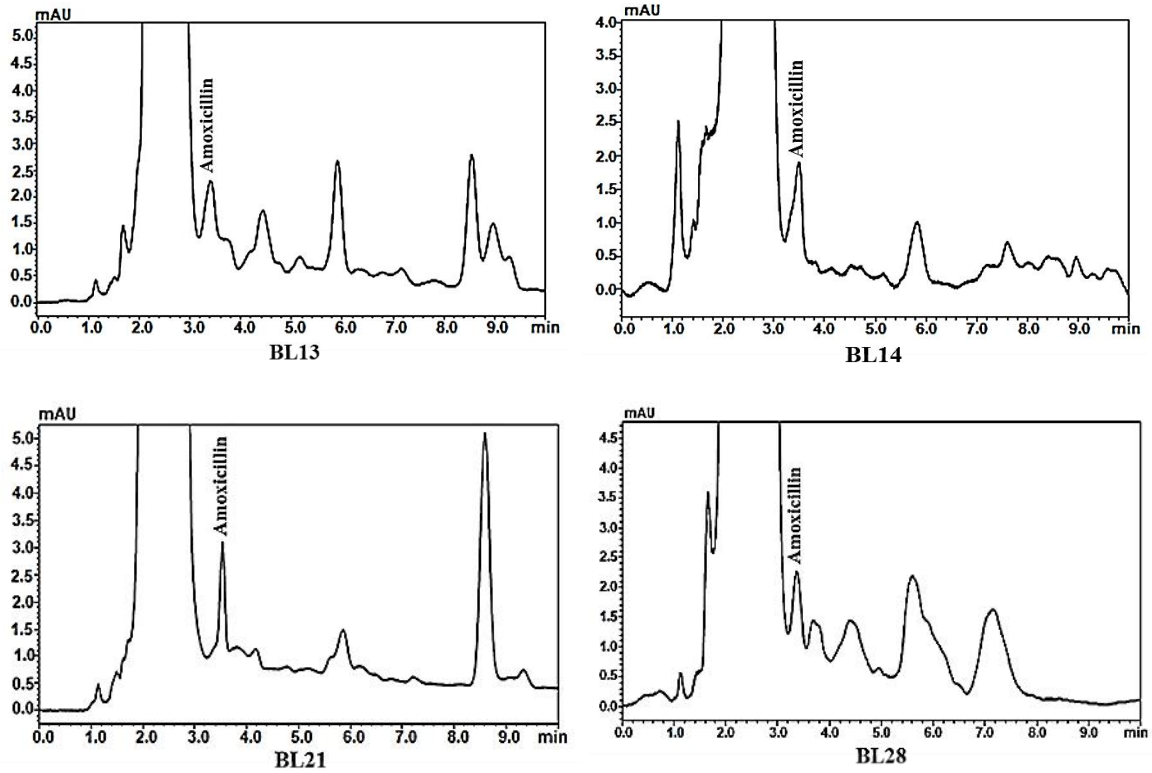


Figure 3.23 Chromatograms of amoxicillin in beef liver

Residual amoxicillin was found in 50 % of total beef liver samples (Figure 3.24).

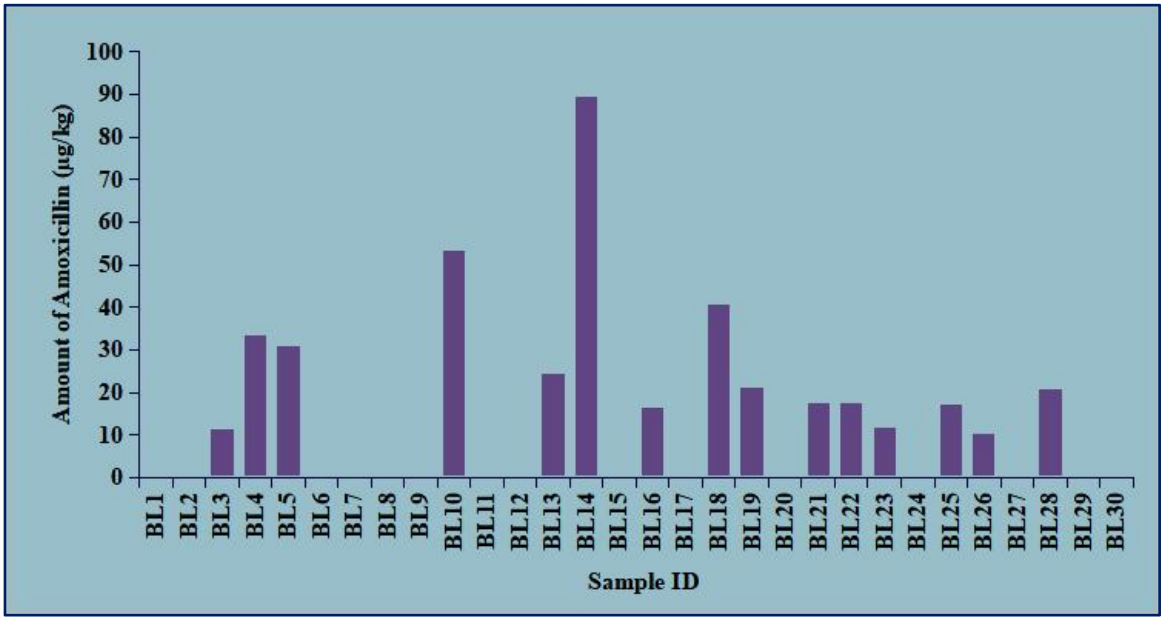


Figure 3.24 Residual amount of amoxicillin in beef liver samples (µg/kg)

3.5 Analysis of Amoxicillin in Broiler Chicken Meat and Liver

3.5.1 Assessment of Residual Amoxicillin in Broiler Chicken Meat and Liver

Broiler chicken meat (n=30) and broiler chicken liver (n=30) samples were purchased from six different local marketplaces in Dhaka North and South City, Bangladesh, for the determination of residual amoxicillin. Each broiler chicken sample weighed 900–1000 g. Homogenized meat samples were kept at -20°C until analysis.

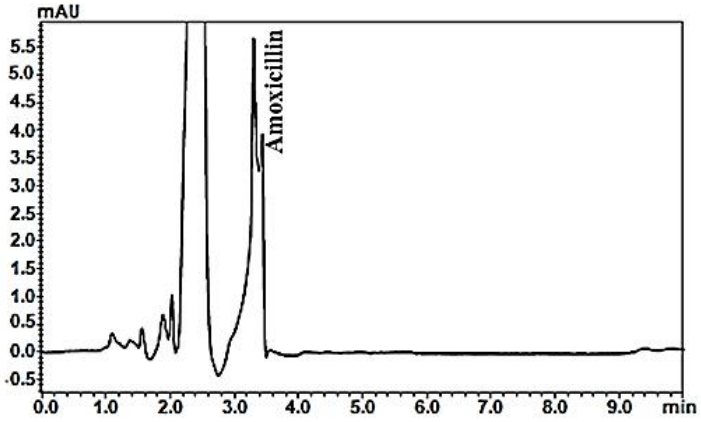


Figure 3.25 Chromatogram of matrix-matched amoxicillin for chicken meat (20 µg/kg)

The matrix-matched calibration for amoxicillin at seven concentrations ranged from 1 to 30 µg/kg and the correlation coefficient (r^2) value was 0.9973 for broiler chicken meat. The recovery percentages ranged from 94.64 to 100.06 and 96.56 to 101.20 for amoxicillin in chicken meat at spiking levels of 10 and 20 µg/kg, respectively (Figure 3.25). The inter-day recoveries were 98.24 and 99.06%, and RSD% were 2.01 and 1.01% for beef meat at spiking levels of 10 and 20 µg/kg, respectively as presented in Table 3.15.

Table 3.15 Recovery percentage and RSD (%) of amoxicillin for broiler chicken meat

Amox (CM) (µg/kg)	**Intra-Day Recovery (%) Day-1 n= 5**	**Intra-Day Recovery (%) Day-2 n= 5**	**Intra-Day Recovery (%) Day-3 n= 5**	**Inter-day Recovery (%) 3 days n= 15**	**RSD (%) Intra-day n= 5**	**RSD (%) Inter-day 3 days n= 15**
10	94.64	100.03	100.06	98.24	2.38 0.99 0.81	2.01
20	96.56	99.41	101.20	99.06	0.48 1.04 1.51	1.01

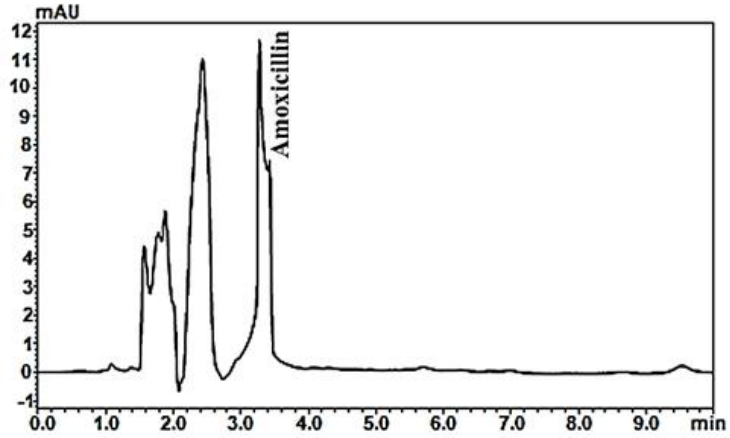


Figure 3.26 Chromatogram of matrix-matched amoxicillin for chicken liver (20 µg/kg)

The matrix-matched calibration for amoxicillin at seven concentrations ranged from 1 to 30 µg/kg and the correlation coefficient (r^2) value was 0.9981 for broiler chicken liver. The recovery percentages ranged from 102.79 to 101.58 and 92.60 to 96.21 for amoxicillin in broiler chicken liver at spiking levels of 10 and 20 µg/kg, respectively (Figure 3.26). The inter-day recoveries were 103.31 and 81.27% and, the relative standard deviation (RSD%) were 2.73 and 1.78% for beef meat at spiking levels of 10 and 20 µg/kg, respectively as presented in Table 3.16.

Table 3.16 Recovery percentage and RSD (%) of amoxicillin for broiler chicken liver

Amox (CL) (µg/kg)	Intra-Day Recovery (%) Day-1 n= 5	Intra-Day Recovery (%) Day-2 n= 5	Intra-Day Recovery (%) Day-3 n= 5	Inter-day Recovery (%) 3 days n= 15	RSD (%) Intra-day n= 5	RSD (%) Inter-day 3 days n= 15
20	102.79	105.57	101.58	103.31	2.98 0.82 4.38	2.73
30	92.60	95.31	96.21	81.27	4.56 0.48 0.31	1.78

3.5.2 Discussion for Residual Amoxicillin in Broiler Chicken Meat and Liver

The correlation coefficients (r^2) for the matrix-matched amoxicillin for broiler chicken meat and liver at concentrations of 1, 5, 10, 15, 20, 25, and 30 µg/kg were 0.9995 and 0.9981, respectively. The LOD and LOQ for amoxicillin in broiler chicken meat and liver were 0.51 and 0.57 µg/kg and, 1.71 and 1.90 µg/kg, respectively (Table 3.17).

Table 3.17 Correlation Coefficient (r^2), standard Deviation, LOD and LOQ of standard amoxicillin and matrix-matched for broiler chicken meat and liver

Antibiotic	Correlation Coefficient (r^2)	Standard Deviation for LOD	LOD (µg/kg)	LOQ (µg/kg)
Amox (STD)	0.9980	0.18	0.55	1.84
CM (Amox)	0.9995	0.17	0.51	1.71
CL (Amox)	0.9981	0.19	0.57	1.90
STD = Standard; Amox = Amoxicillin; CM = Chicken Meat; CL = Chicken Liver				

No residual amoxicillin was found in broiler chicken meat and liver, which might be below the detection level (Figure 3.27 and 3.28).

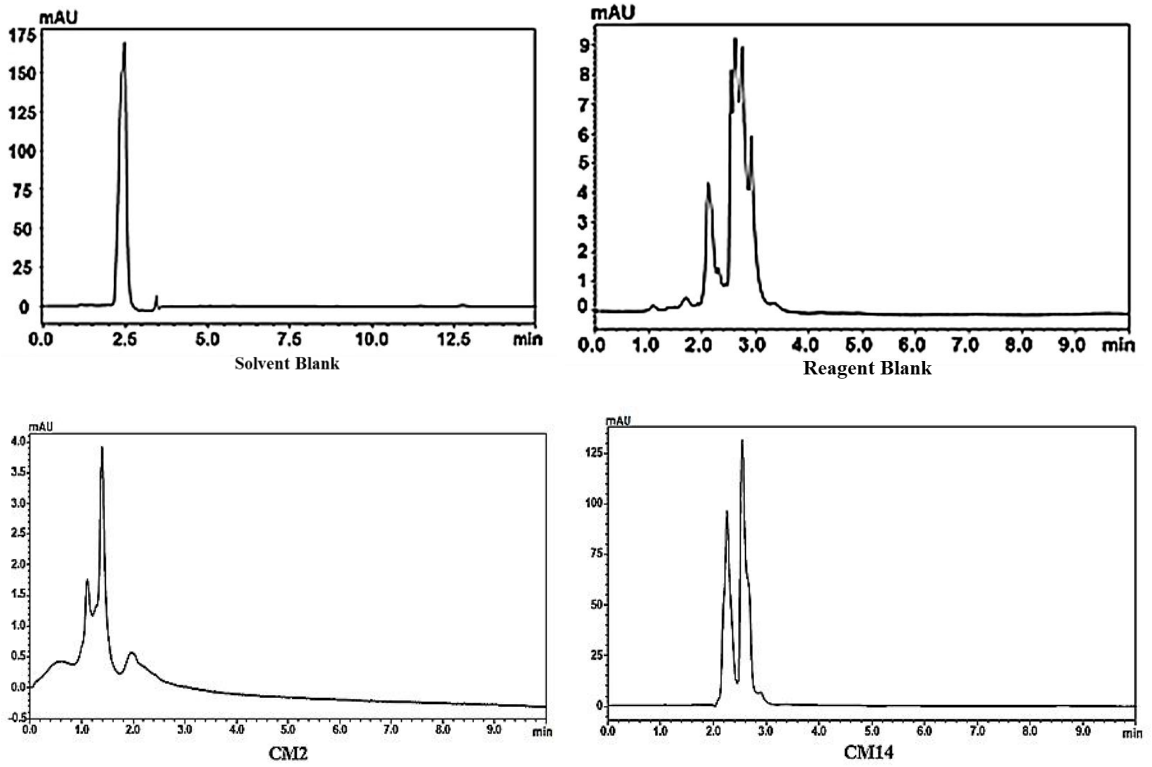


Figure 3.27 Chromatogram of chicken meat containing no amoxicillin

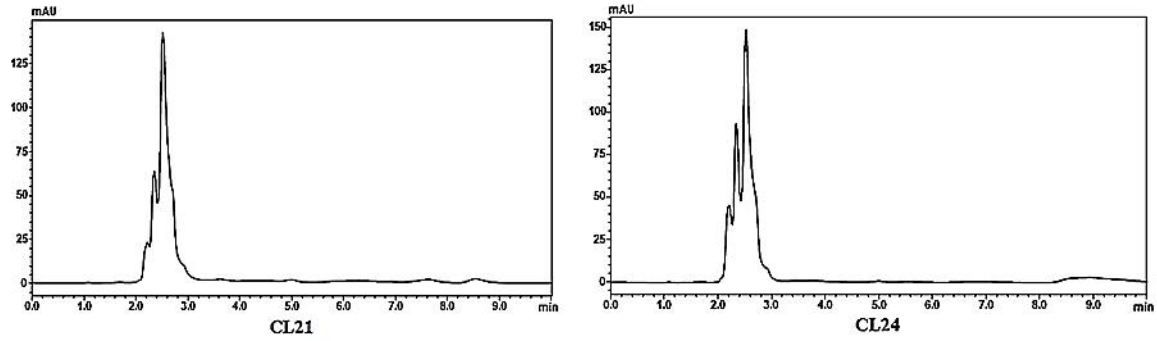


Figure 3.28 Chromatogram of chicken liver containing no amoxicillin

3.6 Analysis of Patulin in Beef Meat and Liver

3.6.1 Assessment of Residual Patulin in Beef Meat and Liver

Samples of beef meat (n=30) and beef liver (n=30) were obtained from six various marketplaces in Dhaka North and South City, Bangladesh, for the assessment of residual patulin. Each sample had a weight ranging from 250 to 500 g. The homogenized meat samples were stored at -20°C prior to analysis.

Patulin concentration in beef liver and meat was determined and quantified using precise and quick reversed-phase HPLC-PDA for efficient separation. At 276 nm, the patulin standard solution was separated on a C-18 column (4.6 × 250 mm, 5µm particle size). The correlation coefficient (r^2) was 0.9991 for the patulin standard at concentrations of 1, 5, 10, 15, 20, 25, and 30 µg/L. The retention time of patulin was 4.9 min (Figure 3.29).

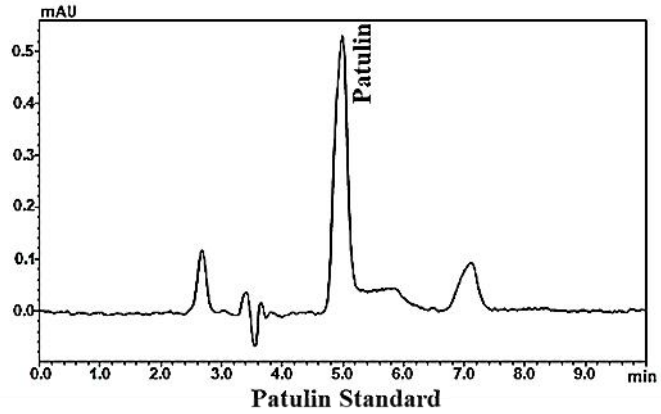


Figure 3.29 Chromatogram of patulin standard

The matrix-matched calibration for patulin at seven concentrations ranged from 1 to 30 µg/kg (Figure 3.30) and the correlation coefficient (r^2) value was 0.9984 for beef meat. The recovery percentages ranged from 94.55 to 93.77 and 96.32 to 97.24 for patulin in beef meat at spiking levels of 10 and 15 µg/kg, respectively. The inter-day recoveries were 94.57 and 96.69%, and RSD% was 0.77 and 2.47% for beef meat at spiking levels of 10 and 15 µg/kg, respectively as presented in Table 3.18.

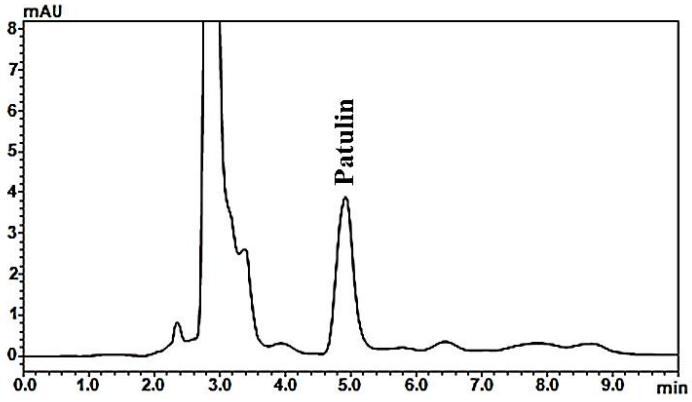


Figure 3.30 Chromatogram of matrix-matched patulin for beef meat (10 µg/kg)

Table 3.18 Recovery percentage and RSD (%) of patulin for beef meat

Patulin (BM) (µg/kg)	**Intra-Day Recovery (%) Day-1 n= 5**	**Intra-Day Recovery (%) Day-2 n= 5**	**Intra-Day Recovery (%) Day-3 n= 5**	**Inter-day Recovery (%) 3 days n= 15**	**RSD (%) Intra-day n= 5**	**RSD (%) Inter-day 3 days n= 15**
10	94.55	95.40	93.77	94.57	0.86 0.87 0.59	0.77
15	96.32	96.51	97.24	96.69	0.26 0.23 0.13	2.47

The matrix-matched calibration for patulin at seven concentrations ranged from 1 to 30 µg/kg and the correlation coefficient (r^2) value was 0.9983 for beef liver. The chromatogram of matrix-matched patulin in beef liver is shown in Figure 3.31. The recovery percentages ranged from 104.31 to 104.66 and 101.99 to 102.38for patulin in beef liver at spiking levels of 10 and 15 µg/kg, respectively. The inter-day recoveries were 104.41 and 102.38% and, the relative standard deviations (RSD%) were 0.38 and 0.71% for beef meat at spiking levels of 10 and 15 µg/kg, respectively, as presented in Table 3.19.

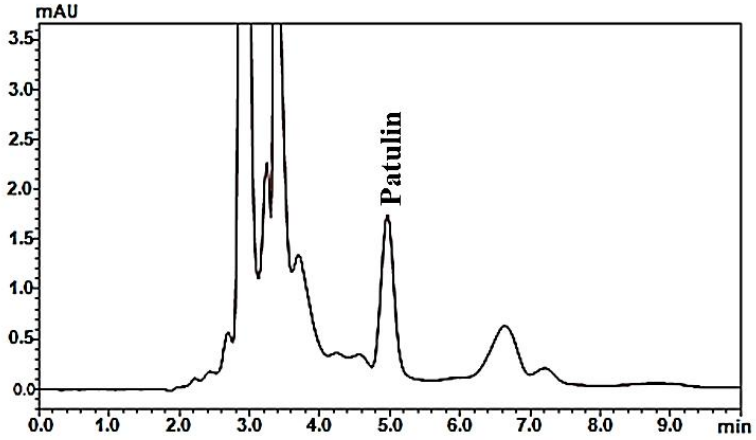


Figure 3.31 Chromatogram of matrix-matched patulin for beef liver

Table 3.19 Recovery percentage and RSD (%) of patulin for beef liver

Patulin (BL) (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
10	104.31	104.26	104.66	104.41	0.30 0.51 0.33	0.38
15	101.99	102.83	102.31	102.38	0.87 0.74 0.53	0.71

Correlation Coefficient (r^2), standard deviation, LOD and LOQ of standard patulin and matrix-matched for beef meat and liver are presented in Table 3.20.

Table 3.20 Correlation coefficient (r^2), standard deviation, LOD and LOQ of standard patulin and matrix-matched for beef meat and liver

Antibiotic	Correlation Coefficient (r^2)	Standard Deviation for LOD	LOD (µg/kg)	LOQ (µg/kg)
Patulin (STD)	0.9991	0.10	0.31	1.03
BM (Patulin)	0.9984	0.13	0.38	1.28
BL (Patulin)	0.9983	0.15	0.45	1.51
STD = Standard; B = Beef Meat; BL = Beef Liver				

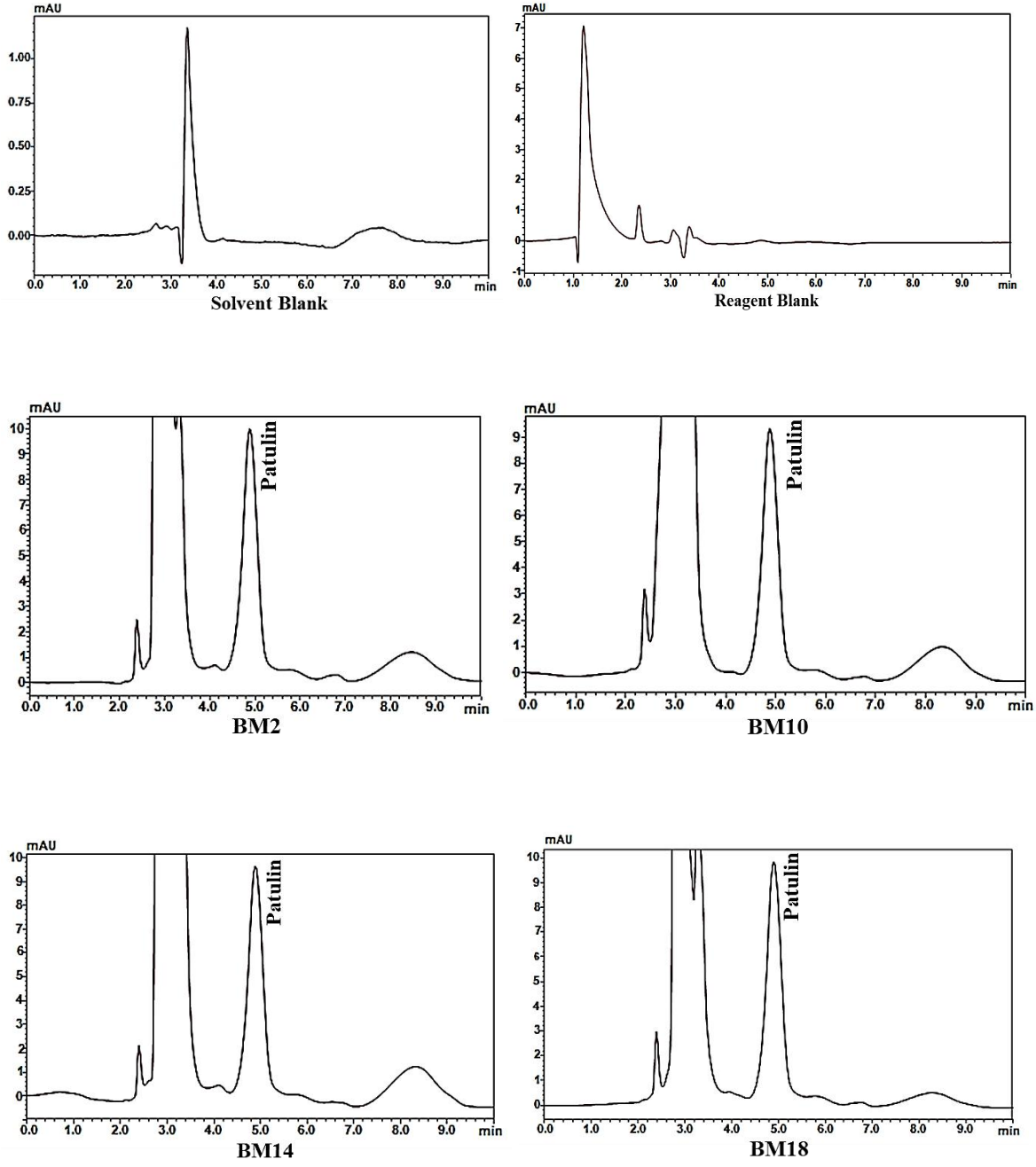
3.6.2 Discussion for Residual Patulin in Beef meat and Liver

Residual patulin antibiotic (as well as mycotoxin) was detected 25 beef samples ranged 46.26 to 193.91 µg/kg and 6 beef liver samples ranged 43.31 to 166.91 µg/kg (Table 3.21). The MRL value for patulin in fruit and fruit juices was 50 µg/kg, but no MRL value was set for patulin in beef meat and liver samples by any regulatory agencies.

Table 3.21 Amount of patulin in beef meat and liver

Sample ID	Amount of Patulin (µg/kg)	Sample ID	Amount of Patulin (µg/kg)
BM1	190.42	BL1	BDL
BM2	189.88	BL2	144.35
BM3	177.92	BL3	BDL
BM4	189.86	BL4	BDL
BM5	177.40	BL5	43.31
BM6	176.21	BL6	161.91
BM7	191.49	BL7	95.52
BM8	185.45	BL8	70.15
BM9	188.65	BL9	126.48
BM10	182.73	BL10	BDL
BM11	193.91	BL11	BDL
BM12	185.20	BL12	BDL
BM13	172.70	BL13	BDL
BM14	187.32	BL14	BDL
BM15	188.18	BL15	BDL
BM16	186.23	BL16	BDL
BM17	188.46	BL17	BDL
BM18	185.22	BL18	BDL
BM19	190.98	BL19	BDL
BM20	187.01	BL20	BDL
BM21	48.93	BL21	BDL
BM22	BDL	BL22	BDL
BM23	46.26	BL23	BDL
BM24	BDL	BL24	BDL
BM25	47.75	BL25	BDL
BM26	BDL	BL26	BDL
BM27	BDL	BL27	BDL
BM28	47.07	BL28	BDL
BM29	BDL	BL29	BDL
BM30	48.40	BL30	BDL
BM = Beef Meat; BL = Beef Liver; BDL = Below Detection Limit			

The chromatograms of residual patulin in beef meat and liver sample are presented in Figure 3.32 and 3.33.



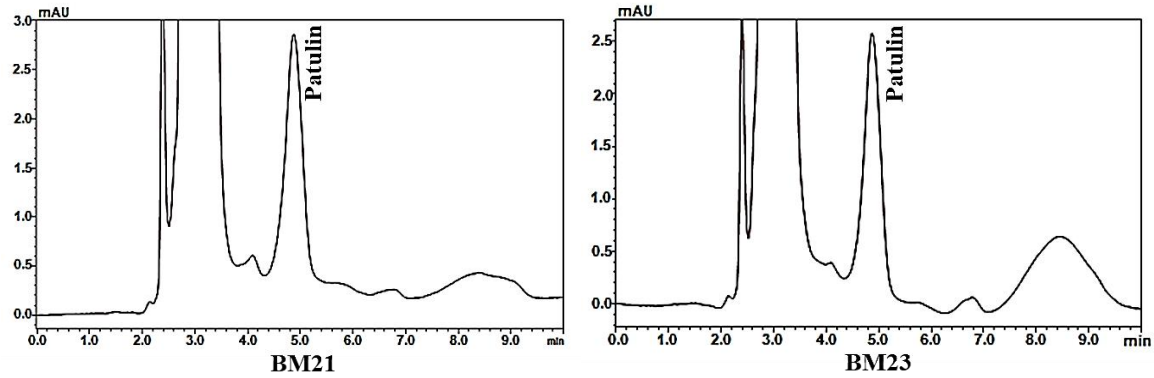


Figure 3.32 Chromatograms of patulin in beef meat

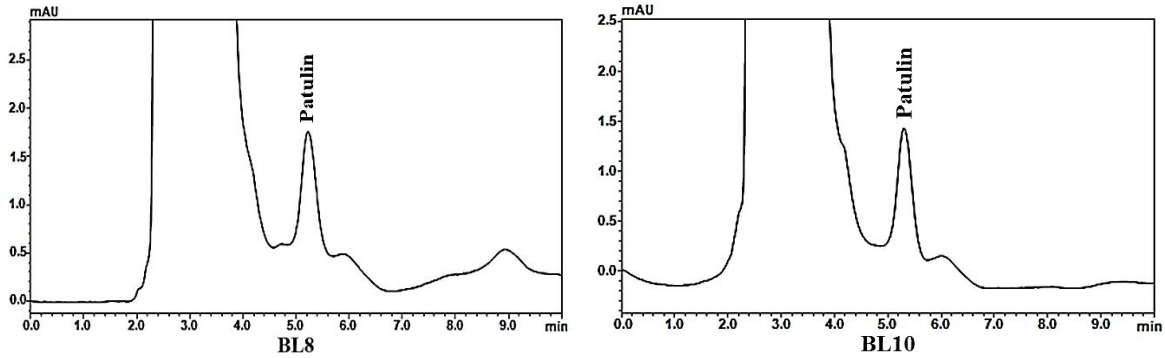


Figure 3.33 Chromatograms of patulin in beef liver

Among the total beef meat samples that were analyzed for determining residual patulin 83.33 % of positive samples contained patulin (Figure 3.34).

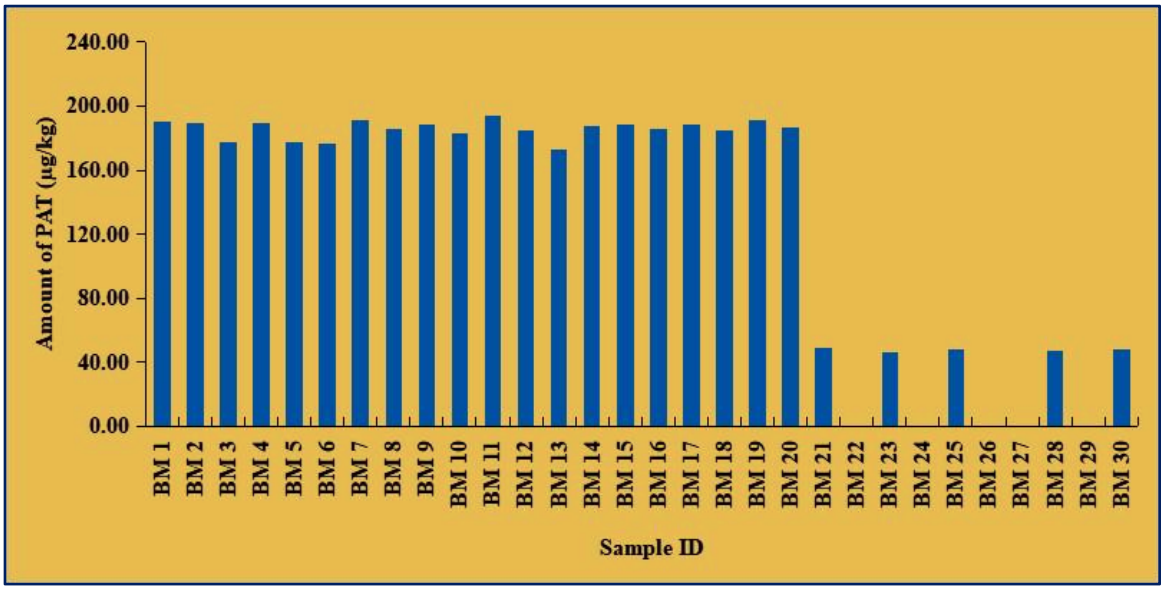


Figure 3.34 Amount of patulin in beef meat samples (µg/kg)

Patulin was found in 20% of beef liver samples among the total beef liver samples ranged 43.31 to 161.91 µg/kg (Figure 3.35) .

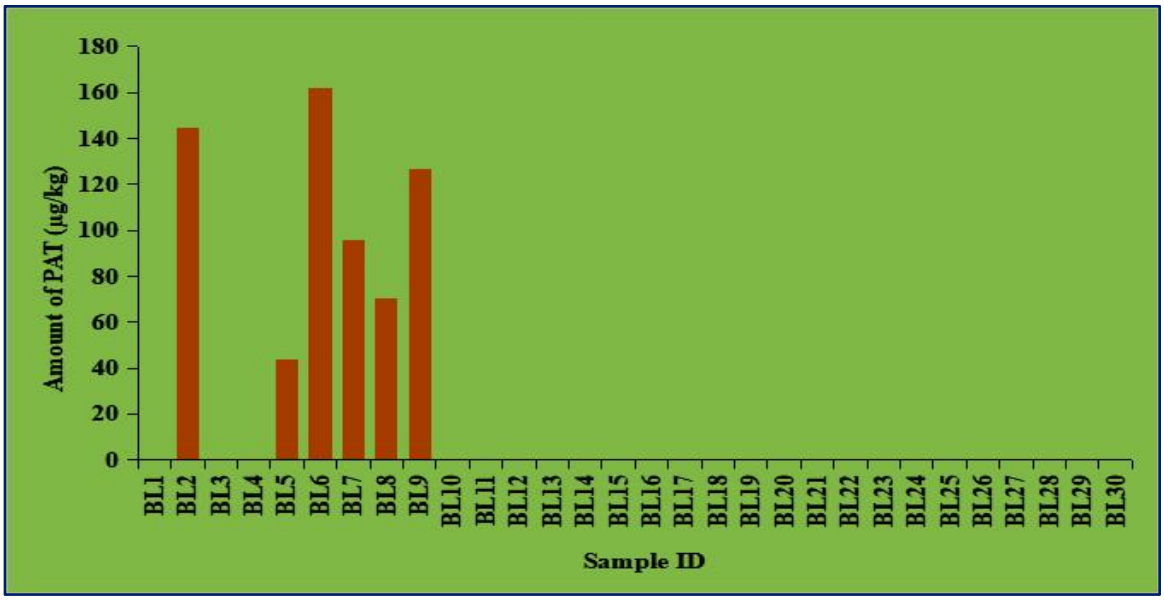


Figure 3.35 Amount of patulin in beef liver samples (µg/kg)

3.7 Analysis of Patulin in Broiler Chicken Meat and Liver

3.7.1 Evaluation of Residual Patulin in Broiler Chicken Meat and Liver

Broiler chicken meat (n=30) and broiler chicken liver (n=30) samples were collected from six different local marketplaces in Dhaka North and South City in Bangladesh for the determination of residual patulin. Each broiler chicken sample weighed 900–1000 g. Homogenized meat samples were kept at -20°C until analysis.

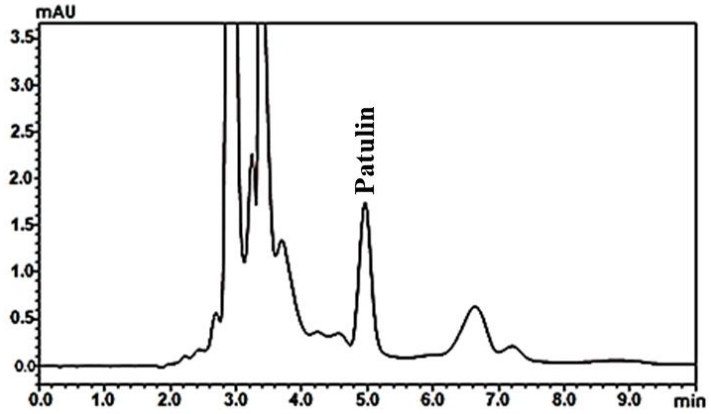


Figure 3.36 Chromatogram of matrix-matched patulin for chicken meat (10 µg/kg)

The matrix-matched calibration for patulin at seven concentrations ranged from 1 to 30 µg/kg and the correlation coefficient (r^2) value was 0.9980 for broiler chicken meat. The recovery percentages ranged from 93.92 to 94.22 and 98.46 to 99.93 for patulin in broiler chicken meat at spiking levels of 10 and 15 µg/kg, respectively (Figure 3.36). The inter-day recoveries were 94.32and 99.47% and, the relative standard deviations (RSD%) were 0.72 and 0.42% for broiler chicken meat at spiking levels of 10 and 15 C, respectively, as presented in Table 3.22.

Table 3.22 Recovery percentage and RSD (%) of patulin for broiler chicken meat

Patulin (CM) (µg/kg)	**Intra-Day Recovery (%) Day-1 (n= 5)**	**Intra-Day Recovery (%) Day-2 (n= 5)**	**Intra-Day Recovery (%) Day-3 (n= 5)**	**Inter-day Recovery (%) 3 days (n= 15)**	**RSD (%) Intra-day (n= 5)**	**RSD (%) Inter-day 3 days (n= 15)**
10	93.92	94.83	94.22	94.32	0.48 0.93 0.74	0.72
15	98.46	100.02	99.93	99.47	0.23 0.40 0.61	0.41

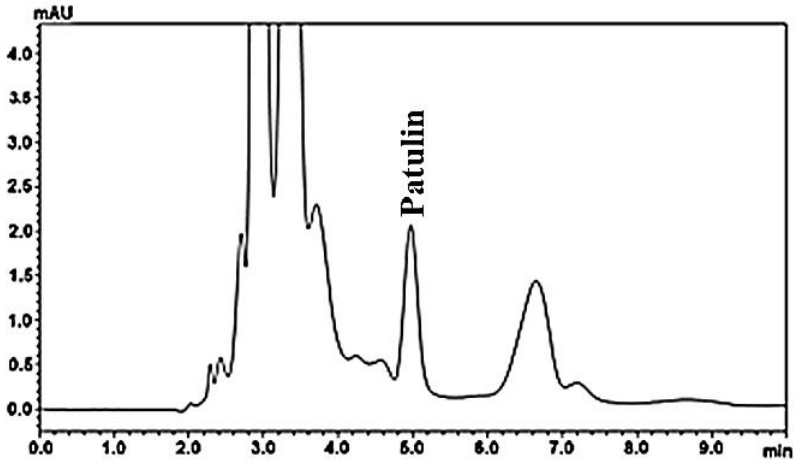


Figure 3.37 Chromatogram of matrix-matched patulin for chicken liver (15 µg/kg)

The matrix-matched calibration for patulin at seven concentrations ranged from 1 to 30 µg/kg (Figure 3.37) and the correlation coefficient (r^2) value was 0.9980 for broiler chicken liver. The recovery percentages ranged from 100.25 to 105.24 and 103.92 to 104.53 for patulin in broiler chicken liver at spiking levels of 10 and 15 µg/kg, respectively. The inter-day recoveries were 103.92 and 104.53% and, the relative standard deviation

(RSD%) was 0.84 and 0.62% for broiler chicken liver at spiking levels of 10 and 15 µg/kg, respectively, as presented in Table 3.16.

Table 3.23 Recovery percentage and RSD (%) of patulin for broiler chicken liver

Patulin (CL) (µg/kg)	Intra-Day Recovery (%) Day-1 (n= 5)	Intra-Day Recovery (%) Day-2 (n= 5)	Intra-Day Recovery (%) Day-3 (n= 5)	Inter-day Recovery (%) 3 days (n= 15)	RSD (%) Intra-day (n= 5)	RSD (%) Inter-day 3 days (n= 15)
10	100.25	105.10	105.24	103.53	0.85 0.81 0.85	0.84
15	103.92	104.32	104.53	104.26	0.69 0.52 0.64	0.62

Correlation Coefficient (r^2), Standard Deviation, LOD, and LOQ of Standard Patulin and Matrix-matched for beef meat and liver samples are presented in Table 3.24.

Table 3.24 Correlation coefficient (r^2), standard deviation, LOD, and LOQ of standard patulin and matrix-matched for beef meat and liver samples

Antibiotic	Correlation Coefficient (r^2)	Standard Deviation for LOD	LOD (µg/kg)	LOQ (µg/kg)
Patulin (STD)	0.9991	0.10	0.31	1.03
CM (Patulin)	0.9980	0.11	0.35	1.18
CL (Patulin)	0.9990	0.19	0.56	1.88
STD = Standard; CM = Broiler Chicken Meat; CL = Broiler Chicken Liver				

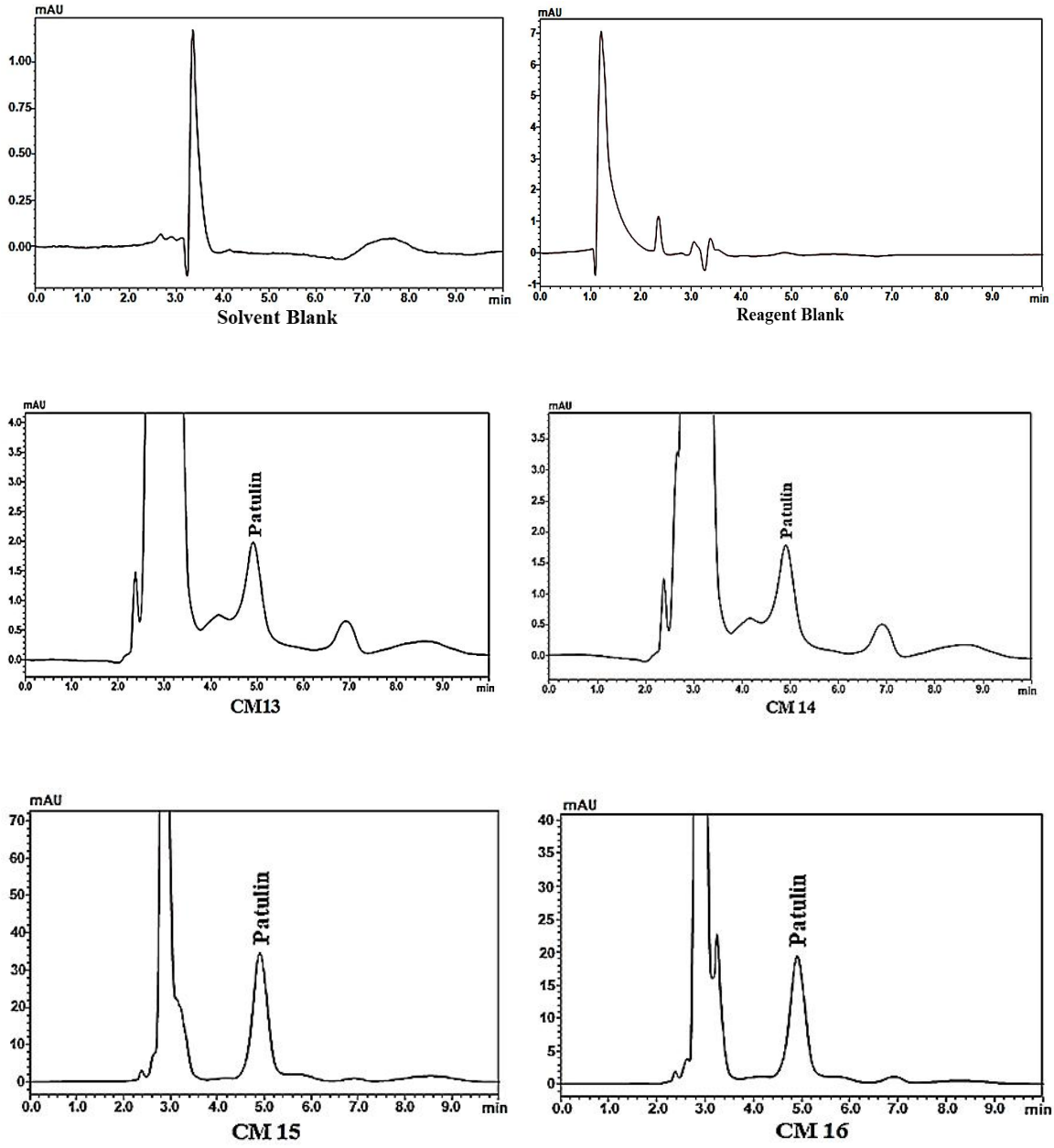
3.7.2 Discussion for Residual Patulin in Broiler Chicken Meat and Liver

Residual patulin antibiotic (as well as mycotoxin) was detected 18 broiler chicken meat samples ranged 16.94 to 310.53 µg/kg and 11 broiler chicken liver samples ranged 14.75 to 52.88 µg/kg (Table 3.24). The MRL value for patulin in fruit and fruit juices was 50 µg/kg, but no MRL value was set for patulin in chicken meat and liver samples by any regulatory agencies.

Table 3.25 Amount of patulin in broiler chicken meat and liver samples

Sample ID	Amount of Patulin (µg/kg)	Sample ID	Amount of Patulin (µg/kg)
CM 1	BDL	CL 1	22.08
CM 2	20.45	CL 2	35.93
CM 3	16.94	CL 3	14.75
CM 4	BDL	CL 4	15.66
CM 5	BDL	CL 5	19.27
CM 6	BDL	CL 6	20.04
CM 7	BDL	CL 7	25.93
CM 8	BDL	CL 8	25.69
CM 9	BDL	CL 9	40.38
CM 10	BDL	CL 10	19.26
CM 11	BDL	CL 11	BDL
CM 12	261.76	CL 12	BDL
CM 13	157.12	CL 13	BDL
CM 14	134.83	CL 14	BDL
CM 15	137.09	CL 15	BDL
CM 16	208.63	CL 16	BDL
CM 17	310.53	CL 17	BDL
CM 18	179.40	CL 18	BDL
CM 19	BDL	CL 19	BDL
CM 20	93.02	CL 20	BDL
CM 21	25.74	CL 21	BDL
CM 22	21.01	CL 22	BDL
CM 23	19.32	CL 23	52.88
CM 24	BDL	CL 24	BDL
CM 25	BDL	CL 25	BDL
CM 26	129.74	CL 26	BDL
CM 27	142.40	CL 27	BDL
CM 28	182.01	CL 28	BDL
CM29	162.41	CL 29	BDL
CM30	162.40	CL 30	BDL
CM = Chicken Meat; CL = Chicken Liver; BDL = Below Detection Limit			

Chromatogram of residual patulin in chicken meat samples with solvent blank and reagent blank are presented in Figure 3.38.



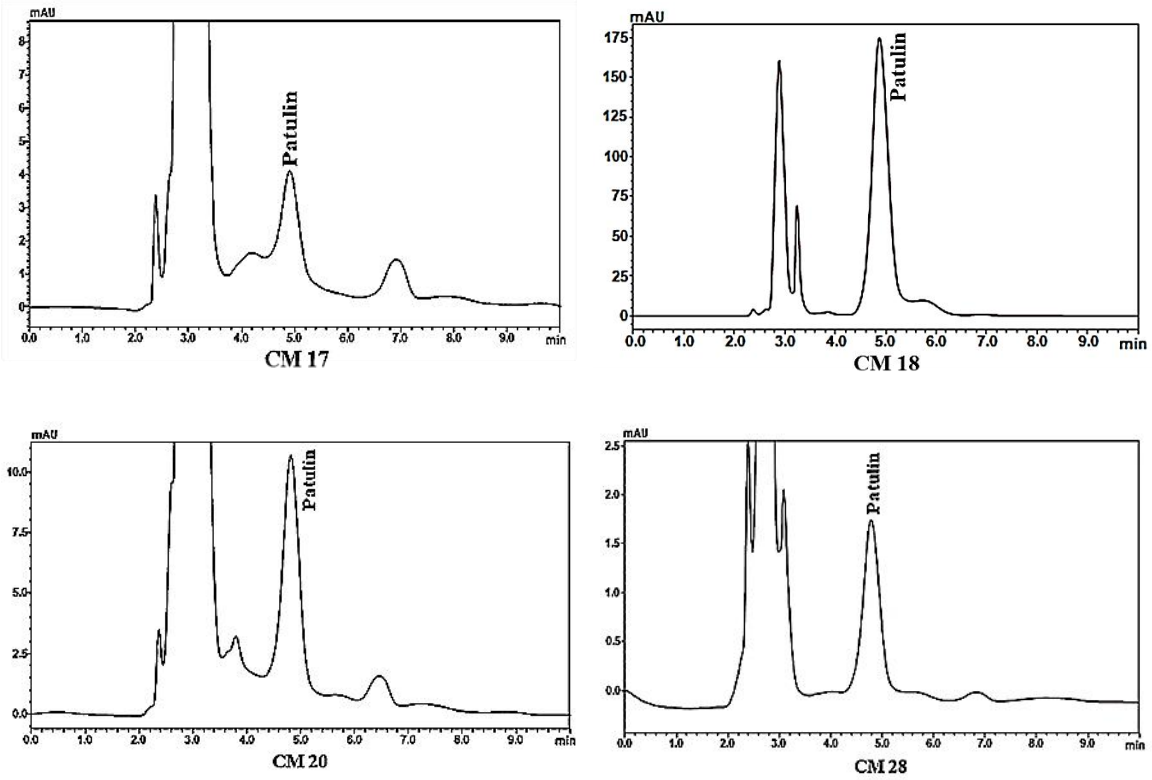


Figure 3.38 Chromatograms of patulin for chicken meat

Patulin was identified and quantified in 18 broiler chicken meat samples. Sixty percent (60%) was the positive samples containing residual patulin among the total broiler chicken meat samples (Figure 3.39).

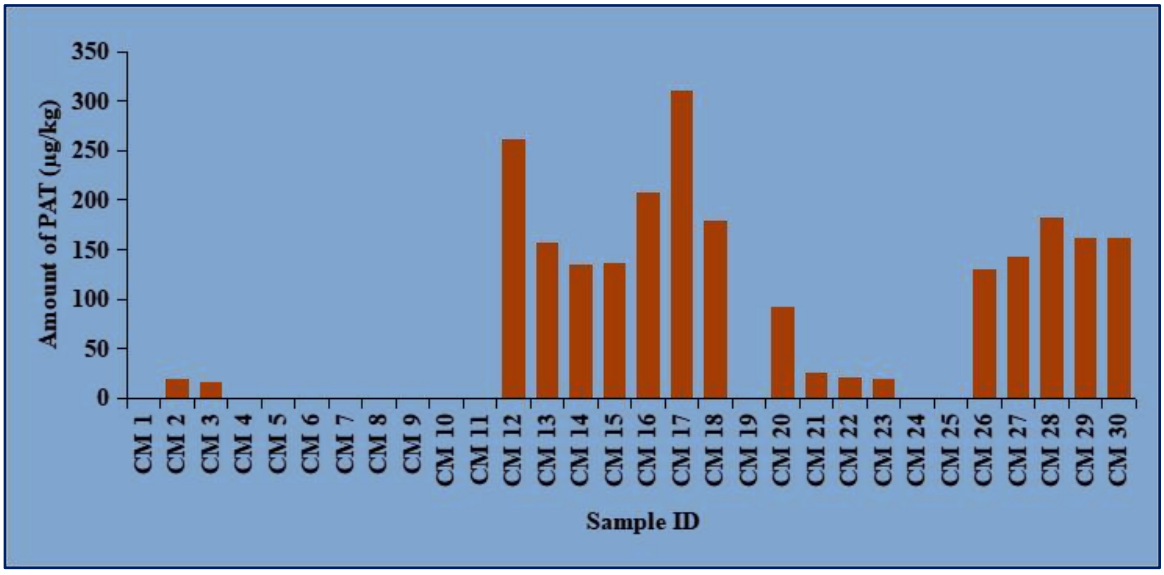


Figure 3.39 Amount of patulin in chicken meat samples (µg/kg)

The Chromatogram of broiler chicken liver samples are displayed in Figure 3.40.

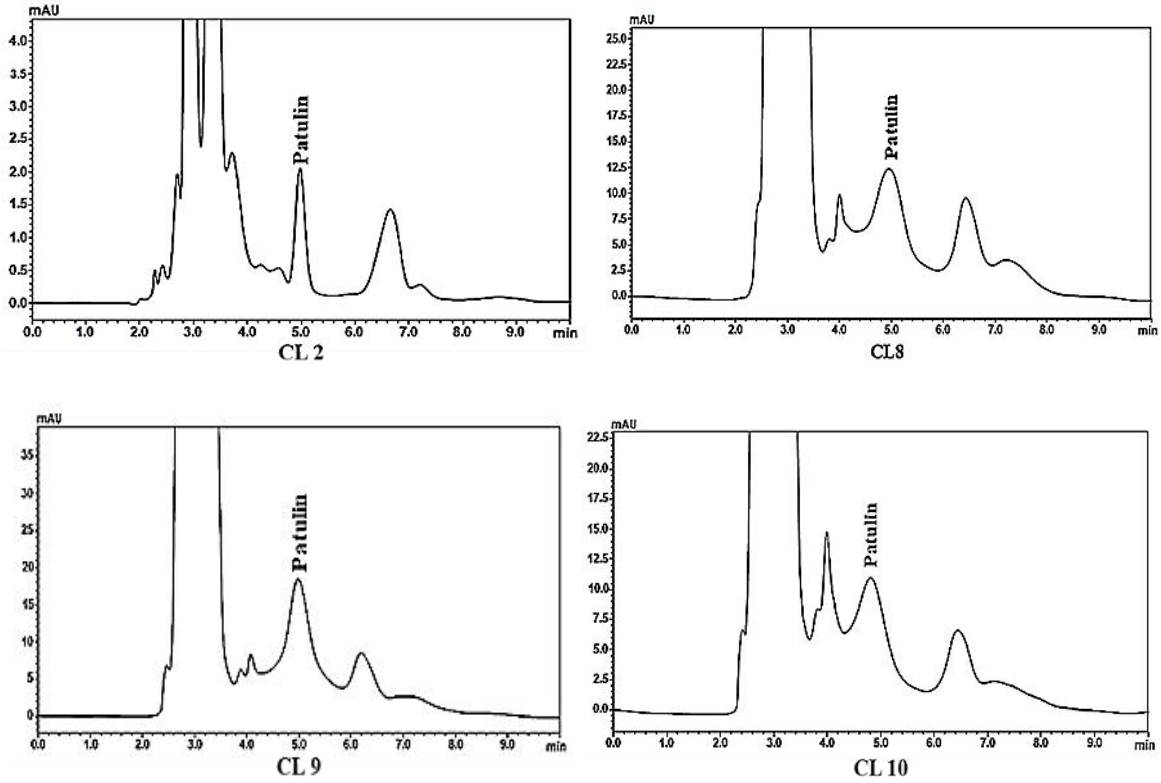


Figure 3.40 Chromatograms of patulin for chicken liver

Patulin was detected and quantified in 11 broiler chicken meat samples. 36.67% was the positive samples containing residual patulin among the total broiler chicken liver samples (Figure 3.41).

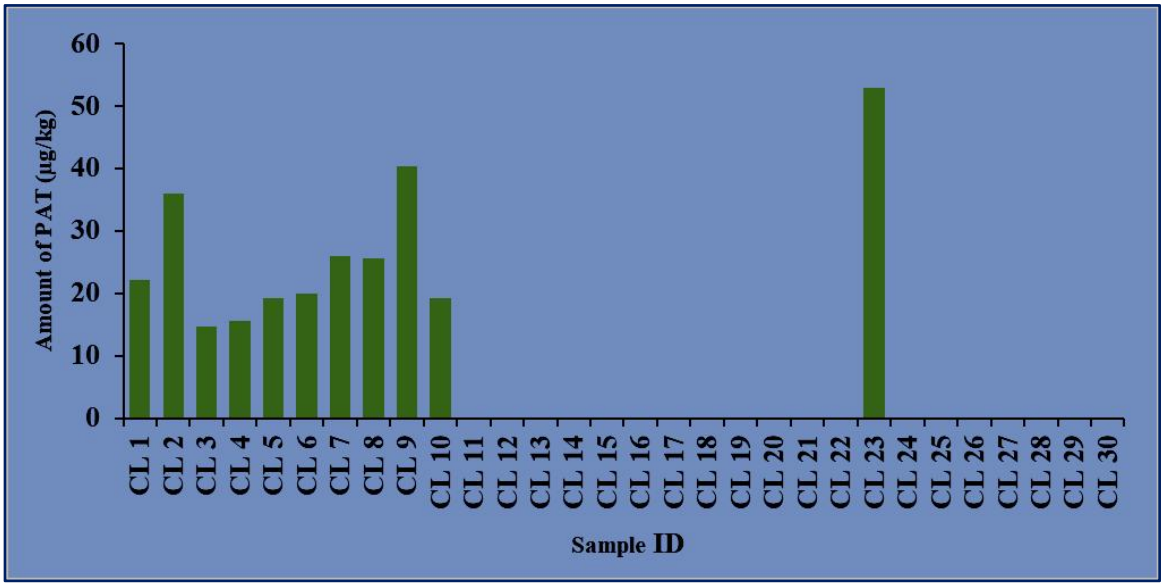


Figure 3.41 Amount of patulin broiler chicken liver samples (µg/kg)

3.8 Health Risk Assessment of Residual Antibiotics

A hazard index (HI) was computed using the formula HI = EDI/ADI in order to evaluate the long-term impacts on health. Beef meat and liver, and broiler chicken meat and liver are considered safe for human consumption if its HI value is less than 1. However, HI > 10 indicates an unacceptable risk for consumption, while $1 \leq HI < 10$ warns of a risk but not an emergency (Table 3.26) [315].

Table 3.26 Estimated daily intake (EDI, µg/kg/bw/day), acceptable daily intake (ADI, µg/kg/bw/day), and associated hazard quotient (HQ) of each antibiotic in all analyzed meat and liver samples (n =120) evaluated for adults

Antibiotics	**Sample ID**	**EDI (µg/kg/bw/day)**	**ADI (µg/kg/bw/day)**	**HI**
Oxytetracycline	BM	ND	30	ND
	BL	9.30	30	3.10×10^{-4}
	CM	43.74	30	1.46×10^{-3}
	CL	ND	30	ND
Tetracycline	BM	ND	30	ND
	BL	ND	30	ND
	CM	195.00	30	6.50×10^{-3}
	CL	ND	30	ND
Chlortetracycline	BM	ND	30	ND
	BL	ND	30	ND
	CM	186.84	30	6.23×10^{-3}
	CL	ND	30	ND
Amoxicillin	BM	0.89	0.07	1.28×10^{-2}
	BL	0.66	0.07	9.47×10^{-3}
	CM	ND	0.07	ND
	CL	ND	0.07	ND
Patulin	BM	19.37	0.4	4.84×10^{-2}
	BL	2.67	0.4	6.68×10^{-3}
	CM	36.24	0.4	9.06×10^{-2}
	CL	0.66	0.4	1.66×10^{-3}
BM = Beef Meat, BL = Beef Liver, CM = Broiler Chicken meat, CL = Broiler Chicken Liver, ND = Not Detected				

3.9 Analysis of Organochlorine Pesticides (OCPs) in Beef Meat and Liver

3.9.1 Determination of Retention Time (RT), LOD and LOQ of Mixed OCPs

To investigate the levels of OCPs, beef meat (n=30) and of beef liver (n=30) samples were purchased from six different local marketplaces in Dhaka North and South City, Bangladesh. Before analysis, the homogenized meat samples were stored at -20°C. Gas chromatography with an electron capture detector (GC-ECD) was used to evaluate the liver and meat of beef for 20 pesticides in a mixed group. The chromatogram for mix-20 OCPs is presented in Figure 3.42. The retention time, correlation coefficient (r^2), regression equation, LOD and LOQ of mixed OCPs have been displayed in Table 3.27.

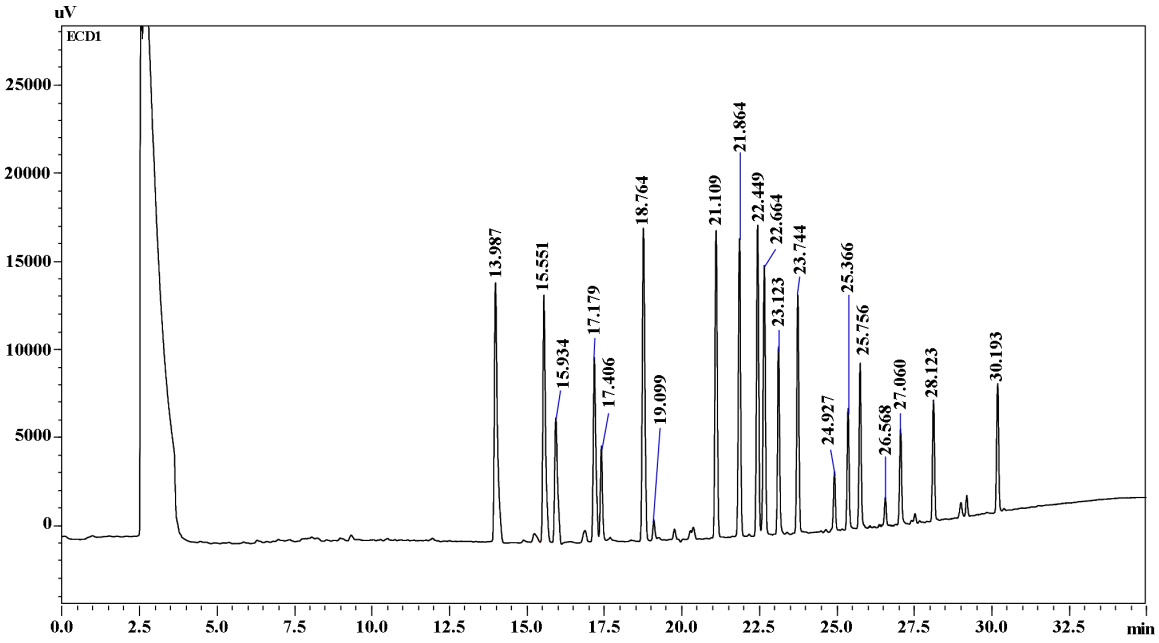


Figure 3.42 Chromatogram of standard OCPs

Table 3.27 Elution order, retention time (RT), correlation coefficient (r^2), regression equation, LOD and LOQ of mixed OCPs

Elution Oder	Pesticides (OCPs)	RT (Mean)	r^2	Regression equation (Y= ax ± b)	LOD (µg/mL)	LOQ (µg/mL)
1	alpha-BHC	13.977	0.9999	y = 4966.4x -318.77	0.0143	0.0430
2	gamma-BHC	15.541	0.9994	y = 4163.4x - 1009.2	0.0145	0.0434
3	beta-BHC	15.925	0.9990	y = 2292x + 1073.6	0.0149	0.0447
4	delta-BHC	17.208	0.9988	y = 3025.6x + 895.59	0.0105	0.0316
5	Heptachlor	17.446	0.9980	y = 1301.1x - 527.08	0.0230	0.0689
6	Aldrin	18.755	0.9997	y = 5061.5x + 1094.1	0.0102	0.0307
7	Heptachlor epoxide	19.107	0.9991	y = 8079.9x + 1028.6	0.0124	0.0371
8	*trans*-Chlordane	21.100	0.9994	y = 4357.7x + 1036.7	0.0355	0.1064
9	*cis*- Chlordane	21.857	0.9993	y = 4145.8x + 1139.8	0.0989	0.2968
10	Endosulfan I	22.440	0.9996	y = 4249.8x + 976.23	0.0252	0.0756
11	4, 4′-DDE	22.658	0.9983	y = 3821.2x + 1880.6	0.0128	0.0383
12	Dieldrin	23.118	0.9978	y = 2371.9x + 1404.9	0.0128	0.0385
13	Endrin	23.739	0.9995	y = 3125.3x + 554	0.0157	0.0471
14	4, 4′-DDD	24.919	0.9985	y = 853.46x + 229.28	0.1110	0.3329
15	Endosulfan II	25.362	0.9990	y = 1798.5x + 550.64	0.0245	0.0734
16	Endrin Aldehyde	25.750	0.9997	y = 2187.7x + 354.44	0.0216	0.0649
17	4, 4′-DDT	26.562	0.9981	y = 348.43x + 197.97	0.0930	0.2790
18	Endosulfan Sulfate	27.049	0.9991	y = 1281.8x - 56.244	0.0105	0.0315
19	Methoxychlor	28.119	0.9994	y = 1491.6x + 412.64	0.0509	0.1527
20	Endrin Ketone	30.187	0.9998	y = 1625.8x + 233.82	0.0256	0.0768

Maximum Residue Limit (MRL) was set by FAO/WHO for pesticides. MRL for OCPs is presented Table 3.28 set by Codex Alimentarius Commission.

Table 3.28 Type of pesticides, ADI and MRL values of OCPs

Pesticides (OCPs)	Type	Acceptable Daily Intake (ADI) (mg/kg bw)	MRLs (Meat) FAO/WHO 2021 (mg/kg)	
			Beef Meat	Chicken Meat
alpha-BHC	Insecticide	0.005	0.01	0.005
gamma-BHC	Insecticide	0.005	0.01	0.005
beta-BHC	Insecticide	0.005	0.01	0.005
delta-BHC	Insecticide	0.005	0.01	0.005
Heptachlor	Insecticide	0.0001	0.2	0.2
Aldrin	Insecticide	0.0001	0.2	0.2
Heptachlor epoxide	Insecticide	0.0001	0.2	0.2
trans-Chlordane	Insecticide	0.0005	0.05	0.5
cis- Chlordane	Insecticide	0.0005	0.05	0.5
Endosulfan I	Insecticide	0.006	0.2	0.03
4, 4′-DDE	Insecticide	0.01	5.0	0.3
Dieldrin	Insecticide	0.0001	0.2	0.2
Endrin	Insecticide	0.0002	0.1	0.1
4, 4′-DDD	Insecticide	0.01	5.0	0.3
Endosulfan II	Insecticide	0.006	0.2	0.03
Endrin Aldehyde	Insecticide	0.0002	0.1	0.1
4, 4′-DDT	Insecticide	0.01	5.0	0.3
Endosulfan Sulfate	Insecticide	0.006	0.2	0.03
Methoxychlor	Insecticide	0.1	n/a	n/a
Endrin Ketone	Insecticide	0.0002	0.1	0.1

3.9.2 Matrix-matched Calibration of OCPs for Beef Meat and Liver

Matrix-matched calibration was performed for the analysis of residual OCPs in beef meat and liver samples to compensate for matrix effects, which are interference from complex sample components that change the analytical signal and interfere with accuracy and precision, This technique confirms that the calibration curve appropriately reflects the actual circumstances and enhances the dependability of quantitative analysis by creating calibration standards in a blank version of the same sample matrix (Figure 3.43 and 3.44).

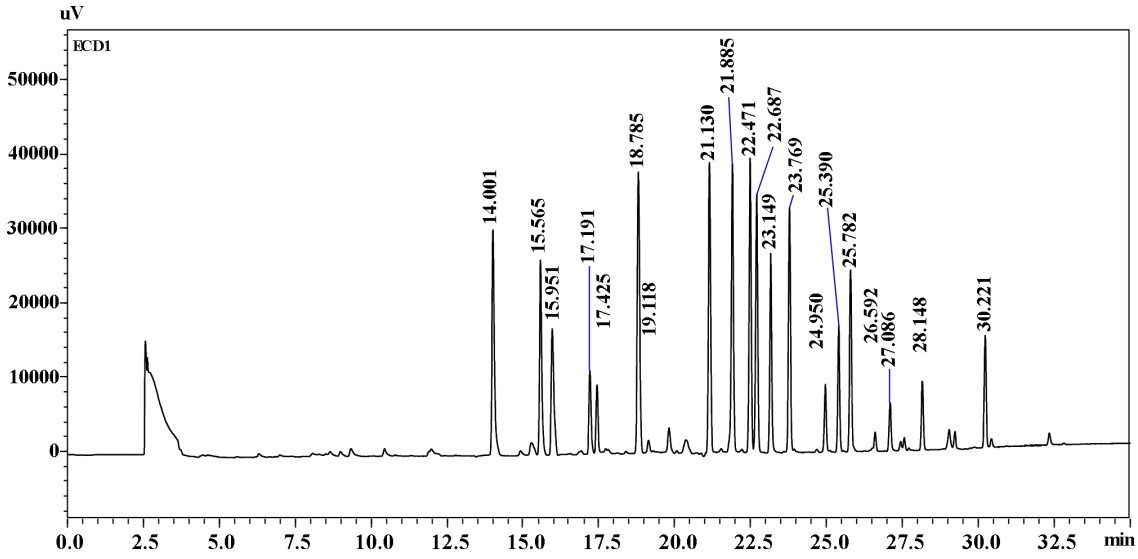


Figure 3.43 Matrix-matched chromatogram of OCPs in beef meat

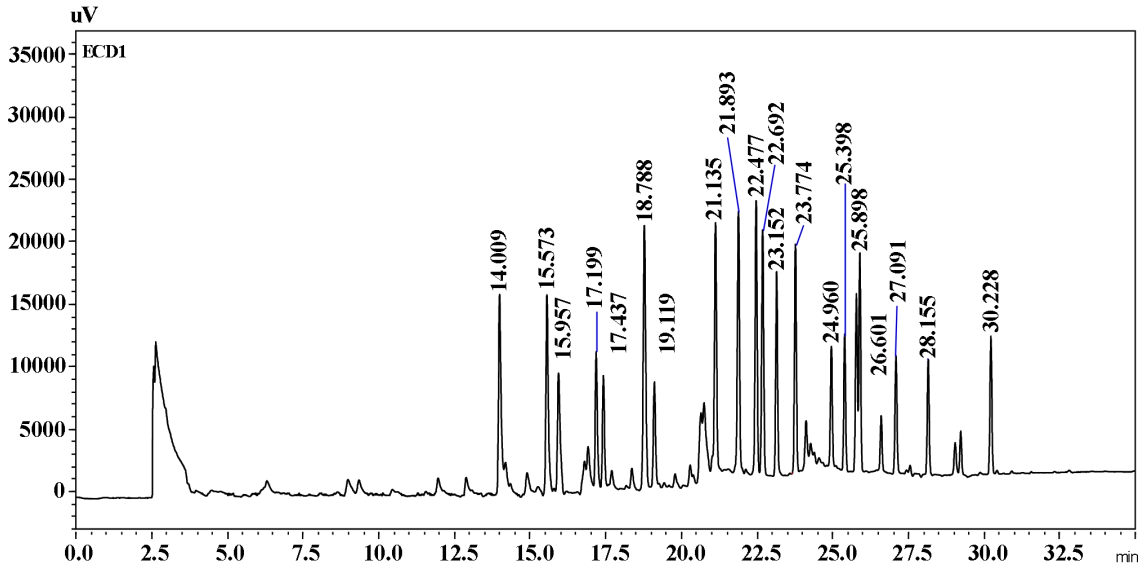


Figure 3.44 Matrix-matched chromatogram of OCPs in beef liver

3.9.3 Estimation of Residual OCPs in Beef Meat and Liver

Among the thirty beef meat samples analyzed, alpha-BHC was detected in eleven samples with a concentration range of 1.01 to 62.49 µg/kg. Gamma-BHC was found in nine samples, with levels ranging from 1.22 to 103.01 µg/kg. Beta-BHC appeared in nine samples where the concentrations varied from 1.13 to 8.94 µg/kg. Delta-BHC was identified in twenty-eight samples, with a range of 84.45 to 329.08 µg/kg. Heptachlor was present in thirteen samples, showing concentrations between 0.97 and 29.64 µg/kg. Aldrin was detected in twenty samples, with levels ranging from 0.96 to 61.71 µg/kg. Heptachlor epoxide was found in twenty-eight samples, with a range of 57.87 to 304.25 µg/kg. *Trans*-chlordane was present in fourteen samples, with concentrations between 0.86 and 7.90 µg/kg. *Cis*-chlordane was detected in eighteen samples, ranging from 0.61 to 6.22 µg/kg. Endosulfan I appeared in twenty-six samples with levels ranging from 1.23 to 22.86 µg/kg. The compound 4, 4′-DDE was found in twenty samples, with concentrations ranging from 0.17 to 21.41 µg/kg. Dieldrin was detected in twenty-two samples, with levels ranging from 0.47 to 36.50 µg/kg. Endrin was identified in seventeen samples, with a concentration range of 0.63 to 16.98 µg/kg. The compound 4, 4′-DDD was found in twenty-three samples, with levels ranging from 0.48 to 38.94 µg/kg. Endosulfan II appeared in twenty-seven samples, with concentrations ranging from 1.61 to 187.29 µg/kg. Endrin aldehyde was detected in nineteen samples, with a range of 0.98 to 24.70 µg/kg. The compound 4, 4′-DDT was found in fifteen samples, ranging from 0.24 to 76.81 µg/kg. Endosulfan sulfate was present in twelve samples, with levels from 0.83 to 11.10 µg/kg. Methoxychlor was detected in eighteen samples, with concentrations ranging from 0.77 to 14.04 µg/kg. Finally, endrin ketone was identified in four samples, with a concentration range of 0.83 to 10.55 µg/kg, respectively (Table 3.29).

Table 3.29 Amount of twenty organochlorine pesticides (µg/kg) in beef meat samples

Sample ID	alpha-BHC	gamma-BHC	beta-BHC	delta-BHC	Heptachlor	Aldrin	Heptachlor epoxide	*trans*-Chlordane	*cis*-Chlordane	Endosulfan-I
BM1	1.86	1.22	1.78	172.63	0.97	2.68	180.00	ND	2.62	2.09
BM2	1.01	ND	ND	ND	14.43	3.46	114.12	ND	1.68	ND
BM3	ND	ND	ND	132.37	6.38	2.11	196.43	ND	1.20	1.31
BM4	ND	ND	ND	143.10	21.57	2.19	186.70	1.84	ND	2.87
BM5	ND	ND	ND	112.26	4.93	ND	ND	ND	2.75	ND
BM6	8.07	3.40	ND	222.61	15.63	3.86	207.46	1.97	24.98	4.02
BM7	6.06	9.79	4.33	84.45	24.27	61.71	60.41	15.49	0.72	22.86
BM8	62.49	ND	ND	ND	29.64	0.99	114.37	ND	2.89	1.83
BM9	ND	ND	ND	180.97	11.94	6.41	100.06	ND	1.54	ND
BM10	ND	ND	ND	220.31	ND	1.46	132.64	1.02	2.00	2.14
BM11	2.88	ND	ND	164.79	ND	2.25	132.89	ND	6.22	2.14
BM12	ND	ND	ND	207.50	ND	ND	115.23	ND	1.28	2.00
BM13	ND	ND	ND	172.01	ND	ND	106.46	ND	0.61	1.70
BM14	ND	ND	ND	157.63	ND	0.96	93.19	ND	1.31	ND
BM15	6.57	10.89	8.94	265.42	ND	6.54	245.20	7.90	ND	8.28
BM16	ND	ND	ND	249.35	ND	ND	186.50	ND	ND	2.23
BM17	ND	ND	ND	206.35	ND	1.95	186.97	ND	0.48	2.31
BM18	ND	ND	1.86	227.85	ND	2.70	241.35	2.92	2.16	2.63
BM19	2.31	9.40	2.18	252.05	ND	4.20	304.25	3.55	1.06	3.34
BM20	2.45	ND	2.49	261.54	ND	4.95	275.72	2.00	1.31	3.66
BM21	ND	ND	ND	299.21	5.10	ND	ND	1.22	1.60	1.86
BM22	ND	ND	ND	220.16	ND	ND	57.87	ND	2.62	1.23
BM23	ND	103.01	ND	215.87	ND	1.36	68.80	0.88	1.68	1.51
BM24	ND	1.58	ND	291.47	ND	1.45	117.53	0.86	1.20	1.79
BM25	9.49	ND	ND	305.52	ND	ND	160.14	1.70	ND	2.68
BM26	2.63	ND	1.13	282.08	17.85	ND	88.98	ND	2.75	1.92
BM27	ND	ND	ND	329.08	ND	ND	128.38	ND	24.98	1.46
BM28	ND	2.86	1.44	314.07	2.94	1.90	134.26	0.91	0.72	1.79
BM29	ND	ND	ND	297.47	7.22	ND	264.82	1.20	2.89	3.39
BM30	ND	2.27	1.57	244.95	ND	1.80	159.19	ND	1.54	1.96

Table 3.29 continuous

Sample ID	4,4´-DDE	Dieldrin	Endrin	4,4´-DDD	Endosulfan II	Endrin aldehyde	4,4´-DDT	Endosulfan sulfate	Methoxychlor	Endrin Ketone
BM1	0.48	2.13	ND	12.63	ND	1.10	0.25	1.83	13.42	ND
BM2	0.19	ND	ND	1.92	ND	ND	ND	ND	ND	ND
BM3	ND	ND	0.99	ND	17.35	ND	2.76	ND	ND	ND
BM4	1.23	1.65	1.26	ND	9.54	2.52	0.95	ND	1.60	1.78
BM5	0.30	ND	0.80	1.53	5.53	ND	ND	1.70	ND	ND
BM6	11.40	5.27	2.28	17.72	27.16	24.70	4.81	11.10	2.12	ND
BM7	21.41	36.50	16.98	38.94	187.29	10.38	76.81	10.47	5.43	10.55
BM8	0.18	0.96	ND	4.36	ND	1.14	ND	ND	ND	ND
BM9	0.45	0.26	ND	2.03	7.39	ND	ND	ND	1.75	ND
BM10	0.52	0.55	0.65	3.56	3.53	1.49	ND	ND	0.97	ND
BM11	0.89	1.10	ND	2.96	10.18	ND	1.03	ND	ND	ND
BM12	ND	1.85	1.64	1.49	5.15	1.86	0.25	0.85	0.86	ND
BM13	0.44	1.14	ND	0.69	1.61	ND	ND	ND	ND	ND
BM14	1.71	ND	ND	7.73	1.69	ND	ND	4.78	ND	ND
BM15	6.75	6.40	6.75	11.39	20.81	10.69	ND	ND	14.04	ND
BM16	0.24	1.91	1.49	0.98	30.38	0.98	2.29	1.11	0.80	ND
BM17	ND	1.56	ND	ND	4.62	0.81	0.18	ND	0.88	ND
BM18	ND	3.82	3.79	ND	119.24	3.68	22.47	ND	ND	ND
BM19	ND	3.30	0.65	0.57	5.34	8.50	0.90	2.80	1.23	ND
BM20	ND	2.54	ND	1.95	1.45	3.65	0.24	ND	1.03	0.83
BM21	0.17	2.07	ND	0.61	6.76	ND	ND	ND	1.42	ND
BM22	0.59	0.47	ND	1.08	4.92	0.98	ND	1.95	ND	ND
BM23	0.21	0.48	ND	ND	3.38	1.54	ND	ND	1.01	ND
BM24	ND	1.23	ND	ND	3.02	ND	0.40	ND	0.90	ND
BM25	3.98	1.06	ND	2.34	5.61	ND	ND	ND	2.45	ND
BM26	2.21	ND	ND	9.53	2.15	26.45	ND	1.91	ND	ND
BM27	ND	ND	2.17	0.48	2.32	6.96	ND	10.35	0.77	ND
BM28	ND	4.90	0.80	ND	56.55	ND	3.06	ND	ND	ND
BM29	ND	4.61	2.67	0.90	13.81	3.75	1.02	ND	0.98	0.89
BM30	0.36	ND	1.07	0.50	5.11	4.44	ND	ND	ND	ND

In a study of thirty beef liver samples, alpha-BHC was identified in twenty-eight samples, with concentrations varying from 17.40 to 340.42 µg/kg. Gamma-BHC was present in sixteen samples, showing a range from 1.75 to 15.79 µg/kg. Beta-BHC was detected in sixteen samples, with levels ranging from 2.87 to 42.82 µg/kg. Delta-BHC appeared in twenty-four samples, with concentrations between 2.24 and 26.41 µg/kg. Heptachlor was found in twenty-two samples, with amounts from 4.67 to 16.67 µg/kg. Aldrin was discovered in twenty-one samples, with levels varying from 0.66 to 20.93 µg/kg. Heptachlor epoxide was present in twenty-eight samples, ranging from 65.92 to 197.61 µg/kg. *Trans*-chlordane was detected in sixteen samples, with concentrations from 0.43 to 29.68 µg/kg. *Cis*-chlordane was found in ten samples, showing a range of 1.19 to 6.22 µg/kg. Endosulfan I was present in twenty-four samples, with amounts ranging from 1.15 to 5.02 µg/kg. The compound 4, 4'-DDE appeared in eleven samples, with a concentration range of 0.10 to 3.38. Dieldrin was found in twenty-one samples, varying from 1.03 to 6.64, while endrin was detected in twenty samples within range of 0.42 to 7.48 µg/kg. The compound 4, 4'-DDD was found in seven samples, with levels from 0.59 to 1.84 µg/kg. Endosulfan II was detected in twenty samples, ranging from 0.22 to 25.55 µg/kg. Endrin aldehyde was found in twenty-six samples, with concentrations from 0.21 to 31.55. The compound 4, 4'-DDT was detected in nine samples, with amounts ranging from 3.08 to 17.75 µg/kg. Endosulfan sulfate was present in two samples, with values ranging from 0.04 to 0.05 µg/kg. Lastly, methoxychlor was found in eight samples, exhibiting levels from 0.26 to 11.67 µg/kg. However, endrin ketone was not identified in the beef liver samples as it was below the detection limit (Table 3.30).

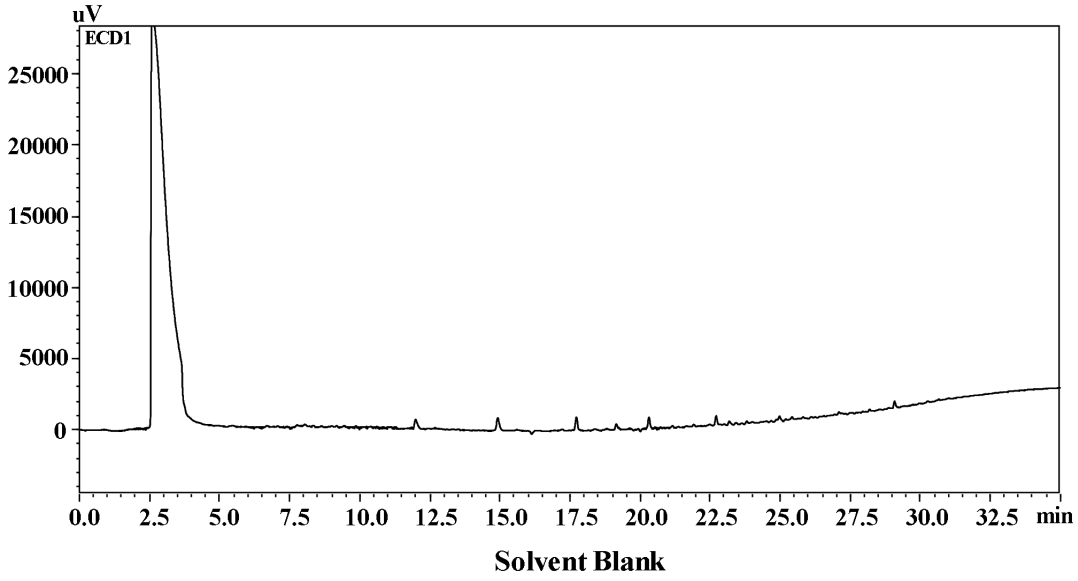
Table 3.30 Amount of twenty organochlorine pesticides (µg/kg) in beef liver samples

Sample ID	alpha-BHC	gamma-BHC	beta-BHC	delta-BHC	Heptac-hlor	Aldrin	Heptachlor epoxide	*trans*-Chlordane	*cis*-Chlordane	Endosulfan-I
BL1	136.09	1.75	3.86	2.24	16.67	1.04	80.19	0.75	1.37	3.09
BL2	51.47	2.08	ND	4.19	9.90	ND	141.22	0.56	ND	3.05
BL3	64.76	ND	ND	7.72	9.30	3.87	ND	4.01	ND	2.28
BL4	82.89	ND	ND	13.06	ND	20.93	65.92	0.96	2.18	1.22
BL5	165.46	7.89	13.50	26.41	11.86	6.10	160.65	4.63	4.16	2.95
BL6	33.58	ND	ND	3.86	7.20	3.12	165.05	1.93	ND	2.96
BL7	36.15	ND	ND	3.21	6.63	2.78	154.81	ND	ND	2.58
BL8	126.73	5.03	9.98	14.13	4.67	5.09	184.36	16.27	1.55	3.93
BL9	87.42	5.68	ND	6.24	ND	15.05	107.93	ND	ND	2.57
BL10	ND	ND	ND	12.88	ND	1.92	138.77	ND	ND	3.36
BL11	63.76	4.34	7.24	7.60	5.29	3.27	131.78	29.68	ND	3.88
BL12	186.90	ND	ND	4.46	19.75	1.55	108.11	5.84	6.22	1.15
BL13	241.88	ND	11.97	5.54	9.05	2.51	144.12	9.18	ND	5.02
BL14	128.25	ND	5.09	5.67	ND	3.28	138.41	ND	ND	ND
BL15	340.42	8.41	ND	ND	12.30	1.04	132.02	5.39	ND	4.22
BL16	92.94	ND	ND	ND	ND	ND	85.50	ND	ND	2.82
BL17	76.72	5.22	13.85	ND	17.93	5.36	150.06	23.30	2.50	3.81
BL18	30.22	6.49	15.48	8.84	ND	2.72	137.58	7.81	2.10	4.53
BL19	23.87	ND	7.00	10.20	14.20	3.93	154.21	ND	1.80	3.04
BL20	144.41	4.18	2.87	4.99	8.99	ND	149.04	ND	ND	4.35
BL21	17.40	ND	ND	ND	6.84	ND	ND	0.43	1.19	ND
BL22	181.00	ND	ND	9.13	10.14	0.66	158.45	6.36	ND	3.00
BL23	85.85	4.66	14.63	4.57	7.55	3.43	125.83	5.32	ND	2.29
BL24	148.09	6.79	ND	ND	9.54	8.85	148.86	ND	ND	2.42
BL25	21.39	4.68	5.10	3.70	ND	ND	106.09	ND	3.16	ND
BL26	ND	13.92	34.87	13.12	7.49	0.70	197.61	ND	ND	1.99
BL27	250.93	ND	4.14	3.97	8.06	ND	177.64	ND	ND	ND
BL28	58.73	ND	6.18	3.47	5.10	0.36	143.50	ND	ND	ND
BL29	33.39	15.79	42.82	9.15	7.05	ND	172.86	ND	ND	2.24
BL30	60.43	7.99	ND	ND	ND	ND	188.35	ND	ND	ND

Table 3.30 continuous

Sample ID	4,4´-DDE	Dieldrin	Endrin	4,4´-DDD	Endosulfan II	Endrin aldehyde	4,4´-DDT	Endosulfan sulfate	Methoxychlor	Endrin Ketone
BL1	3.38	3.01	0.42	ND	0.79	0.59	12.18	ND	ND	ND
BL2	ND	2.52	0.65	0.59	ND	1.13	3.72	ND	ND	ND
BL3	0.10	1.24	ND	ND	ND	1.50	12.76	ND	11.67	ND
BL4	0.64	1.05	2.05	4.46	2.40	1.45	5.51	ND	0.37	ND
BL5	ND	4.15	3.19	ND	ND	4.46	ND	ND	ND	ND
BL6	ND	ND	ND	ND	ND	12.34	ND	ND	ND	ND
BL7	ND	1.03	0.88	ND	9.02	5.75	ND	ND	ND	ND
BL8	ND	4.65	1.32	4.08	17.97	1.58	9.20	ND	ND	ND
BL9	ND	2.21	1.37	ND	1.41	0.21	ND	ND	ND	ND
BL10	0.74	1.55	0.94	1.84	0.22	0.23	ND	ND	ND	ND
BL11	ND	3.28	0.97	ND	4.25	5.44	ND	ND	ND	ND
BL12	ND	1.94	1.08	ND	ND	7.03	ND	ND	ND	ND
BL13	ND	ND	2.69	ND	ND	6.95	ND	ND	ND	ND
BL14	ND	ND	1.76	ND	ND	ND	ND	ND	ND	ND
BL15	0.13	6.43	0.88	ND	8.98	1.62	7.48	ND	1.04	ND
BL16	ND	ND	ND	1.30	0.84	0.33	ND	NQ	0.34	ND
BL17	ND	3.08	ND	ND	ND	0.44	ND	NQ	ND	ND
BL18	0.79	5.64	0.74	ND	22.58	ND	11.48	ND	ND	ND
BL19	ND	ND	ND	ND	0.36	0.03	ND	ND	ND	ND
BL20	0.28	ND	ND	ND	ND	0.16	3.08	ND	1.19	ND
BL21	0.33	ND	ND	ND	ND	0.91	ND	NQ	ND	ND
BL22	0.83	1.17	1.57	ND	1.07	ND	ND	ND	ND	ND
BL23	ND	ND	1.48	ND	4.72	3.56	ND	ND	0.26	ND
BL24	ND	3.08	2.19	ND	3.94	ND	ND	ND	ND	ND
BL25	2.01	2.13	1.30	3.13	0.84	22.76	ND	0.04	0.36	ND
BL26	ND	1.94	ND	ND	2.01	1.49	ND	ND	ND	ND
BL27	1.82	3.30	2.49	1.14	4.45	31.55	ND	0.05	0.59	ND
BL28	ND	6.64	0.93	ND	25.55	2.55	17.75	ND	ND	ND
BL29	ND	ND	ND	ND	4.40	6.25	ND	ND	ND	ND
BL30	ND	2.27	7.48	ND	1.41	4.32	ND	ND	ND	ND

Chromatograms of solvent blank, reagent blank, a few of residual OCPs in beef meat and liver samples are presented in Figure 3.45, 3.46 and 3.47.



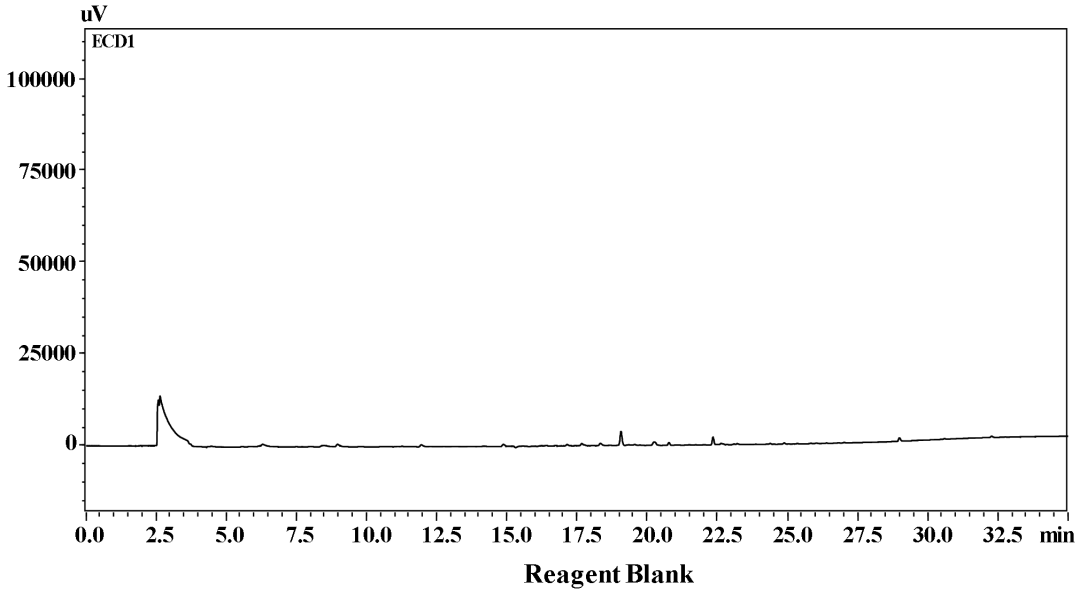


Figure 3.45 Solvent Blank and Reagent Blank for OCPs analysis

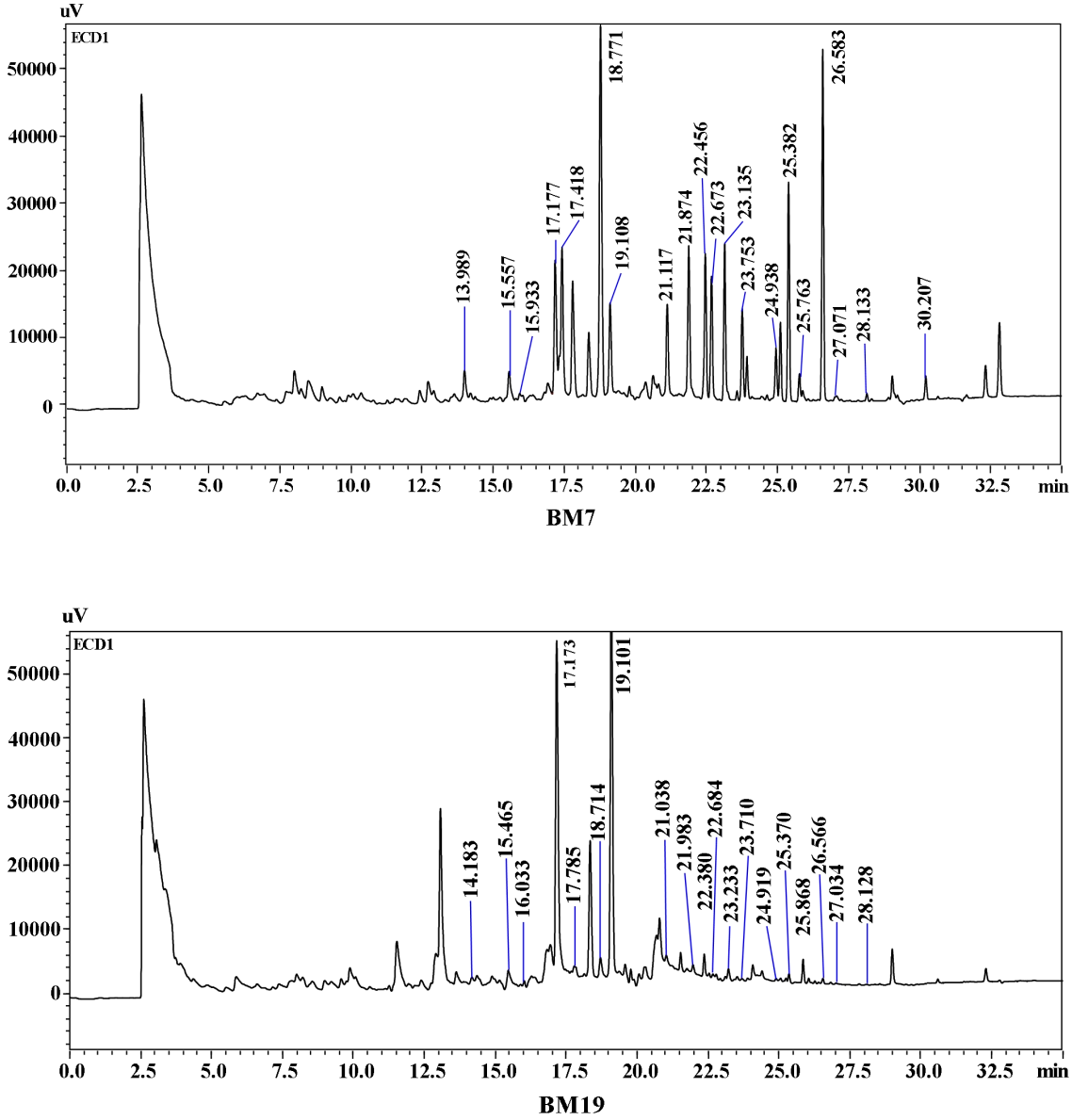


Figure 3.46 Chromagrams of residual OCPs in beef meat

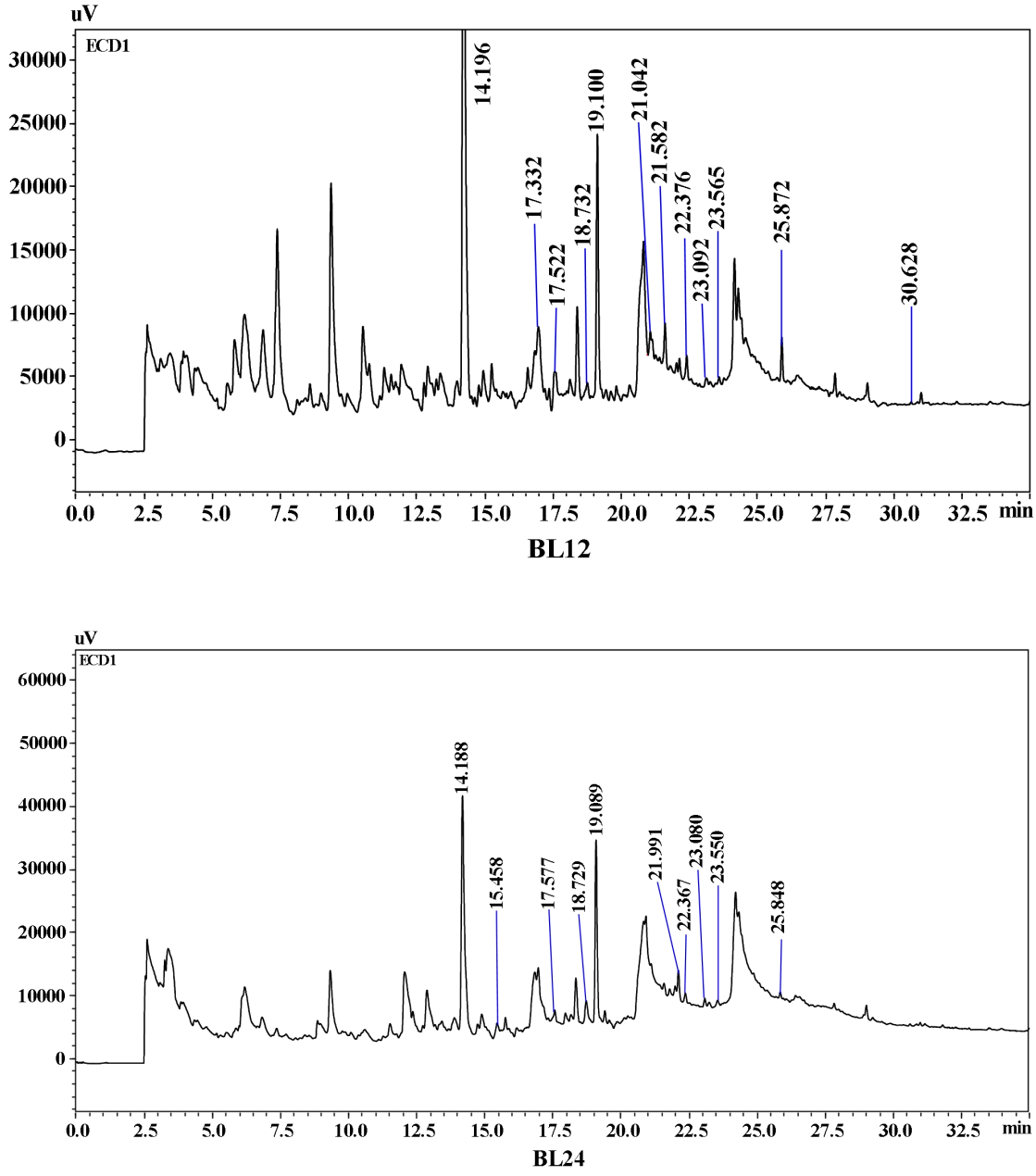


Figure 3.47 Chromagrams of residual OCPs in beef liver

3.9.4 Recovery Percentage of OCPs in Beef Meat and Liver Samples

The percentage of recovery (%) and the relative standard deviation (RSD%) were examined at concentrations of 2.5 and 5 µg/kg for the beef meat and liver samples that were analyzed. The recovery percentages for twenty different pesticides varied from 93.43 to 109.01 and from 99.11 to 107.59% at 2.5 µg/kg, while at 5 µg/kg, they ranged from 99.20 to 108.39 and from 99.50 to 106.68 µg/kg for beef meat and beef liver (Table 3.31).

Table 3.31 Average recovery percentages ± relative standard deviation (RSD) for 20 OCPs extracted from spiking laboratory blank meat and liver samples of beef using QuEChERS technique with two levels of the multi-standards mixture (2.5 and 5 µg/kg)

OCPs	2.5 µg/kg ± RSD %		5 µg/kg ± RSD %	
	Beef Meat	Beef Liver	Beef Meat	Beef Liver
alpha-BHC	90.59 ± 1.30	101.30 ± 1.01	99.49 ± 1.32	101.60 ± 3.09
gamma-BHC	109.01 ± 2.57	104.13 ± 1.52	106.20 ± 2.17	106.17 ± 6.18
beta-BHC	93.43 ± 1.20	99.85 ± 1.58	97.69 ± 5.78	106.68 ± 2.13
delta-BHC	102.49 ± 6.49	103.13 ± 2.06	106.48 ± 4.59	102.81 ± 1.55
Heptachlor	103.85 ± 1.38	99.11 ± 4.09	104.84 ± 1.37	103.52 ± 6.45
Aldrin	100.62 ± 3.28	104.37 ± 1.36	101.37 ± 1.47	105.66 ± 3.23
Heptachlor epoxide	101.83 ± 1.98	103.99 ± 1.82	104.19 ± 1.51	105.10 ± 2.85
trans-Chlordane	102.16 ± 3.99	104.66 ± 5.42	108.39 ± 1.99	102.84 ± 5.11
cis-Chlordane	103.26 ± 6.25	104.66 ± 5.42	101.54 ± 4.37	102.84 ± 5.11
Endosulfan I	102.74 ± 2.02	103.42 ± 2.64	103.06 ± 3.48	104.57 ± 2.67
4,4′ DDE	95.45 ± 1.11	101.13 ± 1.60	102.29 ± 1.76	105.55 ± 2.11
Dieldrin	99.11 ± 1.03	107.59 ± 2.94	99.20 ± 1.39	104.35 ± 5.85
Endrin	102.90 ± 1.58	100.22 ± 2.68	102.42 ± 1.46	105.57 ± 1.21
4, 4′ DDD	102.08 ± 1.53	102.76 ± 1.81	106.99 ± 1.60	103.30 ± 4.69
Endosulfan sulfate	105.72 ± 1.51	99.59 ± 3.51	106.05 ± 4.87	99.50 ± 1.40
Endosulfan II	104.18 ± 1.96	105.63 ± 1.31	102.09 ± 1.40	101.57 ± 3.50
Endrin aldehyde	106.15 ± 1.97	101.66 ± 1.79	107.55 ± 2.27	104.78 ± 2.32
4, 4′ DDT	106.15 ± 2.60	101.35 ± 1.80	103.94 ± 4.37	100.35 ± 1.38
Methoxychlor	105.96 ± 1.41	102.87 ± 1.04	104.71 ± 3.41	103.67 ± 2.05
Endrin Ketone	105.40 ± 3.91	101.00 ± 1.80	107.85 ± 1.05	100.72 ± 3.50

3.9.5 Matrix-matched Calibration of OCPs for Broiler Chicken Meat and Liver

The residual OCPs in broiler chicken meat and liver samples were analyzed using matrix-matched calibration to account for matrix effects, which are interference from intricate sample components that alter the analytical signal and disrupt precision and accuracy. By establishing calibration standards in a blank version of the identical sample matrix, this method improves the reliability of quantitative analysis and verifies that the calibration curve accurately depicts the real situation (Figure 3.48 and 3.49).

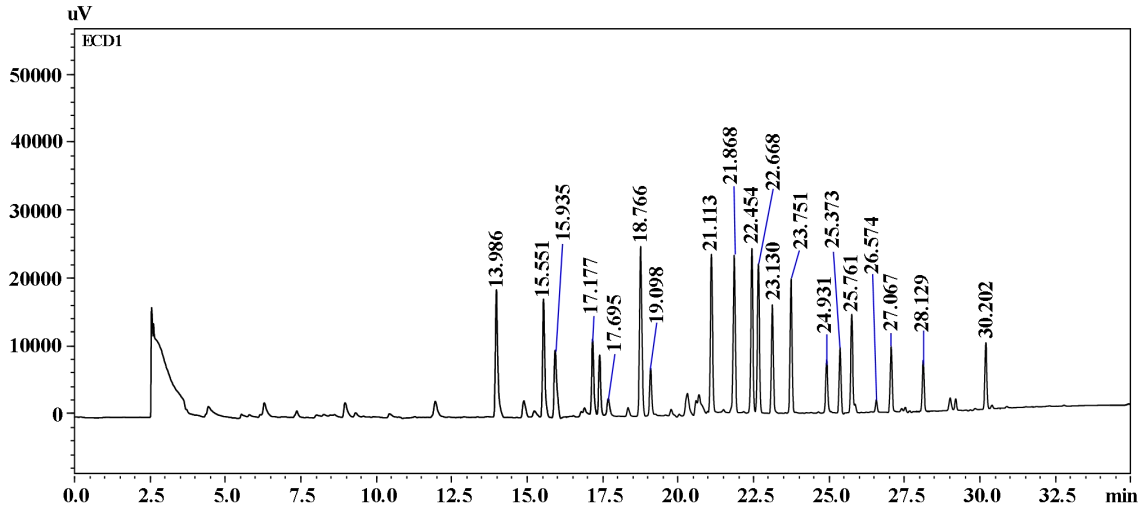


Figure 3.48 Matrix-matched chromatogram of OCPs in chicken meat

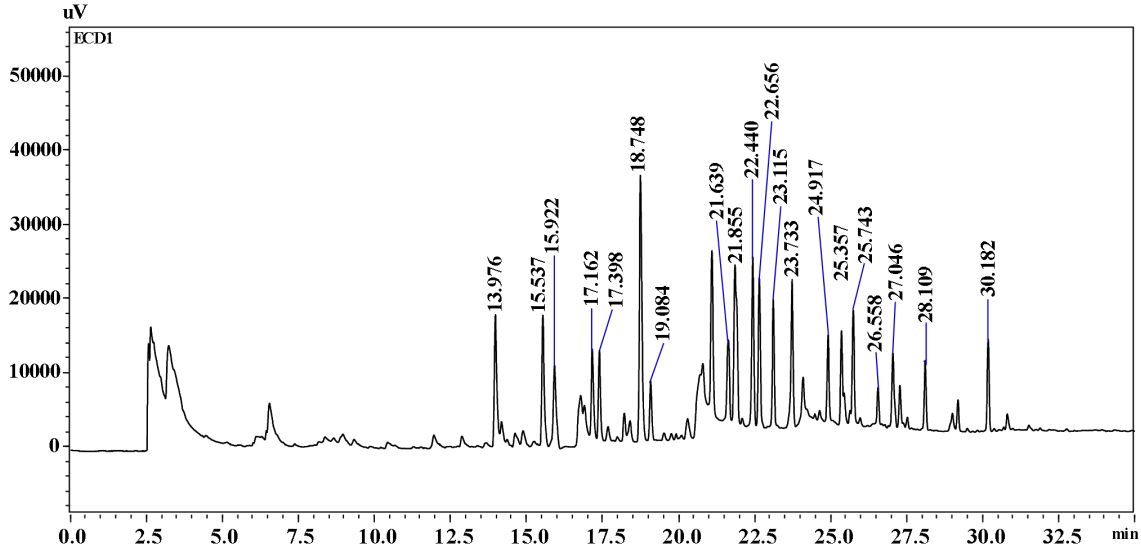


Figure 3.49 Matrix-matched chromatogram of OCPs in chicken liver

3.9.6 Evaluation of Residual OCPs in Broiler Chicken Meat and Liver

In the analysis of thirty broiler chicken meat samples, alpha-BHC was detected in two samples at levels of 1.00 and 2.87 µg/kg. Gamma-BHC was found in one sample at a concentration of 4.94 µg/kg. Delta-BHC was present in twenty-seven samples, with levels varying from 5.91 to 201.65. Heptachlor was identified in four samples, ranging between 6.81 and 8.93 µg/kg. Aldrin was detected in ten samples, with concentrations from 1.60 to 33.93 µg/kg. Heptachlor epoxide appeared in twenty-five samples, with amounts ranging from 137.76 to 270.60. Trans-chlordane was found in thirteen samples, varying from 3.64 to 27.96 µg/kg, while *cis*-chlordane was present in seven samples with levels from 0.44 to 2.24 µg/kg. Endosulfan I was detected in twenty-five samples, with concentrations ranging from 1.72 to 5.91 µg/kg. Two samples contained 4, 4′-DDE at levels of 0.41 and 1.69 µg/kg. Dieldrin was found in twelve samples, ranging from 0.72 to 13.53, and endrin was detected in ten samples, with concentrations from 0.44 to 12.73 µg/kg. Seventeen samples had 4, 4′-DDD, which ranged from 1.48 to 5.75 µg/kg. Endosulfan II was present in nine samples, showing levels from 0.97 to 56.96 µg/kg. Endrin aldehyde was found in sixteen samples, with levels varying from 0.93 to 5.03, while 4, 4′-DDT was detected in seven samples, ranging from 1.39 to 71.84 µg/kg. Endosulfan sulfate was found in six samples, with concentrations between 0.21 and 3.14 µg/kg. Methoxychlor was detected in thirteen samples, with levels ranging from 1.59 to 5.95 µg/kg. However, beta-BHC and endrin ketone were not found in the broiler chicken meat samples as they fell below the detection limit (Table 3.32).

Table 3.32 Amount of twenty organochlorine pesticides (µg/kg) in broiler chicken meat samples

Sample ID	alpha-BHC	gamma-BHC	beta-BHC	delta-BHC	Heptachlor	Aldrin	Heptachlor epoxide	*trans*-Chlordane	*cis*-Chlordane	Endosulfan-I
CM1	1.00	4.94	ND	157.51	8.61	1.61	171.35	13.45	ND	1.75
CM2	ND	ND	ND	165.06	8.93	2.22	183.64	ND	ND	1.84
CM3	ND	ND	ND	186.31	ND	2.76	248.87	ND	ND	4.50
CM4	ND	ND	ND	196.48	ND	ND	196.46	ND	ND	3.57
CM5	ND	ND	ND	201.65	6.81	1.53	203.65	ND	ND	1.93
CM6	2.87	ND	ND	175.76	ND	ND	208.14	ND	ND	2.14
CM7	ND	ND	ND	166.90	ND	ND	ND	ND	ND	ND
CM8	ND	ND	ND	173.15	ND	ND	151.80	ND	ND	2.05
CM9	ND	ND	ND	146.70	ND	ND	175.78	ND	ND	ND
CM10	ND	ND	ND	147.45	ND	1.91	249.43	27.79	4.59	ND
CM11	ND	ND	ND	132.17	ND	ND	ND	13.54	ND	1.81
CM12	ND	ND	ND	175.19	ND	ND	ND	12.53	ND	2.59
CM13	ND	ND	ND	186.33	ND	1.60	182.15	16.57	ND	2.20
CM14	ND	ND	ND	179.44	ND	ND	159.31	11.21	ND	2.57
CM15	ND	ND	ND	173.41	ND	ND	249.14	12.62	2.28	ND
CM16	ND	ND	ND	177.00	ND	2.44	249.49	ND	2.76	ND
CM17	ND	ND	ND	176.78	ND	1.96	258.12	ND	3.28	3.73
CM18	ND	ND	ND	106.48	8.15	ND	270.60	ND	ND	4.88
CM19	ND	ND	ND	ND	ND	ND	157.34	ND	ND	2.79
CM20	ND	ND	ND	6.04	ND	ND	ND	ND	ND	2.59
CM21	ND	ND	ND	ND	ND	ND	137.76	ND	0.44	1.72
CM22	ND	ND	ND	70.77	ND	ND	245.01	ND	20.24	ND
CM23	ND	ND	ND	11.98	ND	ND	210.54	27.96	ND	5.91
CM24	ND	ND	ND	7.86	ND	33.93	204.93	21.09	13.11	3.20
CM25	ND	ND	ND	5.91	ND	ND	195.13	3.64	ND	2.15
CM26	ND	ND	ND	9.79	ND	1.98	ND	12.23	ND	1.90
CM27	ND	ND	ND	7.46	ND	ND	232.29	13.40	ND	3.34
CM28	ND	ND	ND	6.72	ND	ND	245.97	ND	ND	3.01
CM29	ND	ND	ND	7.37	ND	ND	212.50	ND	ND	3.26
CM30	ND	ND	ND	157.51	ND	ND	209.92	26.93	ND	3.38

Table 3.32 continuous

Sample ID	4,4´-DDE	Dieldrin	Endrin	4,4´-DDD	Endosulfan II	Endrin aldehyde	4,4´-DDT	Endosulfan sulfate	Methoxychlor	Endrin Ketone
CM1	ND	0.79	0.44	2.35	4.64	0.93	ND	ND	2.51	ND
CM2	0.41	0.92	ND	5.75	0.97	1.06	1.39	1.21	1.67	ND
CM3	ND	ND	0.67	ND	ND	2.69	ND	ND	5.95	ND
CM4	ND	1.85		5.20	ND	2.35	4.33	ND	ND	ND
CM5	ND	2.03	0.51	ND	3.34	1.63	3.00	ND	2.05	ND
CM6	ND	ND	2.99	ND	ND	ND	ND	ND	ND	ND
CM7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CM8	ND	ND	ND	ND	27.52	ND	44.94	ND	ND	ND
CM9	ND	ND	ND	ND	ND	5.03	ND	ND	ND	ND
CM10	ND	1.32	ND	ND	ND	ND	ND	ND	ND	ND
CM11	ND	ND	ND	1.51	ND	ND	ND	ND	ND	ND
CM12	ND	ND	ND	2.16	ND	ND	ND	ND	1.59	ND
CM13	ND	ND	ND	2.94	ND	1.20	ND	0.48	2.67	ND
CM14	ND	0.72	ND	2.07	ND	1.29	ND	ND	2.52	ND
CM15	ND	ND	ND	ND	ND	ND	ND	ND	2.06	ND
CM16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CM17	ND	ND	ND	1.71	ND	ND	ND	ND	ND	ND
CM18	ND	ND	2.79	ND	38.86	ND	46.69	ND	ND	ND
CM19	1.69	ND	3.12	ND	ND	2.49	ND	ND	ND	ND
CM20	ND	2.58	ND	2.06	ND	ND	ND	0.52	3.10	ND
CM21	ND	3.06	ND	3.42	2.77	ND	ND	0.21	2.45	ND
CM22	ND	ND	1.00	1.29	ND	ND	ND	ND	ND	ND
CM23	ND	ND	ND	2.17	ND	ND	ND	ND	3.92	ND
CM24	ND	ND	12.73	6.42	10.11	ND	ND	3.14	ND	ND
CM25	ND	3.25	ND	1.48	ND	ND	ND	ND	1.78	ND
CM26	ND	2.78	ND	ND	ND	ND	8.24	ND	ND	ND
CM27	ND	ND	ND	2.02	ND	ND	ND	ND	2.36	ND
CM28	ND	13.53	5.25	ND	56.96	ND	71.84	ND	ND	ND
CM29	ND	2.83	ND	2.94	1.18	ND	ND	0.76	ND	ND
CM30	ND	ND	ND	1.62	ND	ND	ND	ND	ND	ND

In a study of thirty broiler chicken liver samples, alpha-BHC was detected in twenty-three of them, with concentrations varying between 2.12 and 159.13. Gamma-BHC appeared in three samples, with levels ranging from 2.17 to 5.85. Beta-BHC was identified in ten samples, showing a range from 13.87 to 69.96. Delta-BHC was present in twenty-five samples, with concentrations from 1.67 to 224.65. Heptachlor was found in three samples, where levels ranged from 3.01 to 7.78. Aldrin was detected in ten samples, with a range from 0.91 to 259.93. Heptachlor epoxide was present in twenty-eight samples, with concentrations between 58.71 and 196.47. *Trans*-chlordane was found in three samples, showing levels from 5.26 to 336.49. *Cis*-chlordane appeared in four samples, with ranges from 0.12 to 280.64. Endosulfan I was detected in twenty-six samples, with concentrations varying from 1.23 to 22.86. The compound 4, 4′-DDE was found in nine samples, ranging from 0.54 to 12.79. Dieldrin appeared in nine samples with concentrations that varied from 0.84 to 9.09. Endrin was found in nine samples ranging from 1.24 to 40.29. The compound 4, 4′-DDD was present in ten samples, showing levels from 0.35 to 28.90. Endrin aldehyde was detected in eighteen samples, with concentrations ranging from 1.12 to 307.65. The compound 4, 4′-DDT was found in twelve samples, ranging from 1.37 to 29.80. Endosulfan sulfate appeared in five samples, with levels between 0.97 and 204.26. Methoxychlor was identified in five samples, with a concentration range of 0.72 to 5.68, and endrin ketone was found in four samples, with levels from 1.95 to 4.47 µg/kg. However, endosulfan II was not detected in any of the broiler chicken liver samples as its levels were below the detection limit (Table 3.33).

Table 3.33 Amount of twenty organochlorine pesticides (µg/kg) in broiler chicken liver samples

Sample ID	alpha-BHC	gamma-BHC	beta-BHC	delta-BHC	Heptachlor	Aldrin	Heptachlor epoxide	*trans*-Chlordane	*cis*-Chlordane	Endosulfan-I
CL1	13.31	ND	11.67	118.67	ND	1.71	119.54	ND	ND	17.73
CL2	26.03	ND	20.93	8.51	3.01	2.23	124.06	ND	ND	26.13
CL3	ND	ND	31.21	47.85	ND	1.81	157.44	ND	ND	ND
CL4	ND	ND	33.50	28.67	ND	1.84	140.28	ND	ND	9.64
CL5	10.37	ND	20.50	29.58	ND	1.60	175.69	336.49	ND	13.21
CL6	3.54	ND	18.14	77.66	ND	1.47	145.95	ND	ND	13.40
CL7	85.72	5.85	20.42	11.64	7.78	0.91	184.14	ND	ND	11.97
CL8	159.13	ND	ND	ND	ND	259.93	149.99	247.47	280.64	8.09
CL9	36.63	7.07	69.96	ND	ND	4.28	154.78	ND	3.21	5.77
CL10	27.62	ND	55.23	ND	4.49	4.14	196.47	ND	ND	18.77
CL11	4.45	ND	ND	ND	ND	ND	76.12	ND	ND	4.13
CL12	4.52	ND	ND	9.56	ND	ND	136.48	ND	ND	4.78
CL13	4.41	ND	ND	5.78	ND	ND	98.68	ND	ND	4.83
CL14	5.39	ND	ND	4.51	ND	ND	157.31	ND	1.69	3.91
CL15	6.64	ND	ND	3.99	ND	ND	174.42	ND	ND	3.73
CL16	5.36	2.17	1.87	3.84	ND	ND	109.74	ND	ND	4.72
CL17	3.91	ND	ND	39.35	ND	ND	72.63	ND	ND	3.70
CL18	ND	ND	ND	6.45	ND	ND	150.61	ND	ND	4.22
CL19	10.59	ND	ND	2.99	ND	ND	130.89	ND	ND	3.79
CL20	ND	ND	ND	5.26	ND	ND	108.36	ND	ND	4.36
CL21	4.45	ND	ND	ND	ND	ND	72.70	ND	ND	4.50
CL22	4.08	ND	ND	3.04	ND	ND	69.78	0.34	0.12	4.84
CL23	4.29	ND	ND	7.55	ND	ND	70.42	ND	ND	ND
CL24	ND	ND	ND	6.03	ND	ND	ND	ND	ND	6.30
CL25	2.67	ND	ND	5.02	ND	ND	58.71	ND	ND	5.44
CL26	2.92	ND	ND	5.85	ND	ND	103.84	ND	ND	6.33
CL27	ND	ND	ND	5.03	ND	ND	97.51	ND	ND	3.82
CL28	5.93	ND	ND	1.67	ND	ND	91.36	ND	ND	5.04
CL29	2.12	ND	ND	2.47	ND	ND	ND	ND	ND	4.82
CL30	ND	ND	ND	224.65	ND	ND	191.97	ND	ND	4.95

Table 3.33 continuous

Sample ID	4,4 ´ - DDE	Dieldrin	Endrin	4,4 ´ - DDD	Endosulfan II	Endrin aldehyde	4,4 ´ - DDT	Endosulfan sulfate	Methoxychlor	Endrin Ketone
CL1	ND	6.77	ND	ND	ND	3.26	ND	40.77	3.13	3.24
CL2	ND	8.10	ND	ND	ND	27.65	8.21	66.64	4.91	ND
CL3	11.61	4.82	ND	ND	ND	7.42	9.17	4.34	ND	ND
CL4	ND	7.84	ND	ND	ND	9.84	29.80	169.48	ND	4.47
CL5	ND	5.25	ND	ND	ND	4.97	6.78	75.68	ND	ND
CL6	ND	4.79	ND	ND	ND	4.28	10.53	61.61	2.13	ND
CL7	ND	7.55	ND	9.81	ND	2.72	ND	34.02	ND	1.95
CL8	12.79	4.51	ND	28.90	ND	15.49	21.86	104.07	5.68	ND
CL9	2.88	7.60	ND	3.60	ND	15.67	11.29	204.26	ND	ND
CL10	ND	8.86	ND	ND	ND	17.16	3.28	153.73	ND	3.63
CL11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CL12	ND	4.93	4.86	ND	ND	307.65	ND	1.94	ND	ND
CL13	0.94	1.27	1.32	1.26	ND	1.47	ND	0.27	ND	ND
CL14	ND	1.20	1.57	0.63	ND	6.23	1.75	2.64	ND	ND
CL15	ND	9.09	2.79	0.44	ND	1.12	ND	ND	ND	ND
CL16	0.54	ND	1.58	ND	ND	7.92	1.37	1.78	ND	ND
CL17	1.38	ND	21.45	2.05	ND	ND	ND	0.97	ND	ND
CL18	ND	ND	ND	ND	ND	ND	9.75	ND	ND	ND
CL19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CL20	ND	1.48	1.52	ND	ND	ND	ND	ND	ND	ND
CL21	ND	1.33	1.46	ND	ND	ND	ND	ND	ND	ND
CL22	ND	0.84	ND	ND	ND	ND	ND	ND	ND	ND
CL23	ND	ND	1.31	ND	ND	ND	ND	ND	ND	ND
CL24	2.86	ND	1.30	ND	ND	4.02	ND	ND	ND	ND
CL25	11.83	0.95	1.24	15.11	ND	2.33	ND	9.13	ND	ND
CL26	2.92	1.41	1.41	0.35	ND	4.54	ND	ND	ND	ND
CL27	ND	0.97	1.33	ND	ND	ND	ND	ND	ND	ND
CL28	ND	11.40	ND	ND	ND	ND	5.31	ND	ND	ND
CL29	ND	1.59	1.52	ND	ND	ND	ND	ND	ND	ND
CL30	ND	0.91	ND	0.99	ND	ND	ND	ND	0.72	ND

A few chromatograms of residual OCPs in broiler chicken meat and liver samples in Figure 3.50 and 3.51.

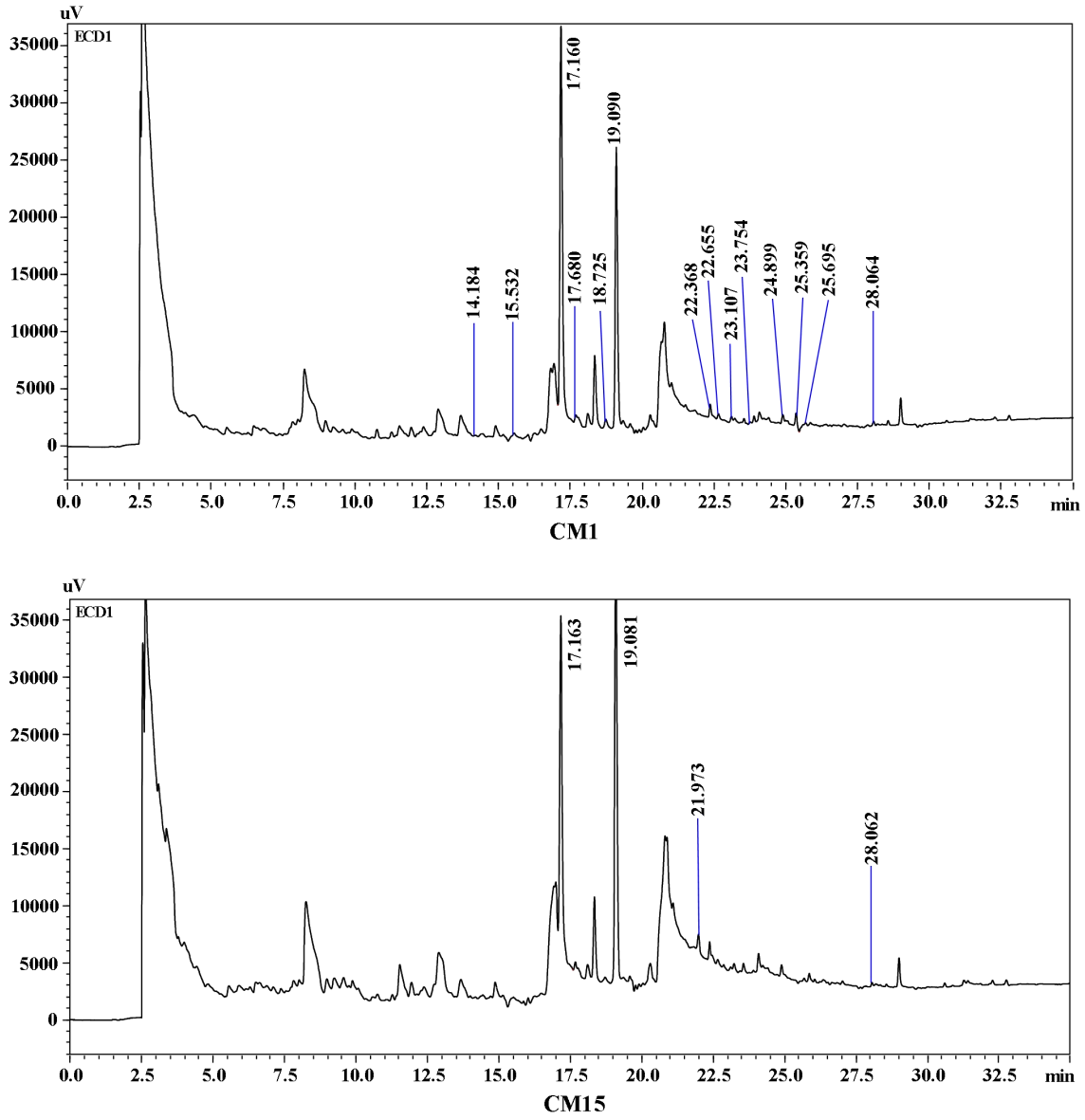
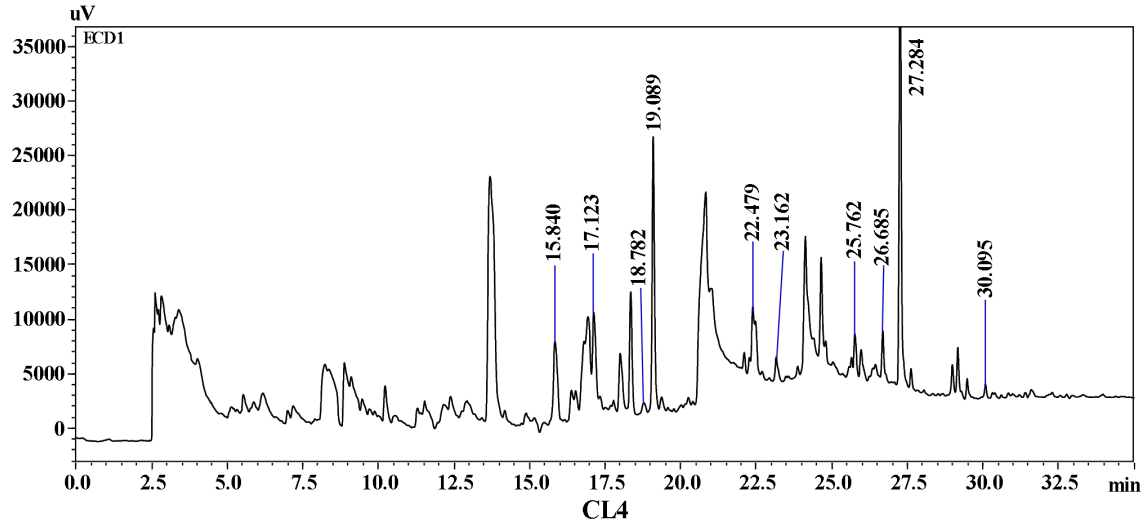


Figure 3.50 Chromagrams of residual OCPs in chicken meat



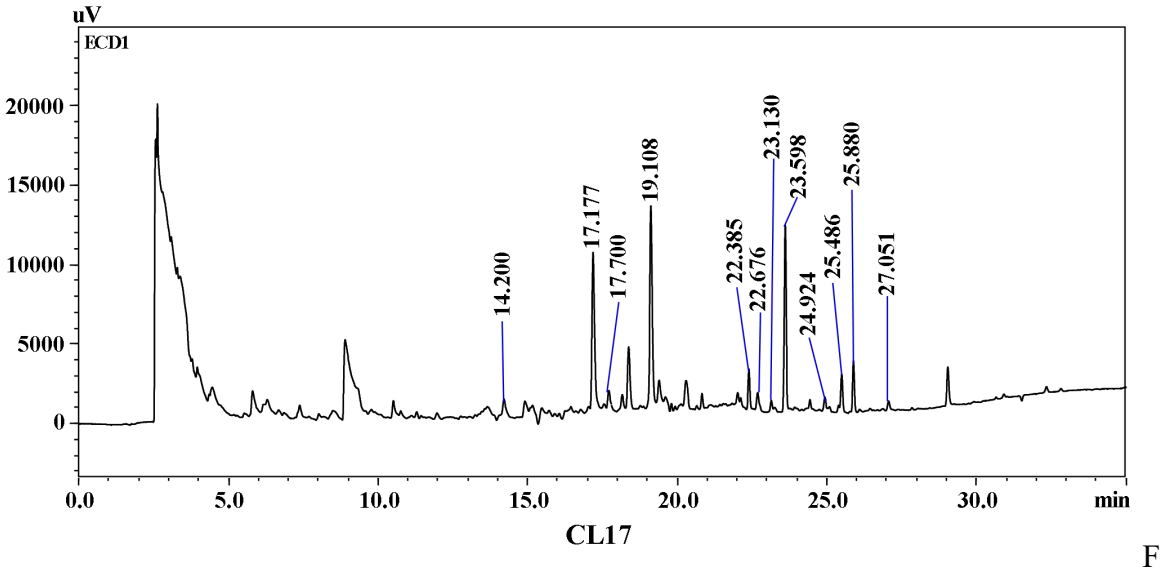


Figure 3.51 Chromagrams of residual OCPs in chicken liver

3.9.7 Recovery Percentage of OCPs in Broiler Chicken Meat and Liver Samples

The percentage of recovery (%) and the relative standard deviation (RSD%) were assessed at concentrations of 2.5 and 5 µg/kg for the beef meat and liver samples that were analyzed. The recovery percentages for twenty different pesticides varied from 98.03 to 108.38 and 93.45 to 104.91% at a concentration of 2.5 µg/kg, and from 97.81 to 106.33 and 99.82 to 106.52 µg/kg at 5 µg/kg for broiler chicken meat and beef liver, respectively (Table 3.34).

Table 3.34 Average recovery percentages ± relative standard deviation (RSD) for 20 OCPs extracted from spiking laboratory blank meat and liver samples of broiler chicken using QuEChERS technique with two levels of the multi-standards mixture (2.5 and 5 µg/kg)

OCPs	**2.5 µg/kg ± RSD %**		**5 µg/kg ± RSD %**	
	Chicken Meat	Chicken Liver	Chicken Meat	Chicken Liver
alpha-BHC	100.25 ± 6.43	99.45 ± 2.63	101.62 ± 2.89	104.82 ± 2.02
gamma-BHC	102.50 ± 7.36	98.13 ± 3.01	101.09 ± 1.09	102.98 ± 6.40
beta-BHC	103.85 ± 7.40	98.28 ± 1.51	97.81 ± 4.55	101.75 ± 7.02
delta-BHC	104.42 ± 3.11	104.40 ± 4.43	103.36 ± 4.30	106.03 ± 1.28
Heptachlor	100.38 ± 6.94	99.74 ± 3.64	105.36 ± 2.91	105.32 ± 1.08
Aldrin	103.54 ± 2.29	103.01 ± 2.95	102.19 ± 2.03	99.82 ± 1.63
Heptachlor epoxide	100.94 ± 1.73	95.24 ± 5.40	102.94 ± 3.66	104.63 ± 4.68
trans-Chlordane	101.78 ± 1.25	93.45 ± 6.65	103.81 ± 1.66	106.33 ± 2.88
cis-Chlordane	105.73 ± 4.04	104.91 ± 3.50	104.47 ± 2.44	100.25 ± 2.88
Endosulfan I	102.90 ± 1.38	104.78 ± 2.16	102.70 ± 1.95	101.44 ± 3.98
4,4′ DDE	105.94 ± 1.65	100.91 ± 1.84	103.19 ± 2.75	104.60 ± 4.87
Dieldrin	108.38 ± 4.78	103.23 ± 1.53	103.50 ± 3.81	100.96 ± 2.81
Endrin	106.99 ± 6.64	100.40 ± 2.25	103.10 ± 3.20	104.54 ± 1.43
4, 4′ DDD	102.09 ± 4.34	95.97 ± 1.29	102.97 ± 3.85	104.25 ± 3.77
Endosulfan sulfate	102.54 ± 1.24	104.88 ± 1.18	106.33 ± 5.54	106.52 ± 4.21
Endosulfan II	102.88 ± 6.06	98.31 ± 1.33	102.02 ± 2.42	101.31 ± 1.52
Endrin aldehyde	100.74 ± 7.14	105.04 ± 1.30	103.49 ± 2.23	102.37 ± 1.56
4, 4′ DDT	98.03 ± 4.11	96.54 ± 1.62	102.98 ± 5.04	105.06 ± 5.59
Methoxychlor	98.01 ± 2.90	103.96 ± 1.01	101.00 ± 1.03	102.22 ± 8.68
Endrin Ketone	100.59 ± 1.02	103.08 ± 4.26	100.93 ± 1.90	102.39 ± 1.39

Number of positive samples in four biological matrices is presented in Table 3.35 and Figure 3.52.

Table 3.35 Number of positive samples in four biological matrices

OCPs	**Number of positive samples**			
	Beef Meat	**Beef Liver**	**Chicken Meat**	**Chicken Liver**
alpha-BHC	11	28	2	23
gamma-BHC	9	16	1	3
beta-BHC	9	16	ND	10
delta-BHC	28	24	27	25
Heptachlor	13	22	4	3
Aldrin	20	21	10	10
Heptachlor epoxide	28	28	25	28
trans-Chlordane	14	16	13	3
cis-Chlordane	18	10	7	4
Endosulfan I	26	24	25	26
4,4´ DDE	20	11	2	9
Dieldrin	22	21	12	9
Endrin	17	20	10	9
4, 4´ DDD	23	7	17	10
Endosulfan sulfate	27	20	9	ND
Endosulfan II	19	26	16	18
Endrin aldehyde	15	9	7	12
4, 4´ DDT	12	2	6	5
Methoxychlor	18	8	13	5
Endrin Ketone	4	ND	ND	4

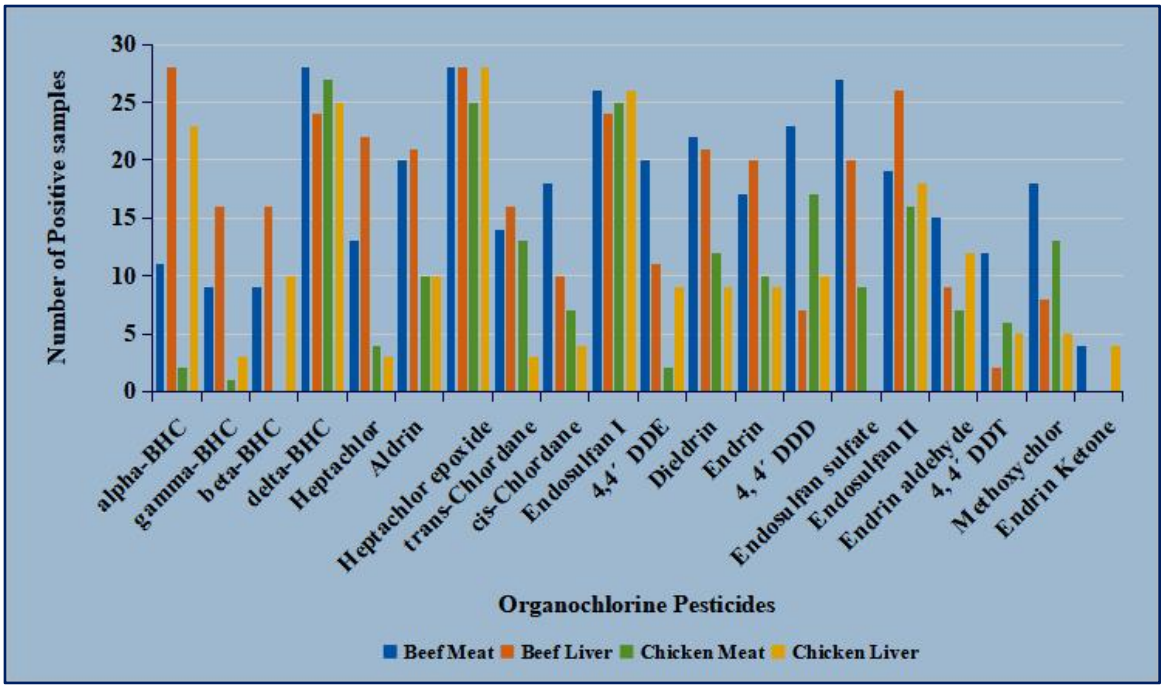


Figure 3.52 Number of positive samples containing OCPs

Mean concentration (µg/kg) of residual OCPs with standard deviation (SD) has been presented in Table 3.36 and Figure 3.53.

Table 3.36 Mean levels (µg/kg) of OCPs in beef and chicken meat and liver samples

OCPs	Mean Concentration ± SD			
	Beef Meat	**Beef Liver**	**Chicken Meat**	**Chicken Liver**
alpha-BHC	9.62 ± 0.18	100.11 ± 8.15	1.93 ± 0.13	18.87 ± 3.56
gamma-BHC	16.05 ± 3.28	6.56 ± 3.76	4.94 ± 0.00	5.03 ± 0.55
beta-BHC	2.86 ± 0.25	12.41 ± 1.12	ND	28.34 ± 2.04
delta-BHC	211.54 ± 7.52	7.85 ± 0.53	109.24 ± 7.94	26.62 ± 4.96
Heptachlor	12.53 ± 8.94	9.80 ± 0.41	8.12 ± 0.93	5.09 ± 0.44
Aldrin	5.50 ± 1.30	4.60 ± 0.49	5.19 ± 0.81	27.99 ± 8.15
Heptachlor epoxide	148.63 ± 6.99	133.89 ± 4.16	198.48 ± 8.10	121.31 ± 4.29
trans-Chlordane	2.69 ± 0.37	7.65 ± 0.84	16.38 ± 4.23	194.77 ± 6.29
cis-Chlordane	2.72 ± 0.53	2.40 ± 0.16	6.67 ± 0.72	71.41 ± 1.54
Endosulfan I	3.09 ± 0.41	3.03 ± 0.98	2.28 ± 0.11	7.44 ± 0.87
4,4´ DDE	2.56 ± 0.51	0.93 ± 0.25	1.05 ± 0.90	5.31 ± 0.52
Dieldrin	3.73 ± 0.74	2.97 ± 0.69	2.97 ± 0.34	4.50 ± 0.34
Endrin	2.44 ± 0.37	1.69 ± 0.53	2.77 ± 0.66	5.66 ± 0.53
4, 4´ DDD	5.24 ± 0.85	2.36 ± 0.53	2.77 ± 0.56	6.31 ± 0.93
Endosulfan sulfate	20.08 ± 0.45	5.58 ± 0.43	16.26 ± 2.02	ND
Endosulfan II	5.32 ± 0.73	4.79 ± 0.73	2.01 ± 0.11	24.65 ± 7.97
Endrin aldehyde	6.20 ± 0.18	9.24 ± 0.48	25.78 ± 2.83	9.93 ± 0.83
4, 4´ DDT	4.14 ± 0.42	0.02 ± 0.01	1.05 ± 0.10	58.21 ± 6.71
Methoxychlor	2.87 ± 0.24	1.77 ± 0.37	2.66 ± 0.17	3.32 ± 0.20
Endrin Ketone	3.51 ± 0.47	ND	ND	3.32 ± 0.14
Range	2.44 - 148.63	0.93 - 133.89	1.05 - 198.48	3.32 - 194.77
Mean ± SD	23.58 ± 2.72	15.88 ± 1.97	20.53 ± 1.94	31.40 ± 2.82
CV	11.54	12.41	9.45	8.98
SD = Standard deviation; CV= Coefficient of variation				

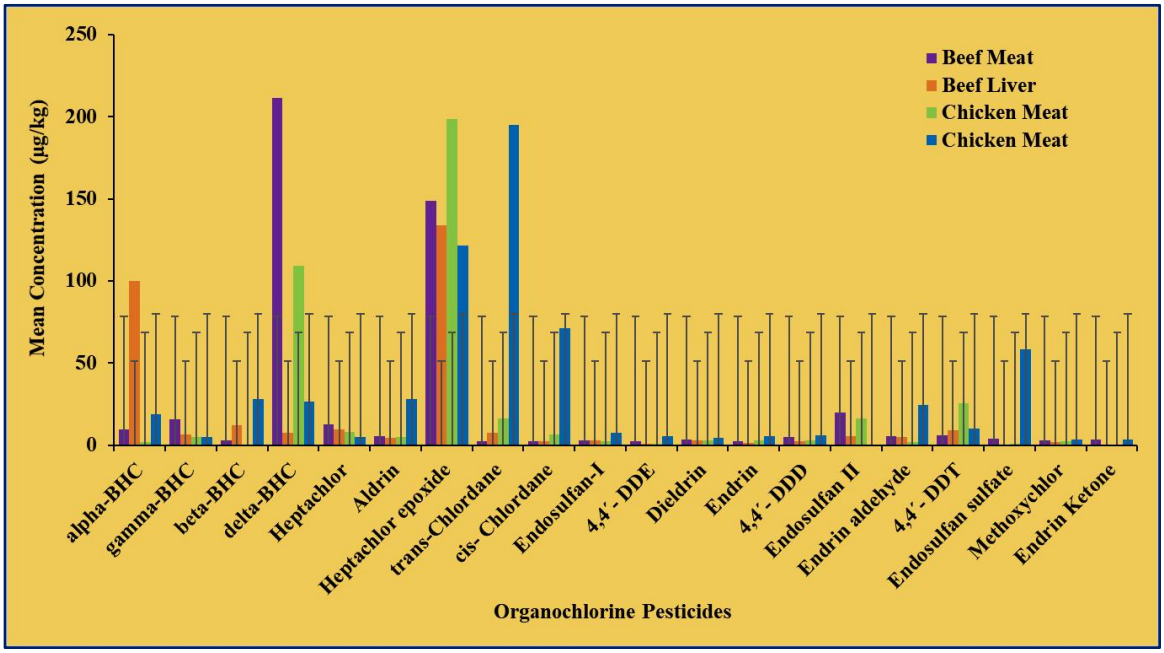


Figure 3.53 Mean Concentration ± SD of OCPs in four matrices

3.9.8 Health Risk Assessment of Organochlorine Pesticides

The beef meat and liver samples contained residual OCPs. Table 3.34 displays the calculated hazard indices (HI) [316] for consuming beef meat contaminated with gamma-BHC, beta-BHC, trans-chlordane, cis-chlordane, endosulfan I, 4,4′ DDE, 4,4′ DDD, endosulfan II, 4,4′ DDT, endosulfan sulfate, and methoxychlor 0.4654, 0.0829, 0.7807, 0.7902, 0.0748, 0.1858, 0.3796, 0.4852, 0.4492, 0.1000, and 0.0042 for adults; 0.4933, 0.0879, 0.8276, 0.8376, 0.0792, 0.1969, 0.4024, 0.5143, 0.4761, 0.1060, and 0.0044 for children, respectively, calculated to be less than 1, indicating no human hazard. In contrast, the HI of alpha-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, endrin aldehyde, and endrin ketone were 0.2790, 6.1346, 18.1667, 7.9704, 215.5200, 5.4062, 1.7697, 3.8587, and 2.5456 for adults; and 0.2957, 6.5028, 19.2572, 8.4489, 228.4567, 5.7307, 1.8760, 4.0903, and 2.6948 for children greater than one (Table 3.37), directly endangering human health (Figure 3.54 and 3.55).

Table 3.37 Estimated daily intake (EDI) and hazardous beef meat indexes were calculated for adults and children

Pesticides	**Average Conc. (mg/kg)**	**EDI (Adult)**	**EDI (Child)**	**ADI mg/kg bw**	**HI (Adult)**	**HI (Child)**
alpha-BHC	0.0048	0.0014	0.0015	0.001	0.2790	0.2957
gamma-BHC	0.0080	0.0023	0.0025	0.005	0.4654	0.4933
beta-BHC	0.0014	0.0004	0.0004	0.005	0.0829	0.0879
delta-BHC	0.1058	0.0307	0.0325	0.005	6.1346	6.5028
Heptachlor	0.0063	0.0018	0.0019	0.0001	18.1667	19.2572
Aldrin	0.0027	0.0008	0.0008	0.0001	7.9704	8.4489
Heptachlor epoxide	0.0743	0.0216	0.0229	0.0001	215.5200	228.4567
trans-Chlordane	0.0013	0.0004	0.0004	0.005	0.7807	0.8276
cis-Chlordane	0.0014	0.0004	0.0004	0.005	0.7902	0.8376
Endosulfan I	0.0015	0.0004	0.0005	0.006	0.0748	0.0792
4,4´ DDE	0.0013	0.0004	0.0004	0.01	0.0372	0.0394
Dieldrin	0.0019	0.0005	0.0006	0.0001	5.4062	5.7307
Endrin	0.0012	0.0004	0.0004	0.0002	1.7697	1.8760
4, 4´ DDD	0.0026	0.0008	0.0008	0.01	0.0759	0.0805
Endosulfan II	0.0100	0.0029	0.0031	0.006	0.4852	0.5143
Endrin aldehyde	0.0027	0.0008	0.0008	0.0002	3.8587	4.0903
4, 4´ DDT	0.0031	0.0009	0.0010	0.01	0.0898	0.0952
Endosulfan sulfate	0.0021	0.0006	0.0006	0.006	0.1000	0.1060
Methoxychlor	0.0014	0.0004	0.0004	0.1	0.0042	0.0044
Endrin Ketone	0.0018	0.0005	0.0005	0.0002	2.5456	2.6984

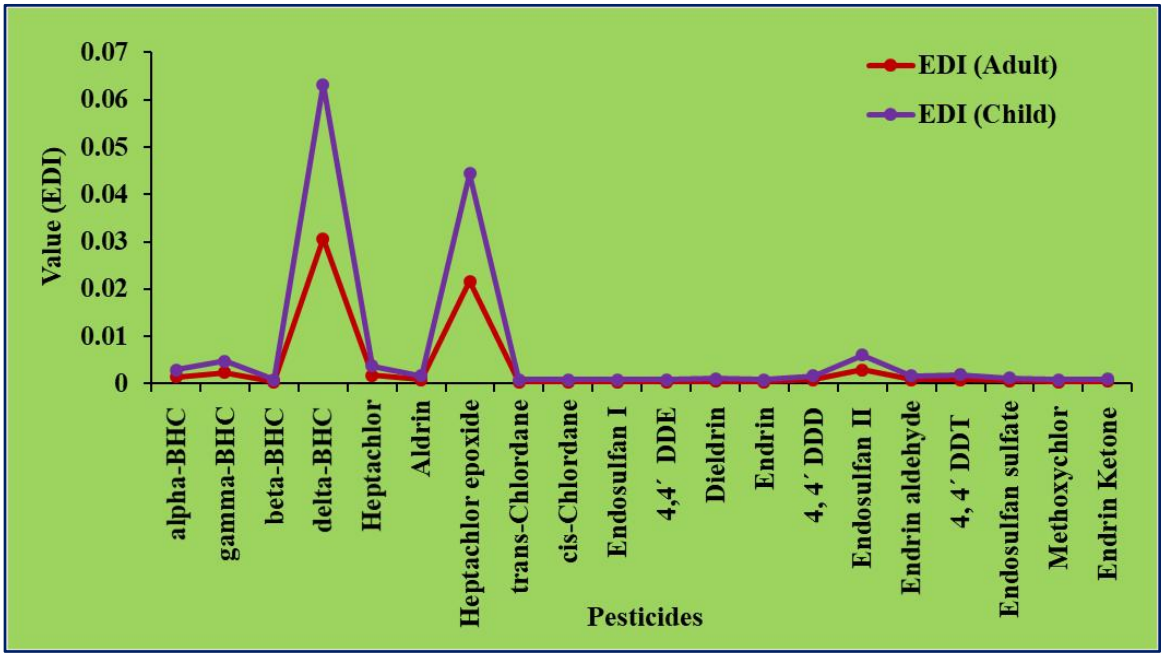


Figure 3.54 Estimated daily intake (EDI) of OCPs for the consumption beef meat

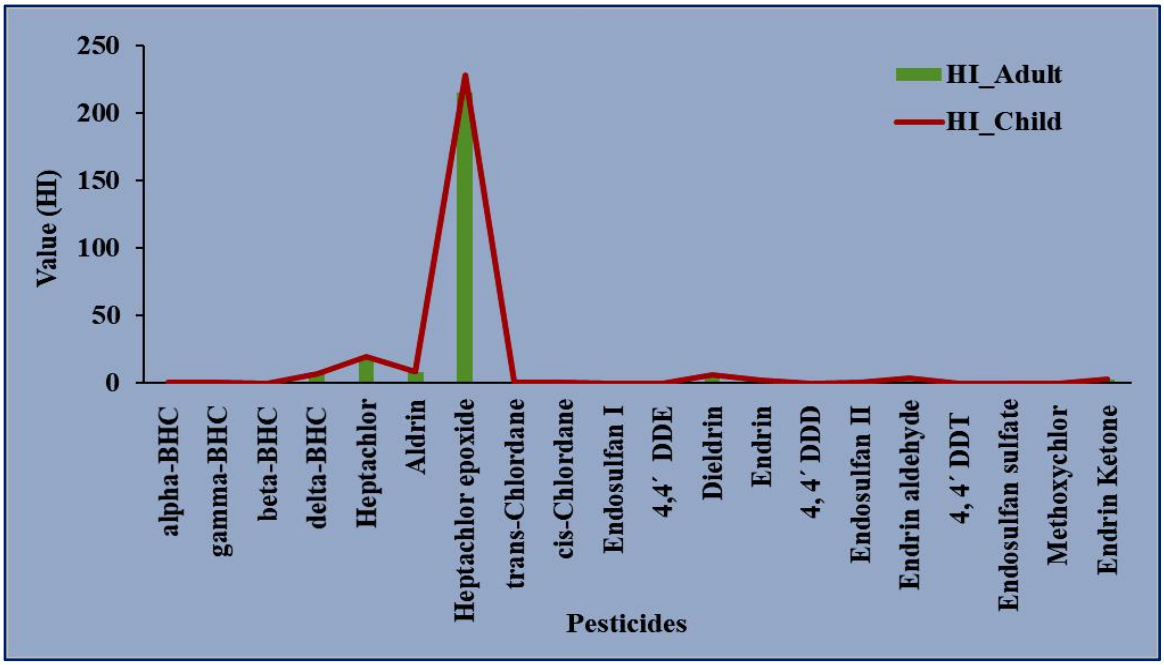


Figure 3.55 Hazardous Index (HI) of OCPs for the consumption beef meat

The samples of broiler chicken meat and liver were found to contain 20 organochlorine pesticides (OCPs) that were analyzed. The calculated hazard indices (HI) for the consumption of broiler chicken meat contaminated with alpha-BHC, gamma-BHC, endosulfan I, 4,4′ DDE, 4,4′ DDD, endosulfan II, endosulfan sulfate, and methoxychlor are presented in Table 3.34. The HI values for adults were 0.2803, 0.1434, 0.0690, 0.0762, 0.2008, 0.3930, 0.0255, and 0.0039, while for children, they were 0.2971, 0.1520, 0.0731, 0.0808, 0.2129, 0.4166, 0.0270, and 0.0041. All these values were less than 1, indicating no risk to human health. Conversely, the HI values for delta-BHC, heptachlor, aldrin, heptachlor epoxide, trans-Chlordane, cis-chlordane, dieldrin, endrin, and endrin aldehyde were 3.1681, 11.7810, 7.5315, 287.8007, 4.7506, 1.9348, 8.6189, 2.0058, 1.4571, and 1.8687 for adults, and 3.3582, 12.4881, 7.9835, 305.0761, 5.0357, 2.0510, 9.1362, 2.1262, 1.5446, and 1.9809 for children, all of which exceeded one, thereby posing a direct threat to human health (Table 3.38, Figure 3.56 and 3.57).

Table 3.38 Estimated daily intake (EDI) and hazardous broiler chicken meat indexes were calculated for adults and children

Pesticides	**Average Conc. (mg/kg)**	**EDI (Adult)**	**EDI (Child)**	**ADI**	**HI (Adult)**	**HI (Child)**
alpha-BHC	0.0010	0.0003	0.0003	0.005	0.0561	0.0594
gamma-BHC	0.0025	0.0007	0.0008	0.005	0.1434	0.1520
beta-BHC	ND	ND	ND	0.005	ND	ND
delta-BHC	0.0546	0.0158	0.0168	0.005	3.1681	3.3582
Heptachlor	0.0041	0.0012	0.0012	0.0001	11.7810	12.4881
Aldrin	0.0026	0.0008	0.0008	0.0001	7.5315	7.9835
Heptachlor epoxide	0.0992	0.0288	0.0305	0.0001	287.8007	305.0761
trans-Chlordane	0.0082	0.0024	0.0025	0.0005	4.7506	5.0357
cis-Chlordane	0.0033	0.0010	0.0010	0.0005	1.9348	2.0510
Endosulfan I	0.0014	0.0004	0.0004	0.006	0.0690	0.0731
4,4´ DDE	0.0005	0.0002	0.0002	0.01	0.0152	0.0162
Dieldrin	0.0030	0.0009	0.0009	0.0001	8.6189	9.1362
Endrin	0.0014	0.0004	0.0004	0.0002	2.0058	2.1262
4, 4´ DDD	0.0014	0.0004	0.0004	0.01	0.0402	0.0426
Endosulfan II	0.0081	0.0024	0.0025	0.006	0.3930	0.4166
Endrin aldehyde	0.0010	0.0003	0.0003	0.0002	1.4571	1.5446
4, 4´ DDT	0.0129	0.0037	0.0040	0.01	0.3737	0.3962
Endosulfan sulfate	0.0005	0.0002	0.0002	0.006	0.0255	0.0270
Methoxychlor	0.0013	0.0004	0.0004	0.1	0.0039	0.0041
Endrin Ketone	ND	ND	ND	0.0002	ND	ND

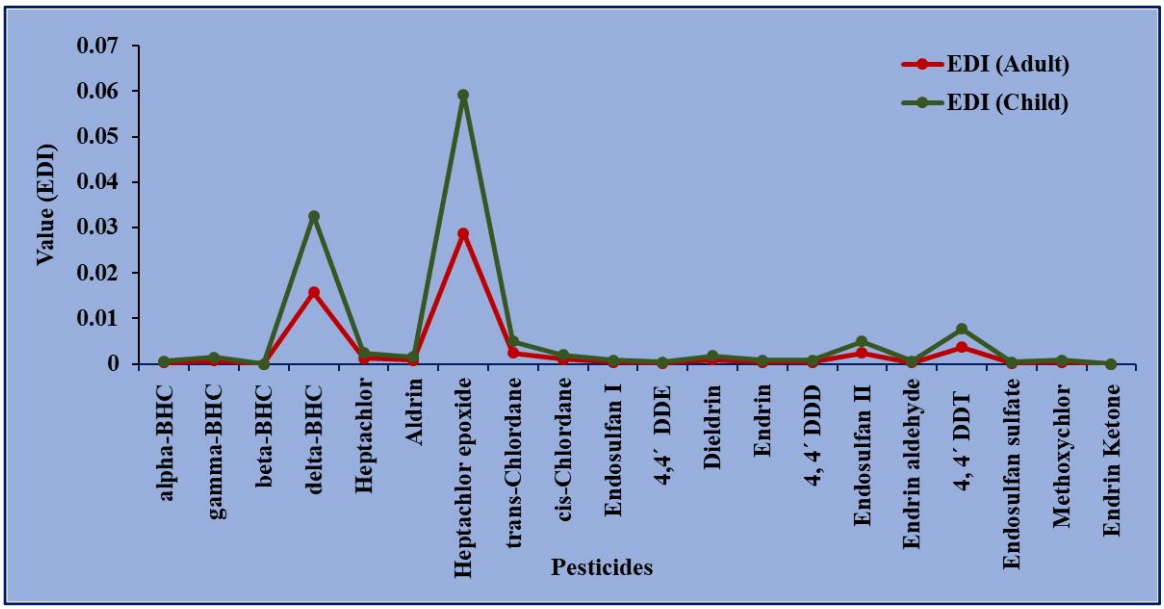


Figure 3.56 Estimated daily intake (EDI) of OCPs for the consumption chicken meat

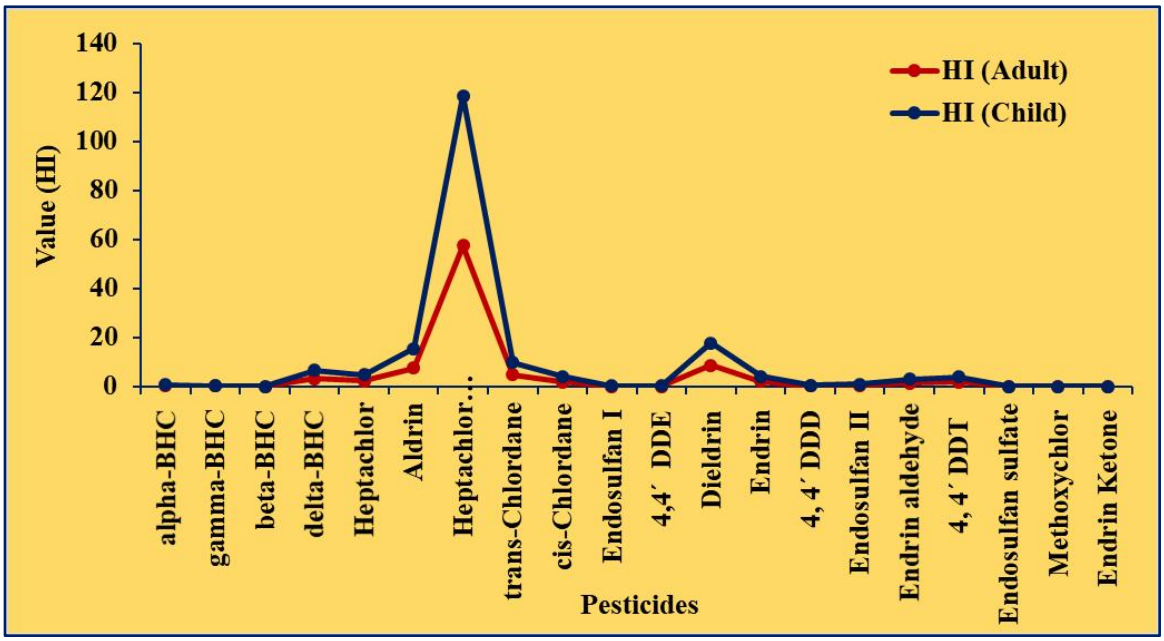


Figure 3.57 Hazardous Index (HI) of OCPs for the consumption chicken meat

3.10 Analysis of Heavy Metals in Beef Meat and Liver

3.10.1 The Linear Regression Equation, Correlation Coefficient, LOD and LOQ

Standard calibration curves were made at four different concentrations using MS Excel software for each metal. LOD and LOQ were calculated for each metals (Table 3.39).

Table 3.39 Regression Equation, correlation coefficient (r^2), LOD and LOQ of heavy metals

Heavy Metals	Regression equation (Y= ax ± b)	r^2	LOD (µg/mL)	LOQ (µg/mL)
Chromium (Cr)	y = 6878.8x + 2372.8	0.9988	0.06	0.20
Nickel (Ni)	y = 2556.5x + 971.36	0.9989	0.06	0.20
Lead (Pb)	y = 9122.2x + 2323.5	0.9990	0.03	0.10
Cadmium (Cd)	y = 1538.3x - 61.83	0.9991	0.005	0.02
Arsenic (As)	y = 612.55x + 169.24	0.9991	0.01	0.03
Copper (Cu)	y = 6396.3x + 1057.8	0.9994	0.05	0.17
Cobalt (Co)	y = 9262.8x + 1486.5	0.9991	0.01	0.03
Manganese (Mn)	y = 4099.2x + 1638.3	0.9994	0.07	0.23
Zinc (Zn)	y = 2214.6x - 227612	0.9998	0.10	0.33
Selenium (Se)	y = 34.969x + 1.4261	0.9993	0.01	0.03

3.10.2 Assessment of Residual Heavy Metals in Beef Meat and Liver

Beef meat (n=30) and beef liver (n=30) samples were purchased from six distinct local markets in Dhaka North and South City, Bangladesh, for the purpose of assessing heavy metal content. The homogenized meat samples were stored at -20°C until they were analyzed. Ten heavy metals were examined using an inductively coupled plasma mass spectrometer (ICP-MS) in both the beef meat and liver. The concentrations of heavy metals found in the beef meat and liver, along with their standard deviations (SD), are presented in Table 3.40 and Figure 3.58.

Table 3.40 The concentration of ten heavy metals (mg/kg fresh weight) in beef meat and liver from different local markets of Dhaka North and South City (mean ± SD)

Sample Type	Basabo	Karwan-bazar	Tongi bazar	Kaptan-bazar	Moulovi-bazar	Moham-madpur	Min - Max	Final Mean ± SD
Cr								
Beef Meat	$1.02 \times 10^{-4} \pm 0.00002$	$1.12 \times 10^{-4} \pm 0.00012$	$1.14 \times 10^{-4} \pm 0.000001$	$7.40 \times 10^{-5} \pm 0.00004$	$1.04 \times 10^{-4} \pm 0.00001$	$1.01 \times 10^{-4} \pm 0.00001$	2.63×10^{-5} - 3.20×10^{-4}	$1.01 \times 10^{-4} \pm 0.00005$
Beef Liver	0.08 ± 0.05	0.19 ± 0.09	BDL	0.13 ± 0.08	0.20 ± 0.09	BDL	0.06 - 0.20	0.12 ± 0.06
Ni								
Beef Meat	$8.07 \times 10^{-5} \pm 0.00001$	$5.98 \times 10^{-5} \pm 0.00002$	$8.19 \times 10^{-5} \pm 0.00001$	$7.00 \times 10^{-5} \pm 0.00002$	$7.43 \times 10^{-5} \pm 0.000009$	$6.68 \times 10^{-5} \pm 0.00002$	2.43×10^{-5} - 9.86×10^{-5}	$7.23 \times 10^{-5} \pm 0.00002$
Beef Liver	0.19 ± 0.08	0.16 ± 0.05	0.39 ± 0.31	0.33 ± 0.35	0.11 ± 0.05	0.13 ± 0.09	0.04 - 0.92	0.22 ± 0.21
Pb								
Beef Meat	$1.28 \times 10^{-4} \pm 0.0001$	$2.98 \times 10^{-4} \pm 0.0003$	$3.50 \times 10^{-4} \pm 0.0003$	$2.08 \times 10^{-4} \pm 0.0002$	$4.02 \times 10^{-4} \pm 0.0003$	$3.63 \times 10^{-4} \pm 0.0003$	1.12×10^{-6} - 7.46×10^{-4}	$2.91 \times 10^{-4} \pm 0.0002$
Beef Liver	2.73 ± 2.14	1.11 ± 0.65	1.24 ±1.42	1.49 ± 0.91	11.77 ± 8.40	11.31 ± 8.70	0.05 - 24.36	5.06 ± 6.70
Cd								
Beef Meat	$1.30 \times 10^{-4} \pm 0.0001$	$2.22 \times 10^{-4} \pm 0.0001$	$2.22 \times 10^{-4} \pm 0.0002$	$1.89 \times 10^{-4} \pm 0.0002$	$2.38 \times 10^{-4} \pm 0.0002$	$4.80 \times 10^{-5} \pm 0.00004$	4.82×10^{-6} - 5.61×10^{-4}	$1.75 \times 10^{-4} \pm 0.0002$
Beef Liver	0.17 ± 0.19	0.10 ± 0.09	0.14 ± 0.10	BDL	0.45 ± 0.35	0.74 ± 0.46	0.04 - 1.17	0.42 ± 0.40
As								
Beef Meat	$1.71 \times 10^{-4} \pm 0.0001$	$5.96 \times 10^{-5} \pm 0.00007$	$1.77 \times 10^{-4} \pm 0.0002$	$8.40 \times 10^{-5} \pm 0.00004$	$2.12 \times 10^{-4} \pm 0.0002$	$1.41 \times 10^{-4} \pm 0.0001$	1.34×10^{-6} - 4.41×10^{-4}	$1.41 \times 10^{-4} \pm 0.0001$
Beef Liver	0.14 ± 0.09	$9.23 \times 10^{-2} \pm 0.06$	$3.17 \times 10^{-2} \pm 0.03$	0.23 ± 0.27	0.23 ± 0.14	$9.68 \times 10^{-2} \pm 0.07$	0.01-0.69	0.14 ± 0.15
Cu								
Beef Meat	$4.32 \times 10^{-2} \pm 0.03$	$1.33 \times 10^{-2} \pm 0.009$	$3.99 \times 10^{-2} \pm 0.17$	$2.81 \times 10^{-2} \pm 0.15$	$4.07 \times 10^{-2} \pm 0.01$	$3.47 \times 10^{-2} \pm 0.01$	1.77×10^{-3} - 6.69×10^{-2}	$3.33 \times 10^{-2} \pm 0.02$
Beef Liver	29.07 ± 12.78	31.72 ± 8.05	15.85 ± 7.42	32.02 ± 11.87	16.47 ± 5.77	16.47 ± 5.77	7.62 - 57.28	25.47 ± 12.60

Co								
Beef Meat	$8.06 \times 10^{-5} \pm 0.00002$	$8.50 \times 10^{-5} \pm 0.00002$	$7.17 \times 10^{-5} \pm 0.00003$	$7.85 \times 10^{-5} \pm 0.00003$	$8.26 \times 10^{-5} \pm 0.00003$	$8.26 \times 10^{-5} \pm 0.00002$	2.82×10^{-5} - 1.24×10^{-4}	$8.13 \times 10^{-5} \pm 0.00002$
Beef Liver	0.18 ± 0.02	0.17 ± 0.08	0.18 ± 0.04	0.16 ± 0.04	0.11 ± 0.01	0.13 ± 0.02	0.09 - 0.23	0.16 ± 0.05
Mn								
Beef Meat	$8.11 \times 10^{-3} \pm 0.005$	$12.1 \times 10^{-2} \pm 0.008$	$9.87 \times 10^{-3} \pm 0.006$	$1.16 \times 10^{-2} \pm 0.007$	$1.06 \times 10^{-2} \pm 0.0107$	$4.60 \times 10^{-3} \pm 0.0012$	2.89×10^{-3} - 2.95×10^{-2}	$9.49 \times 10^{-2} \pm 0.007$
Beef Liver	10.39 ± 0.95	8.33 ± 1.75	8.80 ± 1.66	10.39 ± 1.87	6.21 ± 1.01	6.00 ± 0.50	5.33 - 11.82	8.29 ± 2.13
Zn								
Beef Meat	0.32 ± 0.07	0.38 ± 0.05	0.25 ± 0.11	0.33 ± 0.09	0.37 ± 0.11	0.31 ± 0.09	0.11 – 0.55	0.32 ± 0.09
Beef Liver	131.99 ± 31.83	151.18 ± 15.81	151.29 ± 37.27	140.62 ± 40.46	106.69 ± 18.58	141.87 ± 24.99	81.03 - 203.27	137.27 ± 31.05
Se								
Beef Meat	$1.84 \times 10^{-4} \pm 0.00005$	$2.64 \times 10^{-4} \pm 0.0001$	$1.58 \times 10^{-4} \pm 0.00008$	$1.52 \times 10^{-4} \pm 0.00006$	$9.69 \times 10^{-5} \pm 0.00005$	$1.64 \times 10^{-4} \pm 0.00007$	5.02×10^{-5} - 4.40×10^{-4}	$1.70 \times 10^{-4} \pm 0.00008$
Beef Liver	0.98 ± 0.35	0.92 ± 0.34	1.05 ± 0.25	1.12 ± 0.24	0.54 ± 0.11	1.11 ± 0.36	0.38 - 1.54	0.95 ± 0.33

3.10.3 Estimation of Residual Heavy Metals in Broiler Chicken Meat and Liver

For the analysis of heavy metals, samples of broiler chicken meat (n = 30) and liver (n = 30) were bought from six distinct local marketplaces in Bangladesh's Dhaka North and South City. The standard deviation (SD) of the heavy metal contents in the meat and liver of broiler chickens is displayed in Figure 3.58 and Table 3.41.

Table 3.41 The concentration of ten heavy metals (mg/kg fresh weight) in broiler chicken meat and liver from different local markets of Dhaka North and South City (mean ± SD)

Sample Type	Basabo	Karwan-bazar	Tongi bazar	Kaptan-bazar	Moulovi-Bazar	Moham-madpur	Min - Max	Final Mean ± SD
Cr								
Chicken Meat	0.67 ± 0.09	1.00 ± 0.34	0.82 ± 0.08	0.94 ± 0.41	0.72 ± 0.11	0.90 ± 0.29	0.58 - 1.68	0.84 ± 0.26
Chicken Liver	1.15 ± 0.03	1.33 ± 0.08	1.15 ± 0.06	1.29 ± 0.18	1.05 ± 0.43	1.15 ± 0.11	0.32 - 1.42	1.19 ± 0.20
Ni								
Chicken Meat	0.17 ± 0.07	0.74 ± 0.42	0.76 ± 0.16	0.17 ± 0.03	0.23 ± 0.09	0.23 ± 0.17	0.10-1.45	0.38 ± 0.32
Chicken Liver	0.19 ± 0.12	1.45 ± 2.63	0.18 ± 0.09	0.93 ± 1.31	0.14 ± 0.11	8.66 ± 0.12	0.06 - 42.63	1.92 ± 7.78
Pb								
Chicken Meat	0.56 ± 0.19	0.63 ± 0.49	0.82 ± 0.82	1.45 ± 1.26	0.85 ± 0.76	1.29 ± 0.77	0.05 - 1.71	0.91 ± 0.73
Chicken Liver	0.87 ± 0.54	0.67 ± 0.51	0.38 ± 0.23	0.62 ± 0.47	0.03 ± 0.02	2.55 ± 2.09	0.03 - 5.28	0.95 ± 1.18
Cd								
Chicken Meat	0.14 ± 0.10	BDL	BDL	0.18 ± 0.12	BDL	BDL	0.14 - 0.18	0.16 ± 0.03
Chicken Liver	0.12 ± 0.09	BDL	BDL	BDL	BDL	0.19 ± 0.06	0.04 - 0.24	0.02 ± 0.05
As								
Chicken Meat	0.12 ± 0.15	0.27 ± 0.13	0.13 ± 0.11	0.36 ± 0.22	0.02 ± 0.003	0.14 ± 0.16	0.005 - 0.52	0.19 ± 0.17
Chicken Liver	2.28 ± 3.37	2.35 ± 1.74	0.82 ± 1.22	5.75 ± 4.77	0.42 ± 0.35	1.14 ± 1.39	0.005 -10.79	2.03 ± 2.74
Cu								
Chicken Meat	1.93 ± 0.22	2.47 ± 1.07	2.68 ± 1.03	3.51 ± 1.14	2.65 ± 0.72	2.50 ± 1.06	0.93 - 4.68	2.62 ± 0.97
Chicken Liver	9.88 ± 2.24	7.48 ± 0.86	9.74 ± 1.36	9.34 ± 0.26	8.17 ± 2.43	9.87 ± 2.34	3.94 - 12.52	9.08 ± 1.86
Co								
Chicken Meat	6.03×10^{-3} ± 0.001	1.09×10^{-2} ± 0.008	8.19×10^{-3} ± 0.002	1.14×10^{-2} ± 0.007	6.04×10^{-3} ± 0.001	1.57×10^{-2} ± 0.02	0.005 - 0.05	0.009 ± 0.008
Chicken Liver	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.04 - 0.10	0.06 ± 0.02
Mn								
Chicken Meat	0.49 ± 0.07	0.47 ± 0.14	0.48 ± 0.10	0.86 ± 0.50	0.47 ± 0.15	1.76 ± 2.53	0.03 - 6.28	0.75 ± 1.07
Chicken Liver	5.96 ± 1.84	4.50 ± 0.58	5.94 ± 0.95	7.43 ± 1.36	5.96 ± 2.07	6.01 ± 1.61	2.95 - 9.18	6.05 ± 1.54

Zn								
Chicken Meat	25.69 ± 2.0	31.44 ± 2.89	36.96 ± 2.04	33.70 ± 5.07	32.56 ± 2.58	28.88 ± 2.96	23.70 - 41.20	31.54 ± 4.60
Chicken Liver	79.31 ± 17.84	78.09 ± 14.18	93.92 ± 17.86	88.56 ± 7.84	66.66 ± 17.02	90.96 ± 11.84	41.26 - 152.73	82.92 ± 20.79
Se								
Chicken Meat	0.57 ± 0.75	0.66 ± 0.06	0.66 ±0.06	0.68 ± 0.06	0.69 ± 0.12	0.68 ± 0.05	0.48 - 0.81	0.66 ± 0.08
Chicken Liver	2.13 ± 0.29	1.82 ± 0.22	2.05 ± 0.10	2.11 ± 0.36	2.00 ± 0.55	2.09 ± 0.22	1.12 - 2.54	2.03 ± 0.31

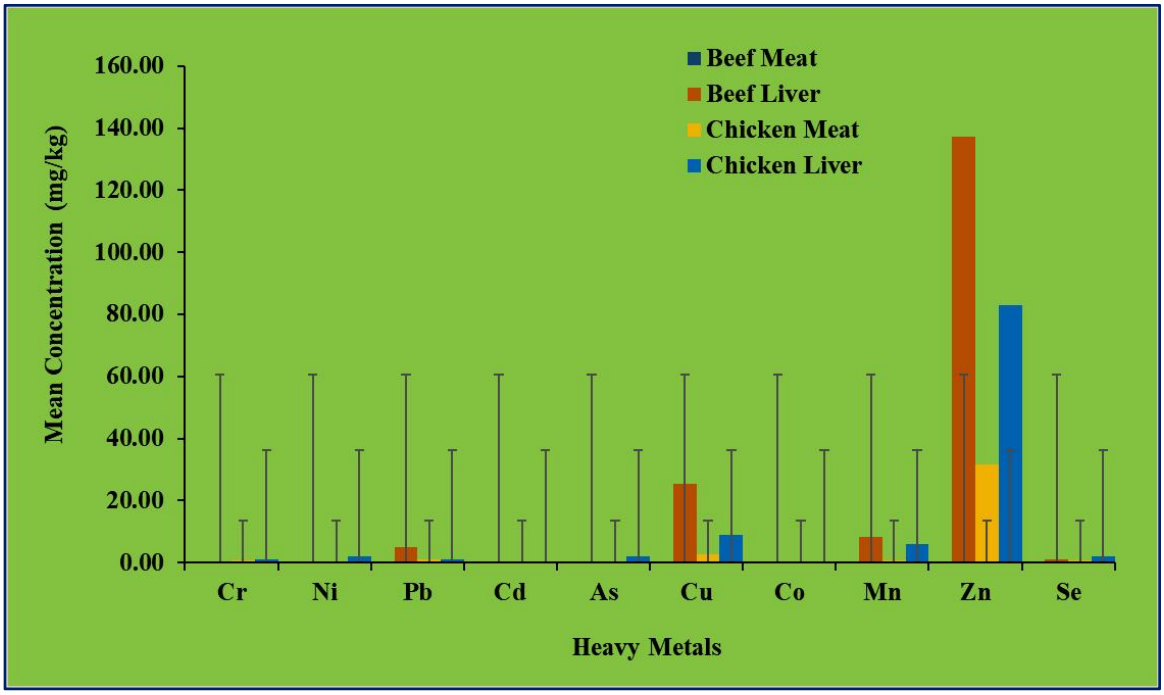


Figure 3.58 Mean Concentration ± SD of heavy metals in four matrices

Maximum Limit (MRL) for toxic heavy metals in cattle and poultry meat that was established by different regulatory bodies is presented in Table 3.42.

Table 3.42 ML value and samples exceeded ML for heavy metals in four matrices

Metal	Matrix	No. of Positive samples	Samples above ML (mg/kg)	Maximum Limit (ML) (mg/kg)
Cr	BM	30	-	0.05 (Codex), 1.0 (FAO/WHO), 1.0 (USDA)
	BL	30	-	-
	CM	9	4	1.0 (FAO/WHO), 1.0 (USDA)
	CL	30	-	-
Ni	BM	30	-	-
	BL	30	-	-
	CM	29	-	-
	CL	30	-	-
Pb	BM	30	-	0.1 (JECFA), 0.05 (EFSA), 0.05 (BFSA), 0.2 (USDA)
	BL	26	26 (28, BFSA)	0.2 (JECFA), 0.05 (BFSA), 0.5 (USDA)
	CM	29	21 (26, BFSA)	0.1 (JECFA), 0.01 (BFSA), 0.1 (EU), 0.2 (USDA)
	CL	22	18 (22, BFSA)	0.1 (JECFA), 0.01 (BFSA), 0.5 (USDA)
Cd	BM	30	-	0.05 (JECFA, EFSA), 0.05 (BFSA), 0.1 (USDA)
	BL	30	3 (10, BFSA)	0.5 (Codex), 0.05 (BFSA), 0.5 (USDA)
	CM	2	2 (2, BFSA)	0.05 (JECFA, EFSA), 0.01 (BFSA), 0.1 (USDA)
	CL	11	-	0.5 (Codex), 0.5 (USDA)
As	BM	30	-	0.1 (USDA), 0.01(BFSA), 0.5 (USDA)
	BL	19	10 (27, BFSA)	0.1 (USDA), 0.01(BFSA)
	CM	28	12 (17, BFSA)	0.1 (JECFA), 0.01(BFSA), 0.5 (USDA)
	CL	24	13 (22, BFSA)	0.5 (USDA), 0.05(BFSA), 0.0003-0.008 (EFSA)
Cu	BM	30	-	1.0 (FAO/WHO)
	BL	30	-	-
	CM	30	29	1.0 (FAO/WHO)
	CL	30	-	-

Co, Mn, Zn and Se were detected and quantified in all beef meat and liver, and broiler chicken meat and liver samples.

3.10.4 Estimated Daily Intake (EDI) for Heavy Metals

The health risk or hazard of a group is determined by its exposure route and intensity. Therefore, it is crucial to establish pollutant routes for target groups to determine exposure levels. The three important routes of ingestion, inhalation, and skin contact are through which metal exposure affects human health. The most important of these pathways is ingestion and the food chain. The current study examined the ingestion route of Cr, Ni, Pb, Cd, As, Cu, Co, Mn, Zn, and Se, which are believed to entail eating beef, chicken meat, and the liver. The average metal content of beef and chicken and each individual's consumption rate were used to estimate the EDI values for particular metals (Figure 3.59 and 3.60). The EDI and preliminary tolerable daily intake (PTDI) values for metals from the consumption of beef and chicken meat by adults and children in Bangladesh are displayed in Table 3.43 and 3.44.

The EDI values of all heavy metals examined through beef meat consumption for adults and children were lower than the FAO/WHO recommended heavy metal intake values. However, the EDI values of beef liver, chicken meat, and liver were higher than the FAO/WHO recommended values, except for manganese (Mn). There appears to be a potential health risk for the average consumption of beef liver, chicken meat, and liver [317].

Table 3.43 Estimated Dietary Intake (EDI) of the heavy metals from the consumption beef and chicken meat by the adults of Bangladesh

Sample ID	Estimated Dietary Intake (EDI) ($mg^{-1}kg^{-1}BW^{-1}day^{-1}$) for Adult									
	Cr	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**
Basabo										
BM	2.51 x 10^{-5}	1.98 x 10^{-5}	3.13 x 10^{-5}	3.19 x 10^{-5}	4.20 x 10^{-5}	0.011	1.98 x 10^{-5}	1.99 x 10^{-3}	0.079	4.50 x 10^{-5}
CM	0.367	0.091	0.311	0.038	0.068	1.063	3.32 x 10^{-3}	0.271	14.172	0.313
BL	0.020	0.048	0.669	0.043	0.034	6.633	0.045	2.546	32.337	0.240
CL	0.634	0.103	0.480	0.010	1.257	5.453	0.033	3.289	43.752	1.174
Karwanbazar										
BM	2.47 x 10^{-5}	1.47 x 10^{-5}	7.31 x 10^{-5}	5.45 x 10^{-5}	1.46 x 10^{-5}	3.27 x 10^{-3}	2.08 x 10^{-5}	2.96 x 10^{-3}	0.090	6.47 x 10^{-5}
CM	0.549	0.411	0.345	BDL	0.148	1.360	6.02 x 10^{-3}	0.261	17.346	0.361
BL	0.024	0.038	0.271	0.026	0.023	7.771	0.043	2.041	37.040	0.226
CL	0.734	0.801	0.369	0.017	1.297	4.125	0.029	2.758	43.080	1.005
Tongibazar										
BM	2.79 x 10^{-5}	2.01 x 10^{-5}	8.57 x 10^{-5}	5.44 x 10^{-5}	4.34 x 10^{-5}	0.010	1.76 x 10^{-5}	2.42 x 10^{-3}	0.062	3.87 x 10^{-5}
CM	0.453	0.417	0.455	BDL	0.073	1.476	4.52 x 10^{-3}	0.266	20.390	0.362
BL	BDL	0.095	0.305	0.018	0.008	3.883	0.040	2.156	37.067	0.258
CL	0.632	0.097	0.209	BDL	0.452	5.371	0.034	3.274	51.814	1.132
Kaptanbazar										
BM	1.81 x 10^{-5}	1.72 x 10^{-5}	5.09 x 10^{-5}	4.64 x 10^{-5}	2.06 x 10^{-5}	6.89 x 10^{-3}	1.92 x 10^{-5}	2.84 x 10^{-3}	0.080	3.73 x 10^{-5}
CM	0.521	0.094	0.794	0.049	0.199	1.936	6.28 x 10^{-3}	0.472	18.593	0.273
BL	0.021	0.081	0.364	BDL	0.057	7.844	0.040	2.458	34.451	0.273
CL	0.709	0.515	0.340	BDL	3.169	5.154	0.029	4.098	48.855	1.165
Moulovibazar										
BM	2.56 x 10^{-5}	1.82 x 10^{-5}	9.85 x 10^{-5}	5.83 x 10^{-5}	5.20 x 10^{-5}	0.010	2.02 x 10^{-5}	2.60 x 10^{-3}	0.090	2.37 x 10^{-5}
CM	0.396	0.125	0.469	BDL	0.011	1.465	3.33 x 10^{-3}	0.258	17.960	0.374

BL	0.024	0.028	2.883	0.111	0.057	4.036	0.027	1.522	26.140	0.131
CL	0.581	0.078	0.009	BDL	0.233	4.509	0.021	3.290	36.775	1.104
Mohammadpur										
BM	2.49×10^{-5}	1.64×10^{-5}	8.90×10^{-5}	1.18×10^{-5}	3.46×10^{-5}	8.50×10^{-3}	2.18×10^{-5}	1.13×10^{-3}	0.075	4.01×10^{-5}
CM	0.496	0.128	0.714	BDL	0.076	1.379	8.69×10^{-3}	0.969	15.934	0.377
BL	BDL	0.031	2.772	0.181	0.024	7.277	0.031	1.471	34.758	0.272
CL	0.635	4.778	1.407	0.106	0.630	5.448	0.041	3.318	50.180	1.152
Mean (BM)	2.48×10^{-5}	1.77×10^{-5}	7.14×10^{-5}	4.29×10^{-5}	3.45×10^{-5}	8.17×10^{-3}	1.99×10^{-5}	2.32×10^{-3}	0.080	4.56×10^{-5}
Mean (CM)	0.464	0.211	0.501	0.086	0.105	1.447	5.33×10^{-3}	0.416	17.399	0.361
Mean (BL)	0.029	0.054	1.240	0.102	0.035	6.240	0.038	2.032	33.632	0.233
Mean (CL)	0.654	1.062	0.526	0.066	0.119	5.010	0.031	3.338	45.743	1.122
PTDI (µg/kg bw/day)	2.8 [a]	4.3 [b]	3.0 [c]	0.66 [c]	1.8 [c]	166.7[d]	RI - 2.4-2.4 - 2.8[e]	UL – 11000 [f]	1000 [c]	DV - 55 [g]
PTDI (mg/kg bw/day)	0.0028	0.0043	0.003	0.00066	0.0018	0.1667	RI - 0.0024 - 0.0028	UL - 11.0	1.0	DV - 0.055

BM = Beef Meat, CM = Chicken Meat, BL = Beef Liver, CL = Chicken Liver, PTDI = Provisional Tolerable Daily Intake, RI = Recommended Intake, UL = Tolerable Upper Intake Level, DV = Daily Value

[a]RDA (1989) [318], [b]WHO (1996, 2001) [319], [c]JECFA (2003) [320], [d]DRI (2001) [321],

[e]National Institutes of Health Office of Dietary Supplements [322]

[f]Institute of Medicine (US) Panel on Micronutrients (2001) [323],

[g]Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds (2000) [324]

Table 3.44 Estimated Dietary Intake (EDI) of the heavy metals from the consumption of beef and broiler chicken meat by the children of Bangladesh

Sample ID	Estimated Dietary Intake (EDI) ($mg^{-1}kg^{-1}BW^{-1}day^{-1}$) for Child									
	Cr	Ni	Pb	Cd	As	Cu	Co	Mn	Zn	Se
Basabo										
BM	3.15×10^{-5}	2.48×10^{-5}	3.92×10^{-5}	4.00×10^{-5}	5.27×10^{-5}	0.013	2.48×10^{-5}	2.49×10^{-3}	0.099	5.65×10^{-5}
CM	0.202	0.051	0.173	0.021	0.038	0.592	1.85×10^{-3}	0.151	7.897	0.175
BL	0.025	0.060	0.840	0.054	0.043	8.322	0.056	3.194	40.574	0.301
CL	0.349	0.057	0.268	0.006	0.701	3.038	0.018	1.833	24.380	0.654
Karwanbazar										
BM	3.43×10^{-5}	1.84×10^{-5}	1.07×10^{-4}	6.84×10^{-5}	1.83×10^{-5}	4.10×10^{-3}	2.61×10^{-5}	3.72×10^{-3}	0.113	8.12×10^{-5}
CM	0.302	0.229	0.192	BDL	0.083	0.758	3.35×10^{-3}	0.145	9.666	0.201
BL	0.029	0.048	0.340	0.032	0.028	9.750	0.054	2.561	46.475	0.284
CL	0.404	0.446	0.205	0.009	0.723	2.299	0.016	1.537	24.005	0.560
Tongibazar										
BM	3.50×10^{-5}	2.52×10^{-5}	1.07×10^{-4}	6.82×10^{-5}	5.45×10^{-5}	0.012	2.20×10^{-5}	3.57×10^{-3}	0.078	4.85×10^{-5}
CM	0.249	0.232	0.253	BDL	0.041	0.823	2.52×10^{-3}	0.148	11.362	0.202
BL	BDL	0.120	0.383	0.022	0.010	4.842	0.051	2.705	46.509	0.324
CL	0.348	0.054	0.116	BDL	0.723	2.993	0.019	1.825	28.873	0.631
Kaptanbazar										
BM	2.27×10^{-5}	2.15×10^{-5}	6.38×10^{-5}	5.82×10^{-5}	2.58×10^{-5}	8.65×10^{-3}	2.41×10^{-5}	3.57×10^{-3}	0.100	4.68×10^{-5}
CM	0.287	0.052	0.442	0.027	0.111	1.079	3.50×10^{-3}	0.263	10.360	0.343
BL	0.027	0.101	0.457	BDL	0.071	9.842	0.051	3.084	43.227	0.343
CL	0.390	0.287	0.190	BDL	1.766	2.872	0.016	2.283	27.224	0.649
Moulovibazar										
BM	3.21×10^{-5}	2.28×10^{-5}	1.24×10^{-4}	7.32×10^{-5}	6.52×10^{-5}	0.013	2.54×10^{-5}	3.27×10^{-3}	0.114	2.98×10^{-5}
CM	0.218	0.069	0.261	BDL	0.006	0.816	1.86×10^{-3}	0.144	10.008	0.208
BL	0.030	0.035	3.617	0.139	0.071	5.064	0.034	1.910	32.799	0.165

CL	0.320	0.043	0.005	BDL	0.130	2.512	0.012	1.833	20.492	0.615
Mohammadpur										
BM	3.12 x 10^{-5}	2.05 x 10^{-5}	1.12 x 10^{-4}	1.47 x 10^{-5}	4.38 x 10^{-5}	0.011	2.47 x 10^{-5}	1.14 x 10^{-3}	0.095	5.03 x 10^{-5}
CM	0.273	0.071	0.398	BDL	0.042	0.769	4.84 x 10^{-3}	0.540	8.879	0.210
BL	BDL	0.038	3.478	0.227	0.030	9.130	0.039	1.845	43.611	0.341
CL	0.350	2.663	0.784	0.059	0.351	3.036	0.023	1.849	27.962	0.642
Mean (BM)	3.11 x 10^{-5}	2.22 x 10^{-5}	8.96 x 10^{-5}	5.38 x 10^{-5}	4.33 x 10^{-5}	1.02 x 10^{-2}	2.50 x 10^{-5}	2.92 x 10^{-3}	0.100	5.22 x 10^{-5}
Mean (CM)	0.255	0.118	0.279	0.048	0.059	0.806	2.97 x 10^{-3}	0.232	9.695	0.201
Mean (BL)	0.036	0.068	1.556	0.129	0.044	7.830	0.048	2.550	42.199	0.293
Mean (CL)	0.360	0.592	0.293	0.037	0.624	2.792	0.017	1.860	25.489	0.625
PTDI (µg/kg bw/day)	2.8 [a]	4.3 [b]	3.0 [c]	0.66[c]	1.8 [c]	166.7[d]	RI - 2.4-2.4 - 2.8[e]	UL – 11000 [f]	1000 [c]	DV - 55 [g]
PTDI (mg/kg bw/day)	0.0028	0.0043	0.003	0.00066	0.0018	0.1667	RI - 0.0024 - 0.0028	UL - 11.0	1.0	DV - 0.055
BM = Beef Meat, CM = Chicken Meat, BL = Beef Liver, CL = Chicken Liver, PTDI = Provisional Tolerable Daily Intake, RI = Recommended Intake, UL = Tolerable Upper Intake Level, DV = Daily Value										

[a]RDA (1989) [318], [b]WHO (1996, 2001) [319], [c]JECFA (2003) [320], [d]DRI (2001) [321],

[e]National Institutes of Health Office of Dietary Supplements [322]

[f]Institute of Medicine (US) Panel on Micronutrients (2001) [323],

[g]Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds (2000) [324]

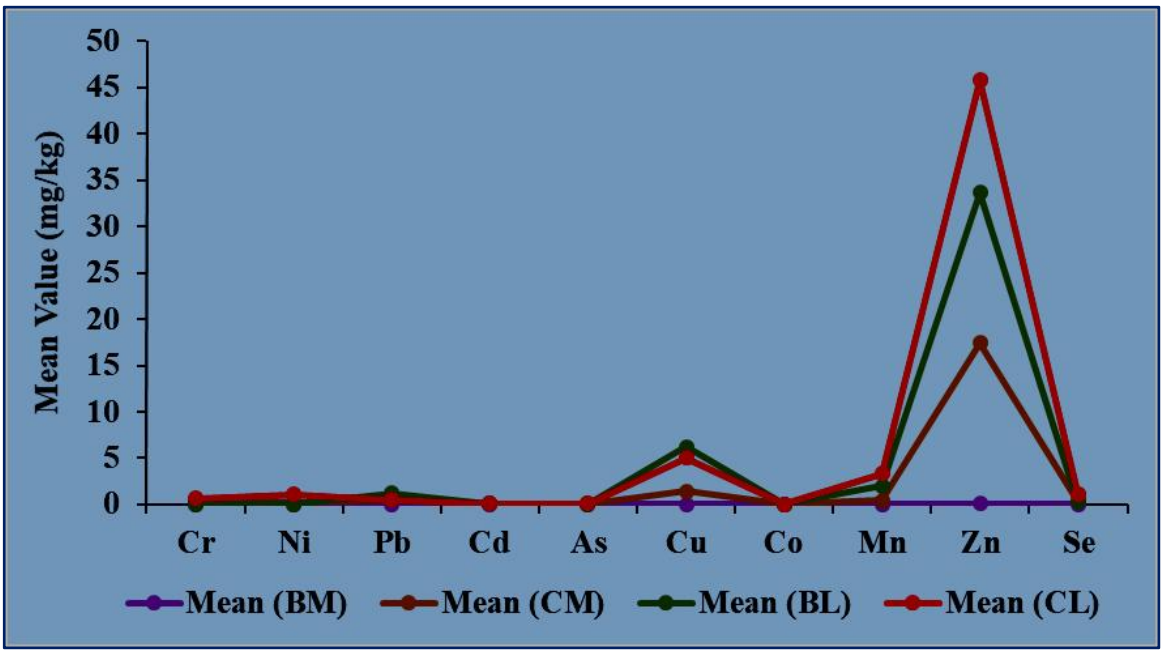


Figure 3.59 EDI of the heavy metals for adults from the consumption beef and chicken meat

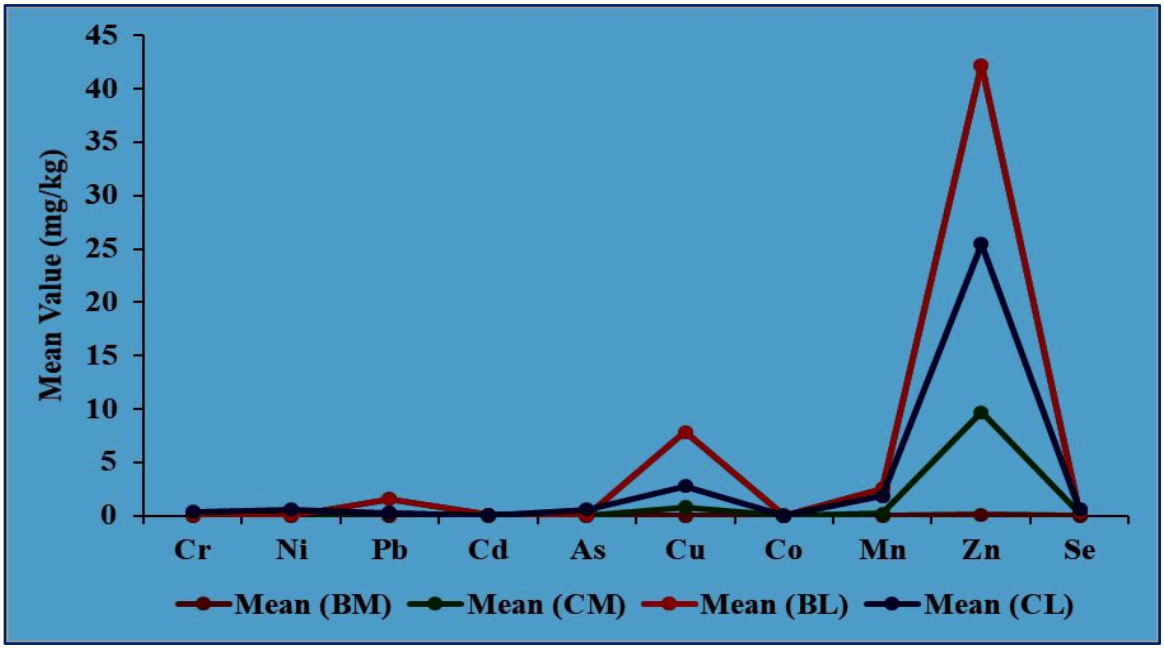


Figure 3.60 EDI of the heavy metals for children from the consumption beef and chicken meat

3.10.5 Non-carcinogenic health risk (THQ) for beef meat

The non-carcinogenic health risks associated with adult residents consuming chicken contaminated with toxic metals were assessed using THQ. Population exposure is considered to have adverse health effects that are not carcinogenic if the THQ, which is the ratio of the dose of the metal under study to the reference dosage level for a comparable metal, is greater than 1. The THQs of the ten metals examined for the consumption of beef and broiler chicken meat are shown in Table 3.45 and 3.46. In this research of the heavy metals analyzed in the beef samples for adults was less than 1, indicating that consuming beef meat does not pose a significant risk to one's health. However, the THQ of manganese and total HI for children are 3.645 and 3.665, respectively, which are greater than 1, indicating that consuming beef meat may pose a significant health risk to children. TTHQ (total of individual metal THQ) varied from 6.53 x 10^{-4} to 2.28 x 10^{-2} for adults and from 0.105to 3.665 for children based on the metals considered in the meat samples under examination (Table 3.43 and 3.44). Beef consumption presents a carcinogenic health risk to children, but not to adults, according to the THQs values found in the research regions. For adults and children, the THQ for metals from the ingestion of beef samples decreased in the following Zn > Cu > Mn > Cd > Co > Pb > As > Se > Ni > Cr and Mn > Zn > Cu > Pb > As > Cd > Se > Co > Ni > Cr, respectively (Figure 3.61 and 3.62).

Table 3.45 Non-carcinogenic (THQ) health risks of heavy metals for adults due to consuming beef meat from Dhaka North and South City of Bangladesh

Non Carcinogenic (THQ) for adults											
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**	**Total TTHQ**
Basabo	1.67 x 10^{-8}	9.89 x 10^{-7}	8.93 x 10^{-6}	6.38 x 10^{-5}	1.40 x 10^{-5}	4.05 x 10^{-4}	1.01 x 10^{-4}	1.27 x 10^{-4}	4.04 x 10^{-4}	9.01 x 10^{-6}	7.74 x 10^{-4}
Karwanbazar	1.82 x 10^{-8}	7.33 x 10^{-7}	2.09 x 10^{-5}	1.09 x 10^{-4}	4.87 x 10^{-6}	1.25 x 10^{-4}	1.06 x 10^{-4}	1.89 x 10^{-4}	4.60 x 10^{-4}	1.29 x 10^{-5}	7.23 x 10^{-4}
Tongibazar	1.86 x 10^{-8}	1.00 x 10^{-6}	2.45 x 10^{-5}	1.09 x 10^{-4}	1.45 x 10^{-5}	3.74 x 10^{-4}	5.86 x 10^{-5}	1.54 x 10^{-4}	3.18 x 10^{-4}	7.74 x 10^{-6}	7.68 x 10^{-4}
Kaptanbazar	1.21 x 10^{-8}	8.58 x 10^{-7}	1.45 x 10^{-5}	9.28 x 10^{-5}	1.45 x 10^{-5}	2.64 x 10^{-4}	6.41 x 10^{-5}	1.81 x 10^{-4}	4.08 x 10^{-4}	7.46 x 10^{-6}	7.44 x 10^{-4}
Moulovibazar	1.70 x 10^{-8}	9.10 x 10^{-7}	2.81 x 10^{-5}	1.17 x 10^{-4}	6.86 x 10^{-6}	3.81 x 10^{-4}	1.03 x 10^{-4}	1.66 x 10^{-4}	4.62 x 10^{-4}	4.75 x 10^{-6}	8.94 x 10^{-4}
Mohammadpur	1.66 x 10^{-8}	8.18 x 10^{-7}	2.54 x 10^{-5}	2.35 x 10^{-5}	1.15 x 10^{-5}	3.25 x 10^{-4}	1.11 x 10^{-4}	1.76 x 10^{-5}	3.85 x 10^{-4}	8.02 x 10^{-6}	6.53 x 10^{-4}
Total (HI)	4.96 x 10^{-7}	2.66 x 10^{-5}	6.12 x 10^{-4}	2.57 x 10^{-3}	3.45 x 10^{-4}	6.12 x 10^{-3}	1.99 x 10^{-3}	2.91 x 10^{-3}	7.96 x 10^{-3}	2.50 x 10^{-4}	2.28 x 10^{-2}

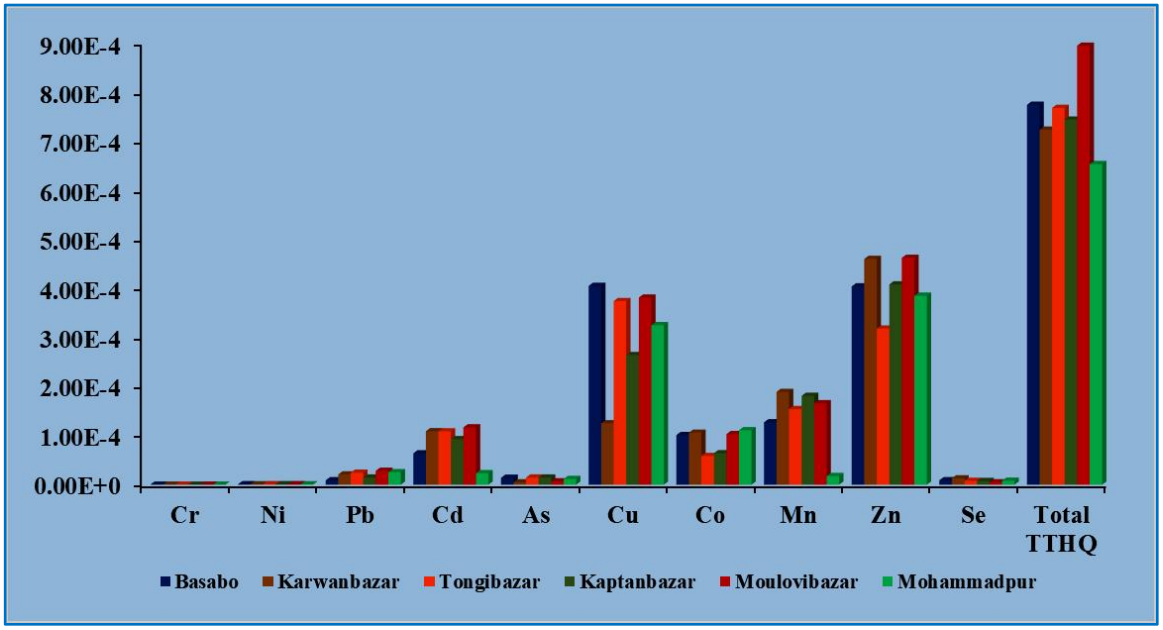


Figure 3.61 Non-carcinogenic (THQ) health risks of heavy metals for adults due to consuming beef meat from Dhaka North and South City of Bangladesh

Table 3.46 Non-carcinogenic (THQ) health risks of heavy metals for children due to consuming beef meat from Dhaka North and South City of Bangladesh

Non Carcinogenic (THQ) for children											
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**	**Total TTHQ**
Basabo	2.10×10^{-8}	1.24×10^{-6}	1.12×10^{-5}	8.01×10^{-6}	1.76×10^{-5}	1.24×10^{-4}	8.26×10^{-6}	0.104	1.24×10^{-4}	1.13×10^{-5}	0.105
Karwanbazar	2.29×10^{-8}	9.20×10^{-7}	2.62×10^{-5}	1.37×10^{-5}	6.10×10^{-6}	1.03×10^{-4}	8.71×10^{-6}	0.155	1.41×10^{-4}	1.62×10^{-5}	0.156
Tongibazar	2.33×10^{-8}	1.26×10^{-6}	3.07×10^{-5}	1.36×10^{-5}	1.82×10^{-5}	1.14×10^{-4}	7.35×10^{-6}	0.126	2.61×10^{-4}	9.71×10^{-6}	0.127
Kaptanbazar	1.52×10^{-8}	1.08×10^{-6}	1.82×10^{-5}	1.16×10^{-5}	8.61×10^{-6}	2.16×10^{-4}	8.04×10^{-6}	0.149	1.25×10^{-4}	9.36×10^{-6}	0.149
Moulovibazar	2.14×10^{-8}	1.14×10^{-6}	3.53×10^{-5}	1.46×10^{-5}	2.17×10^{-5}	1.17×10^{-4}	1.03×10^{-4}	0.136	1.41×10^{-4}	5.96×10^{-6}	0.137
Mohammadpur	2.08×10^{-8}	1.03×10^{-6}	3.19×10^{-5}	2.95×10^{-6}	1.45×10^{-5}	2.67×10^{-4}	9.14×10^{-6}	0.059	1.08×10^{-4}	1.01×10^{-5}	0.057
Total (HI)	6.23×10^{-7}	3.33×10^{-5}	7.68×10^{-4}	3.23×10^{-4}	4.33×10^{-4}	7.68×10^{-3}	2.50×10^{-4}	3.645	9.98×10^{-3}	3.13×10^{-4}	3.665

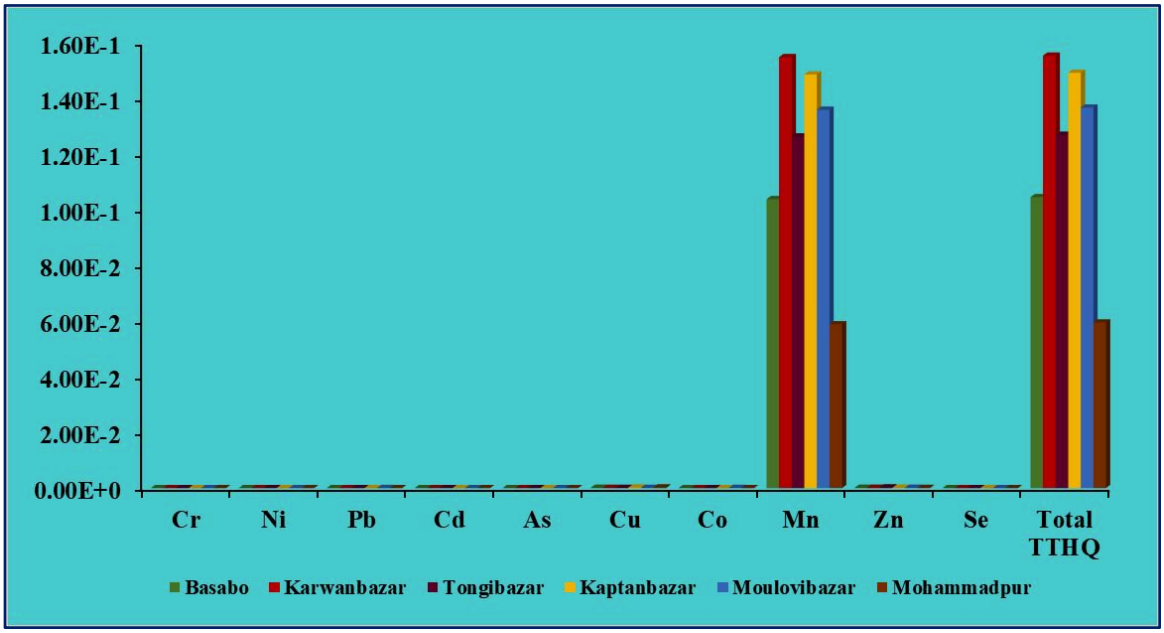


Figure 3.62 Non-carcinogenic (THQ) health risks of heavy metals for children due to consuming beef meat from Dhaka North and South City of Bangladesh

3.10.6 Non-carcinogenic health risk (THQ) for broiler chicken meat

The non-carcinogenic health risks associated with the consumption of broiler chicken meat contaminated with toxic metals were assessed using THQ. Population exposure is considered to have carcinogenic adverse health effects if the THQ, which is the ratio of the dose of the metal in this study to the reference dosage level for a comparable metal, is greater than 1. The THQs of the ten heavy metals examined for the adults and children consuming broiler chicken meat samples are displayed in Table 3.47 and 3.48. The TTHQ of all the heavy metals analyzed in the broiler chicken meat samples and the sum of all TTHQ (HI) for adults in this study was greater than 1, indicating that consuming broiler chicken meat can pose a significant risk to adult health. Consuming broiler chicken meat can also be extremely harmful to children's health, as evidenced by the fact that the TTHQ of nearly all the heavy metals examined in the broiler chicken meat samples and the total TTHQ (HI) in this study were both greater than 1 (Figure 3.63 and 3.64).

Table 3.47 Non-carcinogenic (THQ) health risks of heavy metals for adults due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

Non-carcinogenic (THQ) for adults											
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**	**Total TTHQ**
Basabo	0.001	0.023	0.444	0.151	0.456	0.133	0.055	0.057	0.236	0.313	1.869
Karwanbazar	0.002	0.103	0.394	BDL	1.483	0.170	0.100	0.054	0.289	0.361	2.957
Tongibazar	0.002	0.104	0.650	BDL	0.978	0.185	0.075	0.055	0.340	0.362	2.751
Kaptanbazar	0.002	0.024	0.680	0.195	2.658	0.242	0.105	0.098	0.310	0.374	4.687
Moulovibazar	0.001	0.031	0.535	BDL	0.073	0.183	0.055	0.054	0.299	0.381	1.613
Mohammadpur	0.002	0.032	1.020	BDL	1.014	0.172	0.145	0.202	0.266	0.377	3.230
Total (HI)	0.009	0.316	3.725	0.345	6.663	1.085	0.536	0.520	1.740	2.169	17.108

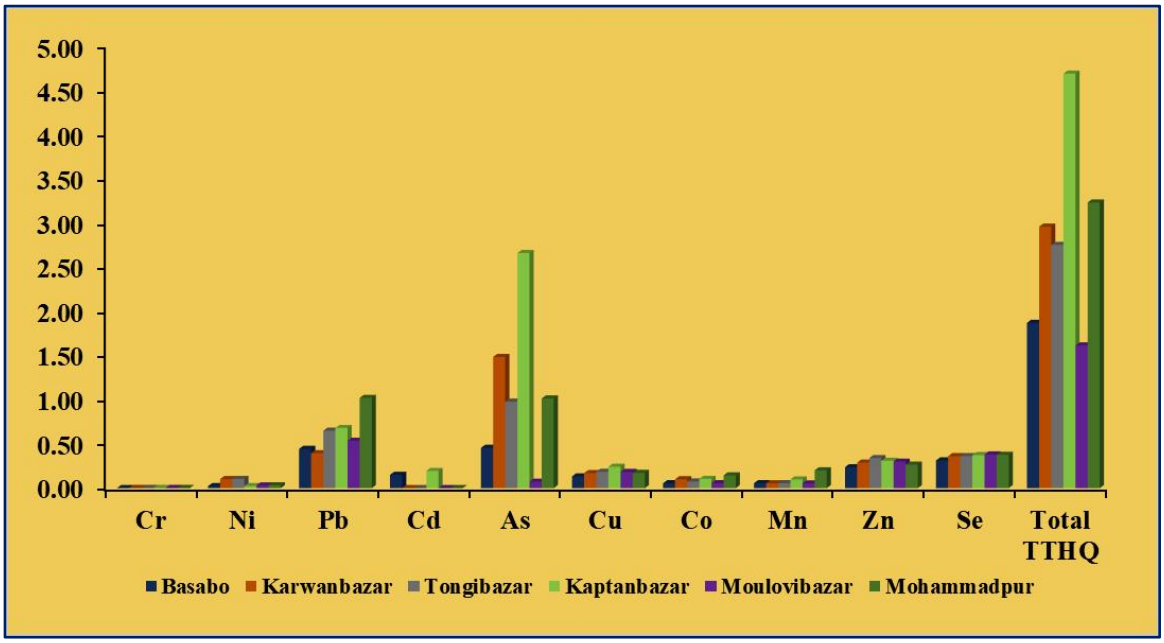


Figure 3.63 Non-carcinogenic (THQ) health risks of heavy metals for adults due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

Table 3.48 Non-carcinogenic (THQ) health risks of heavy metals for children due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

Non-carcinogenic (THQ) for children											
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**	**Total TTHQ**
Basabo	0.001	0.027	0.247	0.084	0.254	0.074	0.031	0.038	0.132	0.175	1.062
Karwanbazar	0.001	0.120	0.220	BDL	0.827	0.095	0.056	0.036	0.161	0.201	1.717
Tongibazar	0.001	0.122	0.362	BDL	0.545	0.103	0.042	0.037	0.189	0.202	1.603
Kaptanbazar	0.001	0.028	0.379	0.108	1.481	0.135	0.058	0.066	0.173	0.208	2.637
Moulovibazar	0.001	0.036	0.298	BDL	0.041	0.102	0.031	0.036	0.167	0.212	0.924
Mohammadpur	0.001	0.037	0.569	BDL	0.565	0.096	0.081	0.135	0.148	0.210	1.842
Total (HI)	0.005	0.370	2.075	0.192	3.713	0.605	0.299	0.348	0.970	1.208	9.784

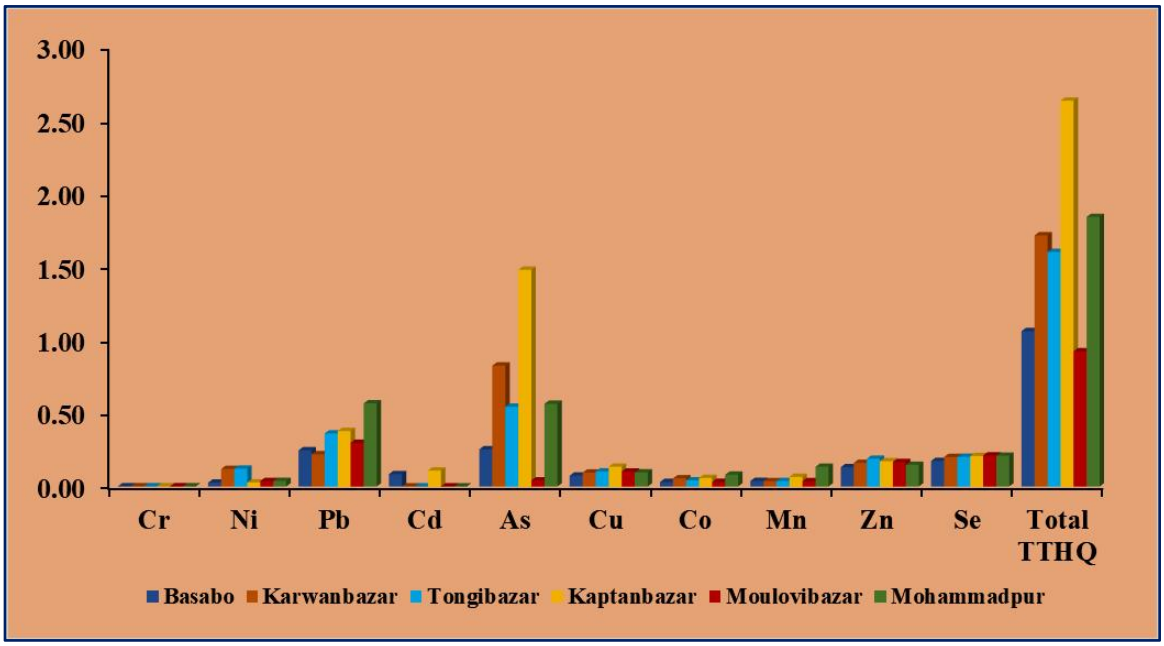


Figure 3.64 Non-carcinogenic (THQ) health risks of heavy metals for children due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

3.10.7 Target Carcinogenic Risk (TCR) for Beef Meat

Food intake was used to calculate the target carcinogenic risks (TCRs) of Cr, Ni, Pb, Cd, As, and Cu because exposure levels to these heavy metals can result in both carcinogenic and non-carcinogenic risks. The TCRs for Cr, Ni, Pb, Cd, As, and Cu linked to the consumption of beef meat were calculated and are shown in Figure 3.65. As a result of consuming beef meat, the mean TCR values for Cr, Ni, Pb, Cd, As and Cu were 1.24×10^{-8}, 3.01×10^{-8}, 6.07×10^{-10}, 1.63×10^{-8}, 2.59×10^{-7} and 6.12×10^{-5} for adults, and 1.56×10^{-8}, 3.78×10^{-8}, 3.14×10^{-10}, 2.04×10^{-5},6.50×10^{-8} and $1.54 \times 10^{-5}$for children (Table 3.49 and 3.50). TCR values less than 1.0×10^{-6} are deemed inconsequential, those more than 1.0×10^{-4} are unsatisfactory, and those between 1.0×10^{-6} and 1.0×10^{-4} are considered acceptable. The TCR values of Cu for adults and children, and Cd for children were within the acceptable range. The carcinogenic risk of Cr, Ni, Pb, Cd, and As from beef meat intake was insignificant to tolerable in this study. On the other hand, long-term cancer risk may result from chronic Cu exposure from consuming beef meat, that has been enhanced with Cu.

Table 3.49 Targeted Cancer Risks (TCR) of heavy metals for adults due to consuming beef meat from Dhaka North and South City of Bangladesh

Targeted Cancer Risks (TCR) for adults						
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**
Basabo	1.25×10^{-8}	3.36×10^{-8}	2.66×10^{-10}	1.21×10^{-8}	3.15×10^{-7}	7.95×10^{-5}
Karwanbazar	1.37×10^{-8}	2.49×10^{-8}	6.21×10^{-10}	2.07×10^{-8}	1.09×10^{-7}	2.45×10^{-5}
Tongibazar	1.39×10^{-8}	3.41×10^{-8}	7.28×10^{-10}	2.07×10^{-8}	3.26×10^{-7}	7.33×10^{-5}
Kaptanbazar	9.07×10^{-9}	2.92×10^{-8}	4.32×10^{-10}	1.76×10^{-8}	1.54×10^{-7}	5.17×10^{-5}
Moulovibazar	1.28×10^{-8}	3.09×10^{-8}	8.37×10^{-10}	2.22×10^{-8}	3.90×10^{-7}	7.47×10^{-5}
Mohammadpur	1.24×10^{-8}	2.78×10^{-8}	7.57×10^{-10}	7.57×10^{-10}	2.59×10^{-7}	6.37×10^{-5}
Mean	1.24×10^{-8}	3.01×10^{-8}	6.07×10^{-10}	1.63×10^{-8}	2.59×10^{-7}	6.12×10^{-5}

Table 3.50 Targeted Cancer Risks (TCR) of heavy metals for children due to consuming beef meat from Dhaka North and South City of Bangladesh

Targeted Cancer Risks (TCR) for children						
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**
Basabo	1.57×10^{-8}	4.22×10^{-8}	1.37×10^{-10}	1.52×10^{-5}	7.91×10^{-8}	1.99×10^{-5}
Karwanbazar	1.72×10^{-8}	3.13×10^{-8}	3.21×10^{-10}	2.07×10^{-8}	2.75×10^{-8}	6.15×10^{-6}
Tongibazar	1.75×10^{-8}	4.28×10^{-8}	3.76×10^{-10}	2.07×10^{-8}	8.17×10^{-8}	1.84×10^{-5}
Kaptanbazar	1.14×10^{-8}	3.66×10^{-8}	2.23×10^{-10}	1.76×10^{-8}	3.87×10^{-8}	1.30×10^{-5}
Moulovibazar	1.60×10^{-8}	3.88×10^{-8}	4.32×10^{-10}	2.22×10^{-8}	9.79×10^{-8}	1.88×10^{-5}
Mohammadpur	1.56×10^{-8}	3.49×10^{-8}	3.91×10^{-10}	4.47×10^{-9}	6.51×10^{-8}	1.60×10^{-5}
Mean	1.56×10^{-8}	3.78×10^{-8}	3.14×10^{-10}	2.04×10^{-5}	6.50×10^{-8}	1.54×10^{-5}

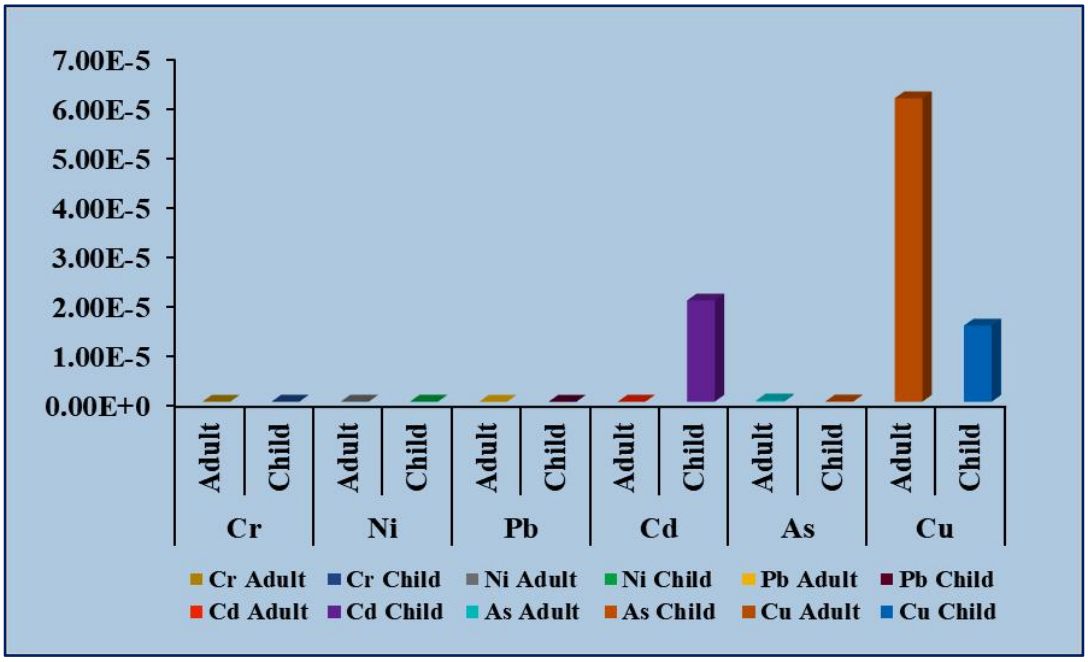


Figure 3.65 Targeted Cancer Risks (TCR) of heavy metals for adults and children due to consuming beef meat from Dhaka North and South City of Bangladesh

3.10.8 Target Carcinogenic Risk (TCR) for Broiler Chicken Meat

The target carcinogenic risks (TCRs) of Cr, Ni, Pb, Cd, As, and Cu were determined using food intake, because exposure to these heavy metals can have both carcinogenic and non-carcinogenic effects. Figure 3.66 displays the computed TCRs for Cr, Ni, Pb, Cd, As, and Cu associated with broiler chicken meat consumption. The mean TCR values for Cr, Ni, Pb, Cd, As, and Cu were 2.32 x 10^{-4}, 3.59 x 10^{-4}, 4.26 x 10^{-6}, 3.28 x 10^{-5}, 1.58 x 10^{-4}, and 2.17 x 10^{-3} for adults and 1.29 x 10^{-4}, 2.00 x 10^{-4}, 2.37 x 10^{-6}, 1.83 x 10^{-5}, 8.79 x 10^{-5} and 1.21 x $10^{-3}$for children as a result of eating broiler chicken meat (Table 3.51 and 3.52). TCR values between 1.0 x 10^{-6} and 1.0 x 10^{-4} are regarded as acceptable, those over 1.0 x 10^{-4} as unsuitable, and those below 1.0 x 10^{-6} as insignificant. The TCR values of Cr, Ni, Pb, Cd, and As for adults and children were within the acceptable range, except copper (Cu) as it was unsatisfactory.

Table 3.51 Targeted Cancer Risks (TCR) of heavy metals for adults due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

Targeted Cancer Risks (TCR) for adults						
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**
Basabo	1.83 x 10^{-4}	1.55 x 10^{-4}	2.64 x 10^{-6}	1.43 x 10^{-5}	1.03 x 10^{-4}	1.59 x 10^{-3}
Karwanbazar	2.75 x 10^{-4}	6.98 x 10^{-4}	2.93 x 10^{-6}	BDL	2.23 x 10^{-4}	2.04 x 10^{-3}
Tongibazar	2.26 x 10^{-4}	3.95 x 10^{-4}	3.87 x 10^{-6}	BDL	1.10 x 10^{-4}	2.21 x 10^{-3}
Kaptanbazar	2.60 x 10^{-4}	1.60 x 10^{-4}	6.75 x 10^{-6}	1.85 x 10^{-5}	2.99 x 10^{-4}	2.90 x 10^{-3}
Moulovibazar	1.98 x 10^{-4}	2.12 x 10^{-4}	3.98 x 10^{-6}	BDL	1.65 x 10^{-5}	2.20 x 10^{-3}
Mohammadpur	2.48 x 10^{-4}	2.18 x 10^{-4}	6.07 x 10^{-6}	BDL	1.14 x 10^{-4}	2.07 x 10^{-3}
Mean	2.32 x 10^{-4}	3.59 x 10^{-4}	4.26 x 10^{-6}	3.28 x 10^{-5}	1.58 x 10^{-4}	2.17 x 10^{-3}

Table 3.52 Targeted Cancer Risks (TCR) of heavy metals for children due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

Targeted Cancer Risks (TCR) for children						
Sample ID	**Cr**	**Ni**	**Pb**	**Cd**	**As**	**Cu**
Basabo	1.02×10^{-4}	8.63×10^{-5}	1.47×10^{-6}	7.99×10^{-6}	5.71×10^{-5}	8.88×10^{-4}
Karwanbazar	1.53×10^{-4}	3.89×10^{-4}	1.31×10^{-6}	BDL	1.24×10^{-4}	1.14×10^{-3}
Tongibazar	1.26×10^{-4}	3.95×10^{-4}	2.15×10^{-6}	BDL	6.13×10^{-5}	1.23×10^{-3}
Kaptanbazar	1.45×10^{-4}	8.92×10^{-5}	3.76×10^{-6}	1.03×10^{-5}	1.67×10^{-4}	1.62×10^{-3}
Moulovibazar	1.10×10^{-4}	1.18×10^{-4}	2.22×10^{-6}	BDL	9.17×10^{-6}	1.22×10^{-3}
Mohammadpur	1.38×10^{-4}	1.21×10^{-4}	3.38×10^{-6}	BDL	6.36×10^{-5}	1.15×10^{-3}
Mean	1.29×10^{-4}	2.00×10^{-4}	2.37×10^{-6}	1.83×10^{-5}	8.79×10^{-5}	1.21×10^{-3}

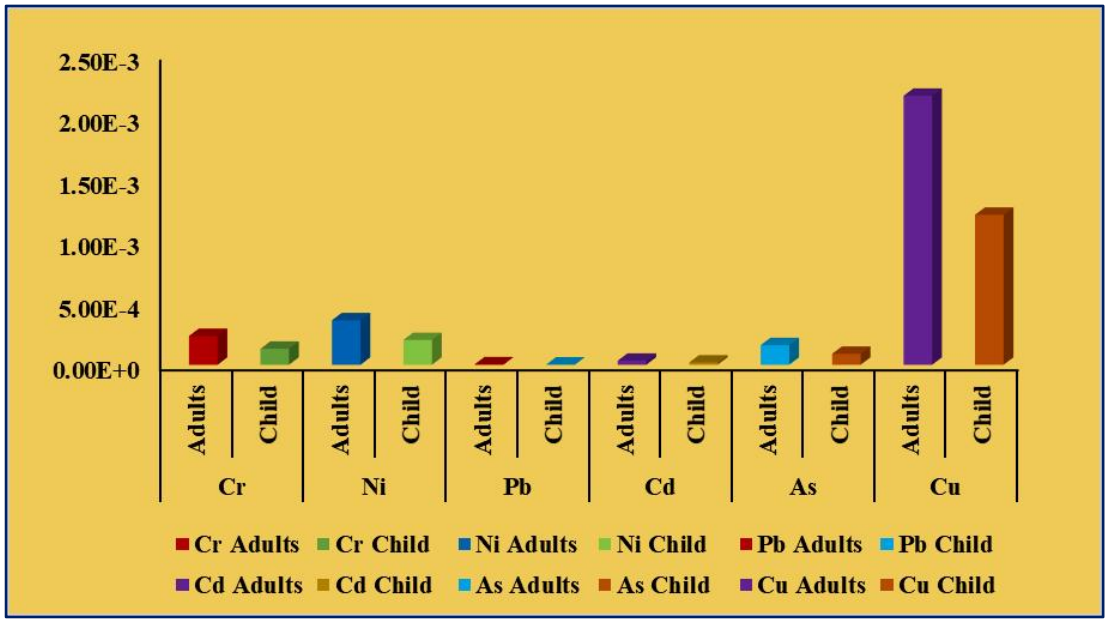


Figure 3.66 Targeted Cancer Risks (TCR) of heavy metals for adults and children due to consuming broiler chicken meat from Dhaka North and South City of Bangladesh

3.10.9 Multivariate Statistical Evaluation for Heavy Metals

A potential tool for evaluating the degree of linear relationship between variable pairs is the Pearson correlation coefficient, which computes a summary index [317]. Table 3.48 presents the evaluation and presentation of the metal to metal correlation data in terms of Pearson product moment correlation coefficients that were significant at the 99% and 95% confidence levels. At a 99% confidence level, the matching pairings of Cr-Ni (0.850), Pd-Cd (0.979), Cr-As (0.799), Ni-As (0.995), Pd-Cu (0.973), Cd-Cu (0.942), Pd-Co (0.966), Cd-Co (0.927), Cu-Co (0.999), Pd-Mn (0.813), Cd-Mn (0.922), Co-Mn (0.928), Pd-Zn (898), Cd-Zn (0.898), Cu-Zn (0.965), Co-Zn (0.963), Mn-Zn (0.978), Cr-Se (0.773), Ni-Se (0.931), As-Se (0.913), Mn-Se (0.676) and Zn-Se (0.593) displayed strong and significant correlations. At the 95% confidence level, the pairs of Ni-Mn (0.398), As-Mn (0.401), Cu-Se (0.364) and Co-Se (0.368) had a weak correlation. The suggestion that the metals might have a comparable source was substantiated by the strong connections (Table 3.53).

Table 3.53 Pearson correlation for heavy metals in four matrices (beef meat and liver; broiler chicken meat and liver)

	Cr	**Ni**	**Pb**	**Cd**	**As**	**Cu**	**Co**	**Mn**	**Zn**	**Se**
Cr	1									
Ni	0.850**	1								
Pb	-0.316	-0.180	1							
Cd	-0.164	-0.120	0.979**	1						
As	0.799**	0.995**	-0.193	-0.152	1					
Cu	-0.204	0.020	0.973**	0.942**	0.017	1				
Co	-0.214	0.030	0.966**	0.927**	0.032	0.999**	1			
Mn	0.099	0.398*	0.813**	0.792**	0.401*	0.922**	0.928**	1		
Zn	0.056	0.271	0.898**	0.898**	0.259	0.965**	0.963**	0.978**	1	
Se	0.773**	0.931**	0.183	0.252	0.913**	0.364*	0.368*	0.676**	0.593**	1
**Correlation is significant at the 0.01 level (2-tailed)										
*Correlation is significant at the 0.05 level (2-tailed)										

3.11 Analysis of Fatty Acids in Beef and Broiler Chicken Meat and Liver Samples

3.11.1 Evaluation of Fatty Acid Profile in Beef and Chicken Meat and Liver Samples

A 20-component FAME Mix standard was used to validate the fatty acids (FAs) determination method. All FA components were identified using chromatograms and retention times (RT) in standard mix standards (Figure 3.67).The FAs were quantitatively analyzed, and calibration curves ranging from 7.81 mg/L to 250 mg/L were generated (Figure 3.50). Six different concentrations were used for the calibration curve. For each FA component, Table 3.54 displayed the retention times, linear equations, correlation coefficients, LOD, and LOQ. The detector's exceptional response was confirmed by correlation coefficients greater than 99%.

Table 3.54 FAME retention time (RT), LOD and LOQ values and linear parameters for the FA calibration curves, of fatty acids

Elution No.	Fatty Acid Components	RT (Mean)	r^2	Calibration Curve Equation	LOD (µg/mL)	LOQ (µg/mL)
1	C14:0	6.461	0.9998	y = 1294.2x - 629.63	0.0145	0.0435
2	C14:1	6.923	0.9994	y = 205.11x - 243.15	0.1632	0.4896
3	C16:0	9.166	0.9999	y = 4221.1x - 7025.1	0.0740	0.2223
4	C16:1	9.548	0.9999	y = 1257.3x - 1367.7	0.0200	0.0597
5	C18:0	12.598	0.9999	y = 2158.9x - 3017.2	0.0912	0.2736
6	C18:1	13.021	0.9999	y = 3684.8x - 6576	0.0198	0.0594
7	C18:1	13.173	0.9999	y = 1004x - 408.4	0.0956	0.2868
8	C18:2	14.084	1.000	y = 512.64x - 319.1	0.1893	0.5679
9	C18:3	15.830	0.9999	y = 491.2x - 209.22	0.1968	0.5904
10	C20:0	18.306	0.9998	y = 251.82x - 129.41	0.4452	1.3356
11	C20:1	19.018	0.9999	y = 2435.5x - 4074	0.0265	0.0795
12	C20:2	20.427	0.9994	y = 254.27x - 922.88	0.2681	0.8043
13	C20:4	22.383	1.000	y = 792.03x - 742.78	0.1898	0.5694
14	C20:3	22.872	0.9999	y = 251.82x - 347.78	0.4379	1.3137
15	C20:5	24.080	0.9999	y = 2831.5x - 4084.1	0.0299	0.0897
16	C22:0	24.650	0.9995	y = 268.78x - 68.337	0.3542	1.0626
17	C22:1	25.054	0.9999	y = 819.48x - 990.82	0.0996	0.2988
18	C24:0	29.005	0.9990	y = 223.6x - 598.72	0.6183	1.8549
19	C22:6	29.216	0.9994	y = 3350.3x - 8665.5	0.0541	0.1624
20	C24:1	29.525	0.9997	y = 275.05x - 478.6	0.3756	1.1268

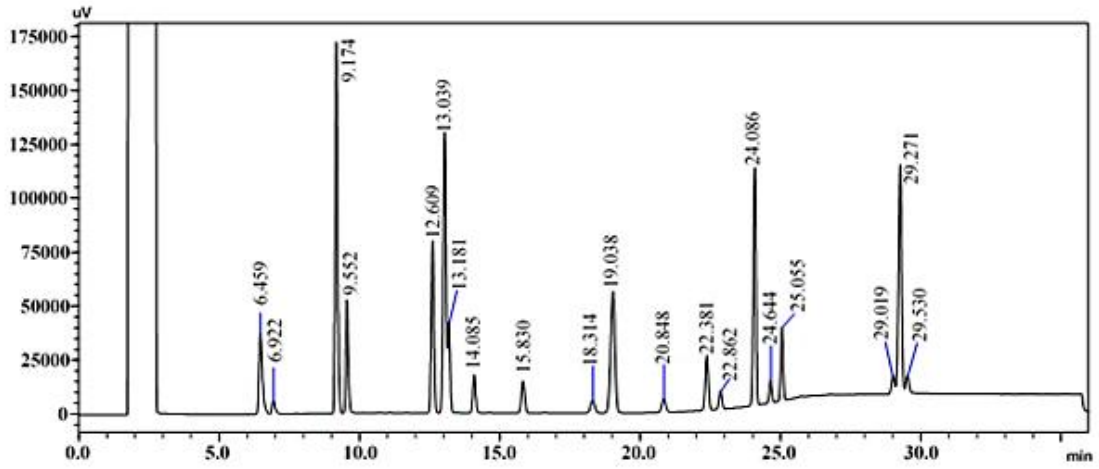


Figure 3.67 Chromatogram of standard methyl esters of twenty fatty acids

The FAME Mix standard was used to assess the accuracy and precision of the method. The repeatability of the standard was no higher than 0.5%, according to the computed RSD% for the retention times. The accuracy of the retention times was no higher than 0.3% when we computed the peak areas that were not higher than 1.0% under the identical circumstances. The sensitivity of the method was demonstrated by the LOD values, which ranged from 0.01 to 0.62 mg/L, and the LOQ values, which varied from 0.04 to 1.85 mg/L. Based on the above information, this technique may be applied to identify FAs in beef and chicken meat in accordance with The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) recommendations.

These fatty acids are divided into three categories: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated (PUFAs). The corresponding formula for calculating these are as follows:

$$\text{SFAs} = \text{C14:0} + \text{C16:0} + \text{C18:0} + \text{C20:0} + \text{C22:0} + \text{C24:0} \ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{MUFAs} = \text{C14:1} + \text{C16:1} + \text{C18:1} + \text{C18:1} + \text{C20:1} + \text{C22:1} + \text{C24:1} \ldots (2)$$

$$\text{PUFAs} = \text{C18:2} + \text{C18:3} + \text{C20:2} + \text{C20:4} + \text{C20:3} + \text{C20:5} + \text{C22:6} \ldots (3)$$

In present research, the obtained highest amount of myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, vaccenic, linoleic, α-linolenic, arachidic, 11-ecosenoic,

11-14 eicosadienoic, arachidonic, eicosatrienoic, behenic, erucic, lignoceric, docosahexaenoic and nervonic acids were found 1.49 (BM), 0.20 (CM), 33.97 (CM), 7.40 (CM), 16.35 (CL), 37.31 (CM), 31.95 (BM), 70.58 (CL), 12.36 (CL), 0.23 (BL), 0.66 (CM), 0.82 (BL), 7.31 (BL), 9.93 (CL), 0.60 (BL), 0.28 (CM), 0.45 (BL), 1.06 (CL) 2.47 (CL) and 1.95% (CL) respectively with acceptable SD%. The total fatty acid (TFAs) were 114.27, 109.21, 150.63, and 198.22% in beef meat, beef liver, broiler chicken meat, and liver, respectively.

The amounts of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were measured in different meats. In beef meat, the percentages were found to be 31.00% SFAs, 60.19% MUFA, and 23.08% PUFA. For beef liver (150 mg), the composition was 23.79% SFAs, 58.51% MUFA, and 26.91% PUFA.

In chicken meat, the percentages were different, with 43.32% SFAs, 73.62% MUFA, and 61.23% PUFA. Chicken liver showed even more variation, having 46.42% SFAs, 54.98% MUFA, and an impressive 98.69% PUFA. The total unsaturated fatty acids (UFSAs) were 83.27, 85.42, 134.85 and 153.67% in beef meat, beef liver, broiler chicken meat and liver, respectively.

3.11.2 Omega-6/Omega-3 Ratio in beef and chicken

The omega-6/omega-3 ratio is used as an indicator of the nutritional value of beef and chicken. The ratio of omega-6 to omega-3 fatty acids in beef is typically higher than the 1:1 ratio recommended for optimal health. The ratio of store-bought grain-fed beef is usually between 15:1 and 20:1. In contrast, the ratio of grass-fed beef is closer to 3:1, which is within the 4:1 range of a nutritious diet. In the present study, the highest omega-6/omega-3 was 8.90 in broiler chicken meat. The omega-6/omega-3 ratios in beef meat, beef liver, and broiler chicken liver were 1.30, 1.80 and 2.93 (Table 3.55).

Table 3.55 Fatty acid composition and main specific ratios of the four matrices

Fatty Acid		FA Content, %, Mean ± SD			
		Beef Meat	Beef Liver	Broiler Chicken Meat	Broiler Chicken Liver
C14:0	Myristic	1.49 ± 0.30	0.59 ± 0.54	0.83 ± 0.19	0.32 ± 0.18
C14:1	Myristoleic	0.11 ± 0.09	0.07 ± 0.06	0.20 ± 0.08	0.13 ± 0.05
C16:0	Palmitic	25.79 ± 6.09	17.03 ± 4.49	33.97 ± 8.58	28.35 ± 3.07
C16:1	Palmitoleic	3.62 ± 0.72	1.48 ± 0.74	7.40 ± 2.15	1.16 ± 0.69
C18:0	Stearic	3.50 ± 1.09	5.50 ± 3.19	7.78 ± 3.44	16.35 ± 3.18
C18:1	Oleic	24.08 ± 4.76	36.50 ± 8.41	37.31 ± 14.19	32.11 ± 11.12
C18:1	Vaccenic	31.95 ± 5.24	19.14 ± 5.03	27.91 ± 6.45	19.13 ± 16.23
C18:2	Linoleic	8.22 ± 2.25	9.17 ± 2.26	49.93 ± 1.58	70.58 ± 2.57
C18:3	Linolenic	0.75 ± 0.33	0.53 ± 0.36	1.96 ± 1.17	12.36 ± 2.16
C20:0	Arachidic acid	0.10 ± 0.07	0.23 ± 0.17	0.08 ± 0.03	0.16 ± 0.06
C20:1	11-Eicosenoic	0.21 ± 0.15	0.23 ± 0.19	0.66 ± 0.23	0.35 ± 0.20
C20:2	11-14 Eicosadienoic	0.44 ± 0.11	0.82 ± 0.37	0.81 ± 0.25	0.79 ± 0.49
C20:4	Arachidonic	4.37 ± 0.88	7.31 ± 3.21	4.30 ± 3.00	2.19 ± 0.52
C20:3	Eicosatrienoic	8.22 ± 0.01	7.09 ± 0.03	3.80 ± 0.33	9.93 ± 0.64
C20:5	Eicosapentaenoic	0.56 ± 0.13	0.60 ± 0.32	0.18 ± 0.07	0.37 ± 0.15
C22:0	Behenic	0.12 ± 0.07	0.26 ± 0.12	0.28 ± 0.03	0.17 ± 0.09
C22:1	Erucic	0.04 ± 0.03	0.45 ± 0.09	0.04 ± 0.01	0.14 ± 0.04
C24:0	Lignoceric	0.02 ± 0.01	0.08 ± 0.03	0.38 ± 0.03	1.06 ± 0.12
C22:6	Docosahexaenoic	0.51 ± 0.08	1.40 ± 0.95	0.25 ± 0.12	2.47 ± 0.14
C24:1	Nervonic	0.18 ± 0.03	0.64 ± 0.30	0.09 ± 0.02	1.95 ± 0.15
SFAs		31.00 ± 7.62	23.79 ± 8.54	43.32 ± 12.30	46.42 ± 6.70
USFAs		83.27 ± 14.81	85.42 ± 19.02	134.85 ± 16.75	153.67 ± 15.68
MUFAs		60.19 ± 11.02	58.51 ± 11.52	73.62 ± 9.85	54.98 ± 6.34
PUFAs		23.08 ± 3.79	26.91 ± 7.5	61.23 ± 6.52	98.69 ± 6.67
Omega-6		13.04 ± 3.24	17.29 ± 5.84	55.04 ± 4.83	73.13 ± 3.58
Omega-3		10.04 ± 0.55	9.62 ± 1.66	6.19 ± 1.69	25.13 ± 3.09
PUFA/SFA		0.74 ± 0.50	1.13 ± 0.88	1.41 ± 0.53	2.13 ± 0.99
Omega-6/ Omega-3		1.30 ± 5.89	1.80 ± 3.52	8.90 ± 2.86	2.93 ± 1.16

Chromatogram of solvent blank, analyzed 20 fatty acids in beef meat and liver, broiler chicken meat and liver are presented in Figure 3.68, 3.69, 3.70 3.71 and 3.72 .

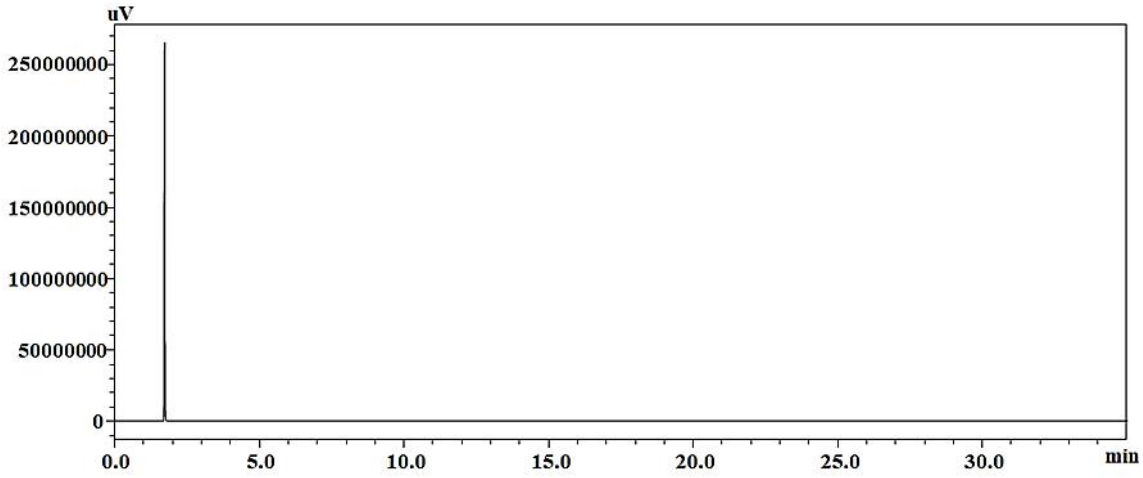


Figure 3.68 Chromatogram of solvent blank for fatty acid analysis

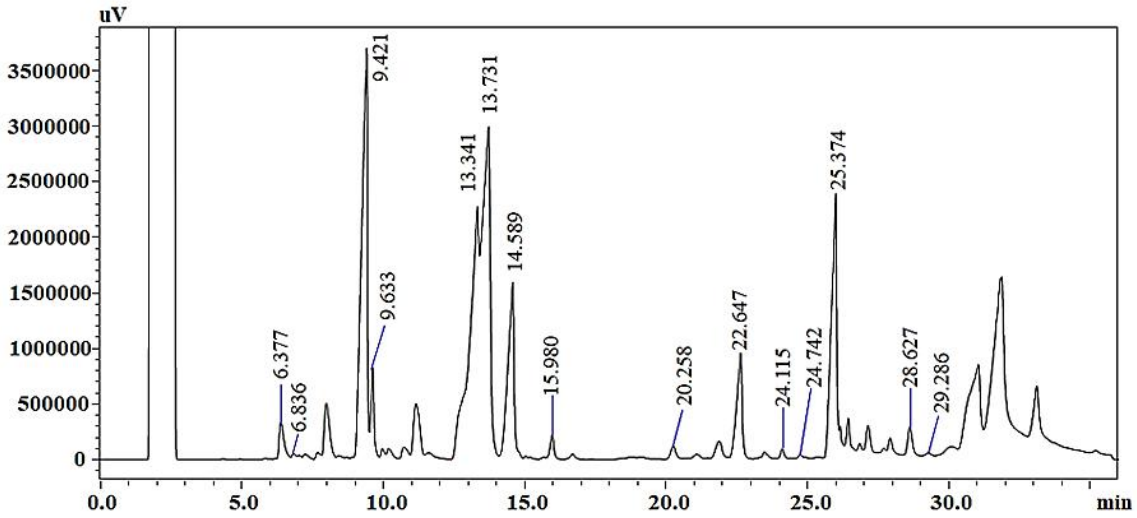


Figure 3.69 Chromatogram of analyzed fatty acids in beef meat

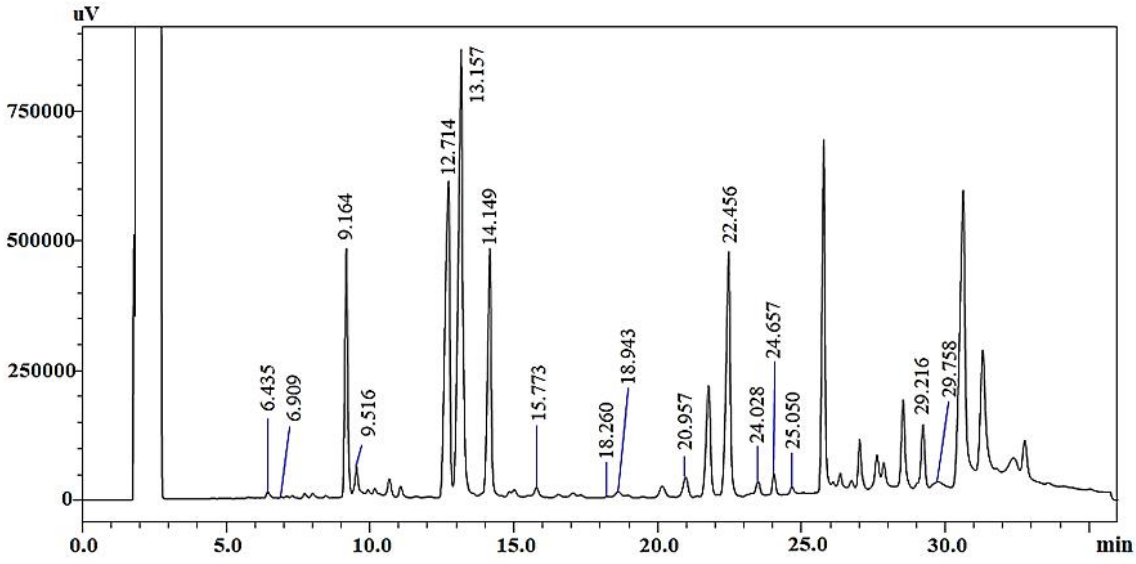


Figure 3.70 Chromatogram of analyzed fatty acids in beef liver

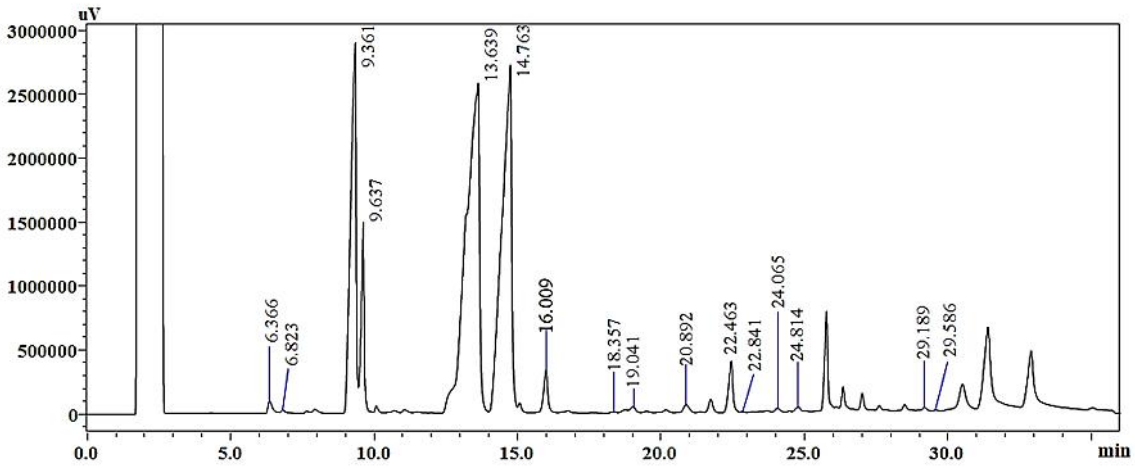


Figure 3.71 Chromatogram of analyzed fatty acids in chicken meat

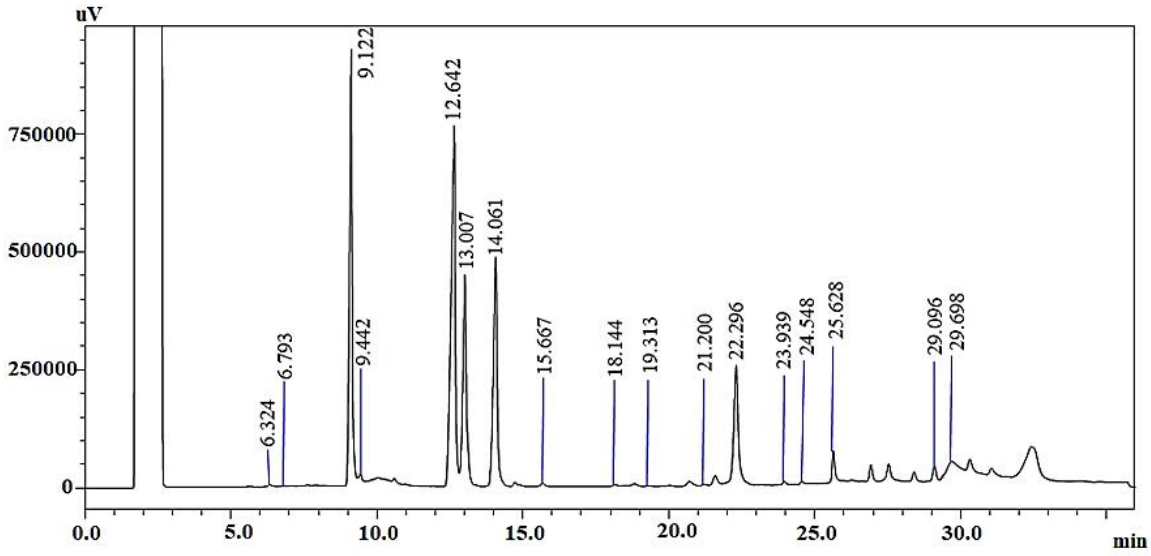


Figure 3.72 Chromatogram of analyzed fatty acids in chicken liver

Graphical presentation of fatty acid profile in beef meat and liver, and broiler chicken meat and liver have been displayed in Figure in 3.73, 3.74, 3.75 and 3.76.

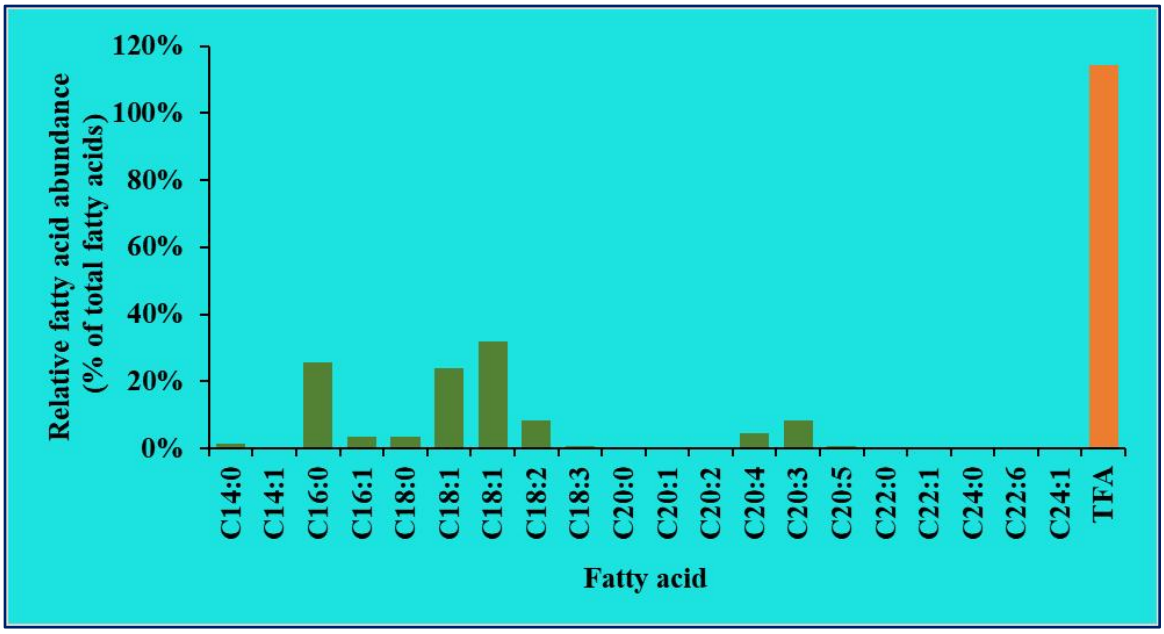


Figure 3.73 Fatty acid profile in beef meat

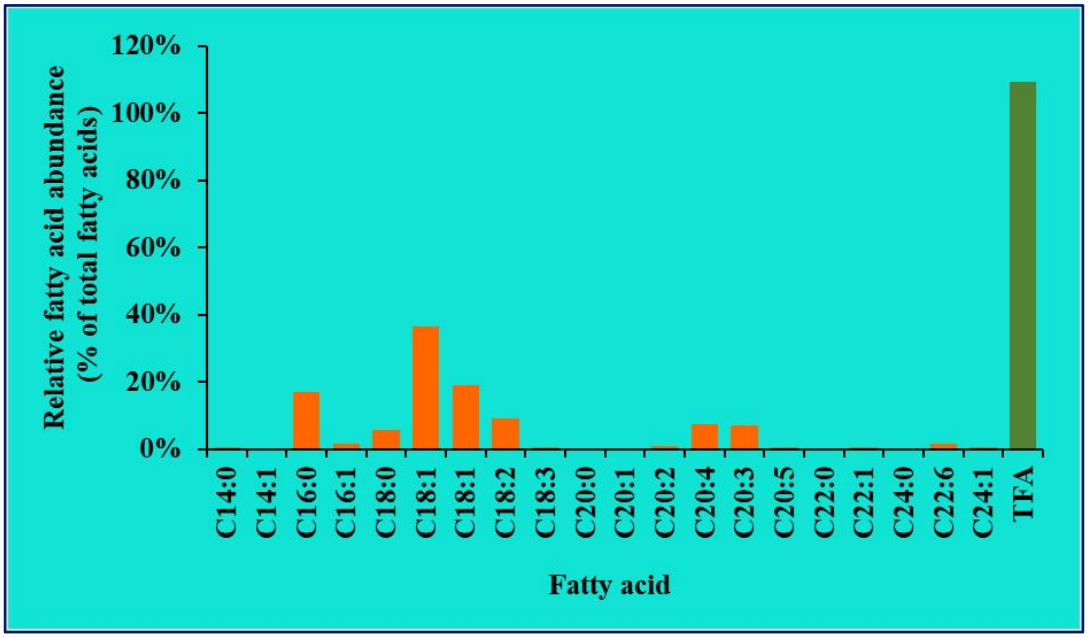


Figure 3.74 Fatty acid profile in beef liver

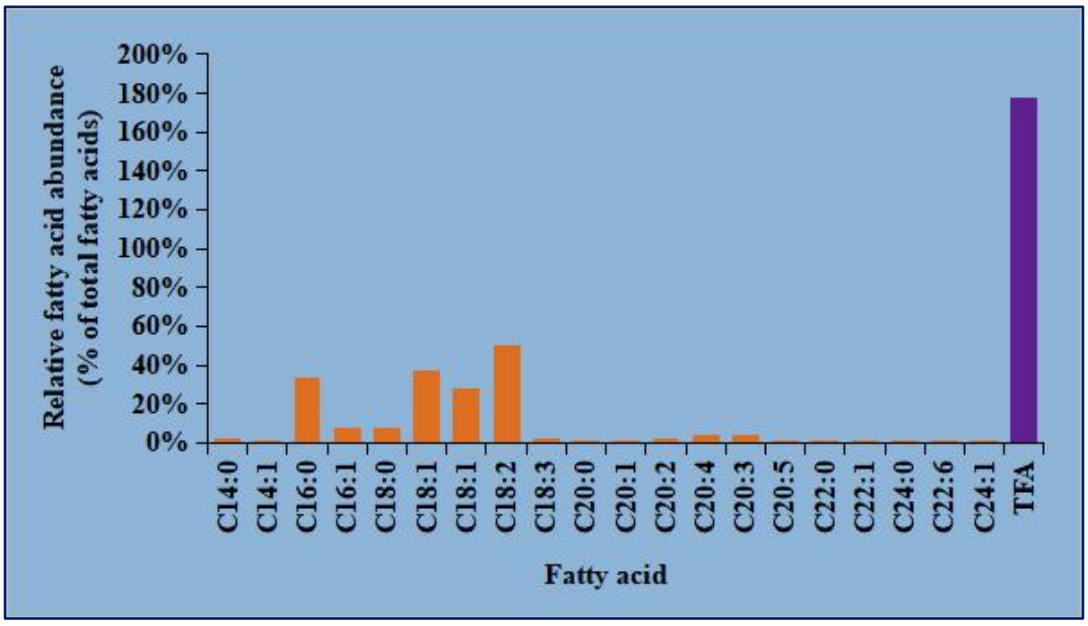


Figure 3.75 Fatty acid profile in chicken meat

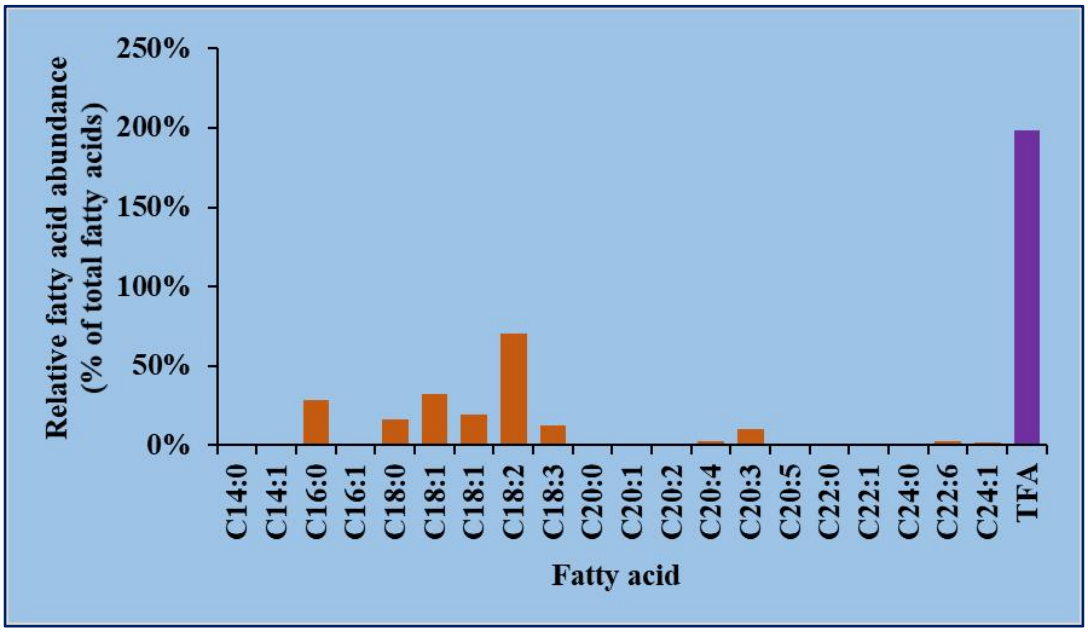


Figure 3.76 Fatty acid profile in chicken liver

3.11.3 Fat Content (%)

The calculated average fat % were 1.57 and 6.19 % in beef meat and liver (Table 3.56 and Figure 3.77).

Table 3.56 Fat% in beef meat and liver

Sample ID	W1 (g)	W2 (g)	Total Fat %	Average
BM1	63.92	63.98	0.6	1.57
BM2	69.93	69.97	0.4	
BM3	63.56	63.96	4	
BM4	63.92	63.99	0.7	
BM5	69.93	69.98	0.5	
BM6	63.56	63.96	4	
BM7	69.92	69.95	0.3	
BM8	63.92	63.97	0.5	
BM9	63.56	63.99	4.3	
BM10	69.92	69.96	0.4	
BL1	63.91	64.31	4	6.19

BL2	69.97	70.46	4.9	
BL3	63.91	64.41	5	
BL4	69.97	71.07	11	
BL5	63.91	64.56	6.5	
BL6	69.97	70.71	7.4	
BL7	63.91	64.39	4.8	
BL8	69.97	70.43	4.6	
BL9	63.91	64.74	8.3	
BL10	69.97	70.51	5.4	

The calculated average fat % were 1.57 and 6.19 % in broiler chicken meat and liver (Table 3.55).

Table 3.57 Fat % in broiler chicken meat and liver

Sample ID	W1 (g)	W2 (g)	Total Fat %	Average
CM1	63.92	64.05	1.3	0.78
CM2	69.93	70.02	0.9	
CM3	63.92	63.94	0.2	
CM4	69.93	70.03	1.0	
CM5	63.92	64.01	0.9	
CM6	69.93	69.98	0.5	
CM7	63.92	63.98	0.6	
CM8	69.93	69.97	0.4	
CM9	63.92	64.01	0.9	
CM10	69.93	70.04	1.1	
CL1	63.92	63.99	0.7	2.95
CL2	63.92	64.17	2.5	
CL3	69.93	70.16	2.3	

CL4	69.93	70.14	2.1	
CL5	63.92	64.13	2.1	
CL6	69.93	70.38	4.5	
CL7	63.92	64.3	3.8	
CL8	69.93	70.25	3.2	
CL9	63.92	64.27	3.5	
CL10	72.94	73.42	4.8	

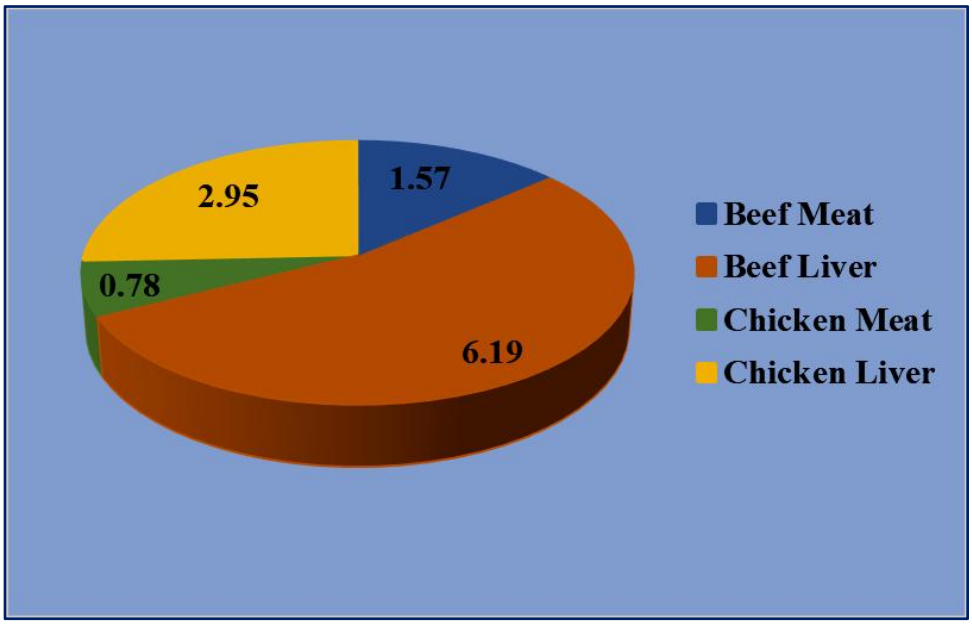


Figure 3.77 Total fat content (%) in beef meat, beef liver, chicken meat and liver

CONCLUSION

In Bangladesh, raw meat has been found to contain chemical contaminants such as heavy metals, antibiotics, and pesticides, which primarily stem from polluted feed, water, and environmental factors. The contamination of the food chain is exacerbated by industrial activities, ineffective waste management, and insufficient monitoring systems. This research aimed to detect and measure these residues utilizing highly sensitive and validated analytical methods, which include High-Performance Liquid Chromatography with Photodiode Array Detection (HPLC-PDA), Gas Chromatography with Electron Capture Detection (GC-ECD), and Flame Ionization Detection (GC-FID), as well as Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All analytical instruments were calibrated correctly, and the methods were verified for their accuracy, precision, sensitivity, and selectivity. Throughout the study, meticulous analytical documentation, such as chromatograms, logbooks, and experimental records, was kept. The analysis of residual antibiotics, organochlorine pesticides, heavy metals, and fatty acid composition was conducted separately using six distinct methods, with each method meeting the established validation criteria. The analysis utilized the approved modified extraction techniques.

Antibiotics are predominantly used in cattle and poultry in Bangladesh for the purposes of enhancing growth, disease prevention, and illness treatment. The commercial farming of chickens and cattle in Bangladesh can contribute to antibiotic resistance in both humans and animals due to the inappropriate and excessive use of these drugs. The growth of the cattle and commercial chicken industries in Bangladesh significantly influences the food value chain. Consequently, it is crucial to examine antibiotic residues to mitigate the risk of antibiotic resistance affecting both animals and humans. A total of 120 samples (including beef meat, beef liver, broiler chicken meat, and broiler chicken liver) were analyzed for antibiotic residues. Oxytetracycline (OTC) residues were found in 26.67% of the samples, with one specific sample (BL18) surpassing the EU Maximum Residue Limit (MRL) of 100 µg/kg, measuring at 368.97 µg/kg. Residues of oxytetracycline, tetracycline, and chlortetracycline were identified in three broiler chicken meat samples (CM18, CM21, and CM25), two samples (CM17 and CM20), and one sample (CM23), respectively, four of which exceeded the Codex Alimentarius MRL, and six surpassed the EU MRL. Amoxicillin was detected in seven beef meat samples (ranging from 4.89 to 9.36 µg/kg) and fifteen beef liver samples (ranging from 10.39 to 89.47 µg/kg), with two liver samples (BL10 and BL14) exceeding the Codex and EU MRL of 50 µg/kg. Patulin, which is both an antibiotic and a mycotoxin, was found in 83.33% of beef meat samples (ranging from 46.26 to 193.91 µg/kg), 20% of beef liver samples (ranging from 43.31 to 166.91 µg/kg), 60% of chicken meat samples (ranging from 16.94 to 310.53 µg/kg), and 36.67% of

chicken liver samples (ranging from 14.75 to 52.88 µg/kg). There are currently no regulatory MRLs established for patulin in meat or liver products. The health risk assessment indicated that the hazard index (HI) for all antibiotics remained below 1, suggesting that current exposure levels do not pose notable risks to human health (Figure 3.78) .

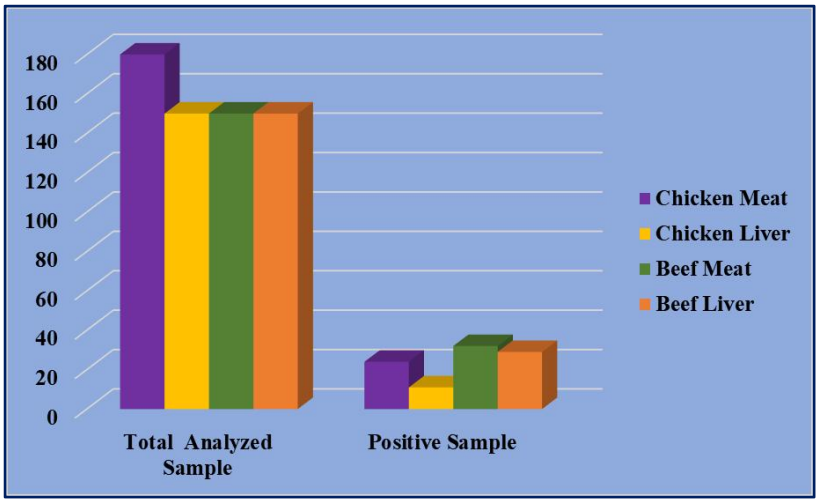


Figure 3.78 Comparison of positive samples with total analyzed samples of four matrices

The use of pesticides in agriculture is on the rise due to the growing global demand for food. However, the presence of pesticide residues in agricultural products has emerged as a significant health issue for consumers, contributing to concerns about food safety. In Bangladesh, there is particular worry regarding residual pesticides, notably organochlorine pesticides, found in raw meat from cattle and poultry. A total of 120 samples were evaluated, consisting of 30 beef meat, 30 beef liver, 30 broiler chicken meat, and 30 broiler chicken liver, using GC-ECD to analyze 20 OCPs. Alpha-BHC residues were detected in one beef meat sample (BM1 = 0.0623 mg/kg), in 28 beef liver samples (ranging from 0.0174 to 0.3404 mg/kg), and in 9 broiler chicken liver samples (ranging from 0.0066 to 0.1591 mg/kg), all of which exceeded the MRL established by FAO/WHO. Eleven samples of chicken liver (with values from 0.0058 to 0.1187 mg/kg), 28 beef meat samples (ranging from 0.0845 to 0.3291 mg/kg), and 28 broiler chicken samples (from 0.0059 to 0.2017 mg/kg) exhibited delta-BHC levels that surpassed the MRL. Two beef liver samples (BL18 = 0.0155 mg/kg and BL29 = 0.0428 mg/kg) and nine chicken liver samples (with values from 0.0117 to 0.0700 mg/kg) contained beta-BHC residues, while one beef liver sample (BL29 = 0.0158 mg/kg) had gamma-BHC level that exceeded the MRL. Endosulfan II was detected in one sample of chicken meat (CM18 = 0.0389 mg/kg), while

endosulfan sulfate was found in another chicken meat sample (CM13 = 0.4800 mg/kg), and hexachlor epoxide was noted in three beef meat samples (BM19 = 0.3043, BM = 0.2757, and BM29 = 0.2648 mg/kg) that were above the MRL (Figure 3.79).

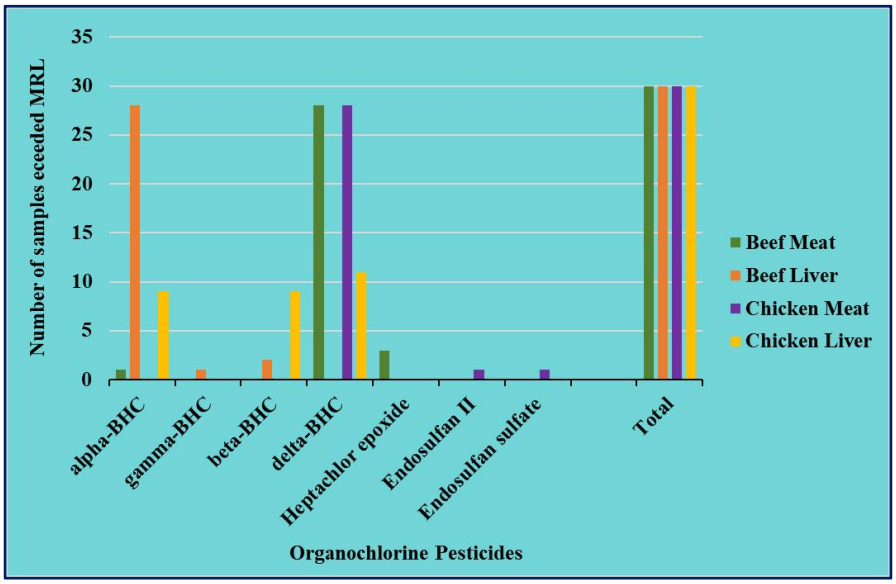


Figure 3.79 Number of samples exceeded MRL of OCPs

The mean concentrations of total analyzed OCPs were 471.32 µg/kg in beef meat, 317.75 µg/kg in beef liver, 410.55 µg/kg in chicken meat, and 628.08 µg/kg in chicken liver (Figure 3.80).

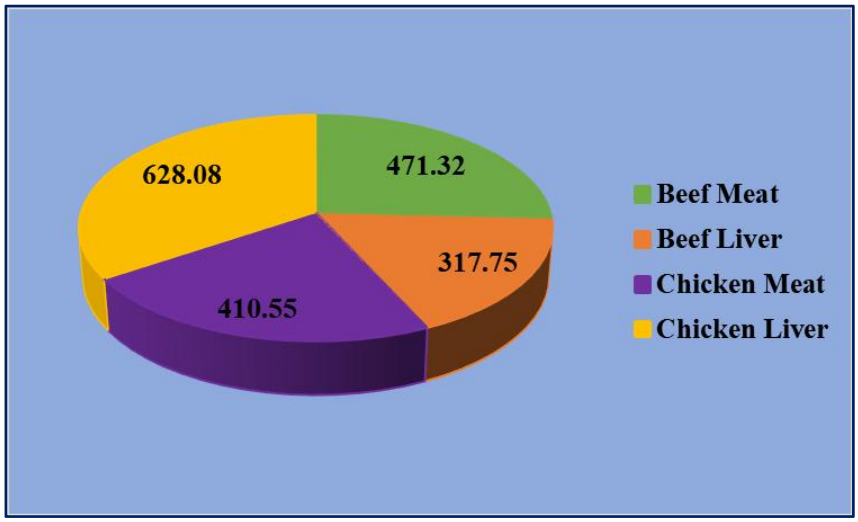


Figure 3.80 Mean concentrations of analyzed OCPs in four matrices

Health risk evaluation was conducted for 20 OCPs associated with the consumption of beef and chicken. The assessment indicated that the hazard index (HI) values for δ-BHC, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, and endrin ketone surpassed 1 for both adults and children who consume beef, suggesting possible health risks. The health risk assessment further revealed that both adults and children consuming broiler chicken meat had HI values for delta-BHC, heptachlor, aldrin, heptachlor epoxide, trans-Chlordane, cis-chlordane, dieldrin, endrin, and endrin aldehyde greater than 1, indicating considerable health threats.

Heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), and copper (Cu) have been detected in raw meat in Bangladesh and high levels of these metals in food may elevate the likelihood of liver or kidney disease and cancer, particularly when consumed in large amounts. It is recommended that monitoring and control programs be implemented to ensure food safety. A total of ten heavy metals (Cr, Ni, Pb, Cd, As, Cu, Co, Mn, Zn, Se) were examined in 120 samples (30 beef meat, 30 beef liver, 30 broiler chicken meat, and 30 broiler chicken liver). The mean concentrations of heavy metals were 0.37, 178.11, 38.06 and 106.35 in beef meat, beef liver, chicken meat and liver, respectively (Figure 3.81).

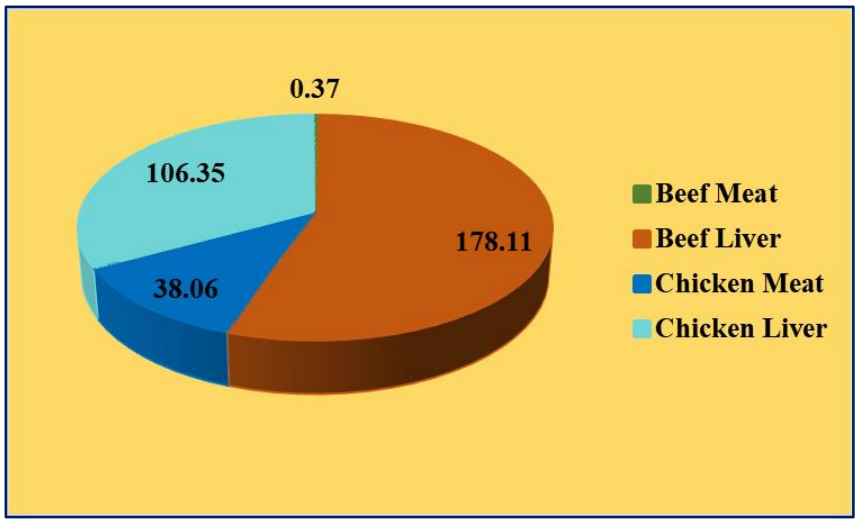


Figure 3.81 Mean concentrations of heavy metals in four matrices

In the analysis of 30 beef meat samples, all detected metals were identified and quantified, remaining within the maximum limits defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Bangladesh Food Safety Authority (BFSA). Out of 30 beef liver samples, lead (Pb) was found in 26 samples, exceeding the maximum limit set

by JECFA, and in 28 samples above the limit established by BFSA. Three samples exhibited cadmium levels surpassing the maximum limit defined by JECFA, while ten samples had cadmium exceeding the limit set by BFSA. Additionally, it was observed that 27 samples were above the maximum limit established by BFSA, and 10 samples exceeded the maximum limit set by JECFA. In the evaluation of 30 broiler chicken meat samples, chromium (Cr) was detected in 4 samples above the levels recommended by the FAO/WHO; lead (Pb) was identified in 21 and 26 samples; cadmium (Cd) in 2 and 2 samples; arsenic (As) in 12 and 17 samples, above the maximum limit set by JECFA and BFSA, respectively. Copper (Cu) was found in 29 samples, exceeding the maximum limit established by FAO/WHO. Among the 30 broiler chicken liver samples, lead (Pb) was detected in 18 and 22 samples, and arsenic (As) was found in 13 and 22 samples, above the maximum limits defined by JECFA and BFSA, respectively.

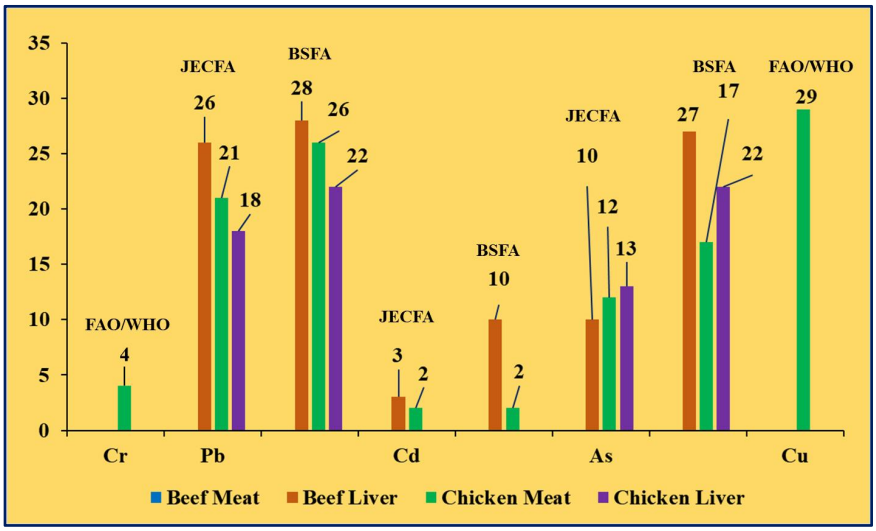


Figure 3.82 Positive samples exceeded ML by JECFA and BFSA

Health risk assessment indicated that the total hazard index (HI) for adults consuming beef was below 1, suggesting minimal risk. However, the HI for children (3.665) consuming beef, and both adults and children consuming broiler chicken meat exceeded 1, indicating potential non-carcinogenic health risks. Targeted cancer risks (TCR) was evaluated for the heavy metals due to consuming beef and broiler chicken meat. The TCR value for Cu was within the acceptable range (1×10^{-4} to 1×10^{-6}), but it was insignificant for Cr, Ni, Pb, Cd and As for consuming beef meat. TCR value of Cr, Ni, Pb, Cd and As were within the acceptable range, but in case of Cu it was unsatisfactory and may pose a cancer risk.

Forty samples (beef meat and liver, chicken meat and liver) were analyzed for fatty acid profiles. Total fat contents were recorded 1.57, 6.19, 0.78 and 2.95% in beef meat, beef liver, broiler chicken meat and liver samples, respectively. Mean concentrations of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) were within normal biological ranges. The sum of total analyzed fatty acids was 114.29, 109.21, 177.36 and 200.09 (composition%) in beef meat, beef liver, broiler chicken meat and liver, respectively (Figure 3.83).

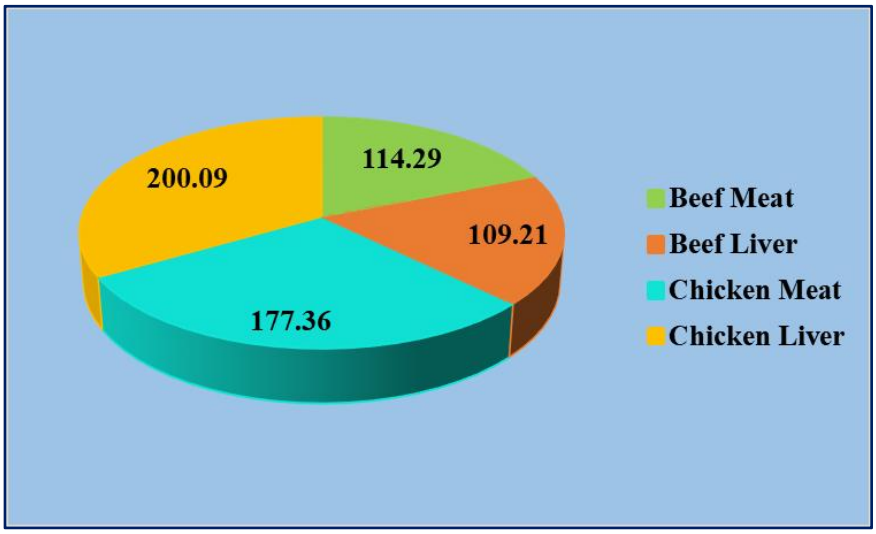


Figure 3.83 Total analyzed fatty acids content (%) in four matrices

The results suggest that the accumulation of chemical contaminants can influence fatty acid profiles, underscoring the importance of monitoring both contaminant levels and nutritional quality parameters.

The findings emphasize the urgent need for establishing national MRLs for chemical contaminants in meat and liver by the Bangladesh Food Safety Authority (BFSA), regular monitoring programs for antibiotic, pesticide, and heavy metal residues in food products, regulatory enforcement on the prudent use of veterinary drugs and agricultural pesticides, improved waste management and feed quality control to prevent contamination at the source and public awareness campaigns promoting food safety and responsible antibiotic use in animal farming. This study provides critical baseline data to guide policymakers, researchers, and stakeholders in strengthening Bangladesh's food safety framework. Implementing effective surveillance, monitoring, and control measures will be essential to ensure safe and sustainable meat production, safeguard public health, and align national practices with global food safety standards.

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PUBLICATIONS

Publications during PhD Research

1. **Nargis Parvin**, Mohammad Shoeb, Nilufar Nahar, Rafiza Islam (2022). Investigation of tetracycline residues in poultry meat samples from Dhaka city by high-performance liquid chromatography, Current Research on Biosciences and Biotechnology 3 (2), 227-232. doi: 10.5614/crbb.2022.3.2/P89JJ95N

2. Tasnim Ahmed, Rafiza Islam, **Nargis Parvin**, Mohammad Shoeb (2022). Determination of age-dependent endogenous indole-3 acetic acid (IAA) level in different organs of tomato plants, Current Research on Biosciences and Biotechnology 3 (2), 202-207. doi: 10.5614/crbb.2022.3.2/WFP0HMM1

3. Mohammad Shoeb, Farhana Sharmin, Md. Nazrul Islam, Lutfun Nahar, Rafiza Islam and **Nargis Parvin** (2022). Assessment of Physico-Chemical Parameters of Water Samples Collected from the Southern Part of Bangladesh, Dhaka University Journal of Sciences, 70(1), 49-57. doi: 10.3329/dujs.v70i1.60381

4. Mohammad Shoeb, Murshid Hossain, M. Mazharul Islam, M. Shahed Reza, Nilufar Nahar, and **Nargis Parvin** (2022). Determination of some preservatives and sweeteners in fruit drinks by high-performance liquid chromatography, Journal of the Bangladesh Chemical Society 33(2):83-86.

5. Mohammad Shoeb, Md. Farhanul Alam, Md. Mazharul Islam, **Nargis Parvin**, Md. Nazrul Islam and Md. Iqbal Rouf Mamun (2021). Assessment of Quality of Azithromycin, a Macrolide Antibiotic by NMR Spectroscopy, Bangladesh Pharmaceutical Journal 24(1), 37-44. doi: 10.3329/bpj.v24i1.51634

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Book Chapter

1. Shoeb, M., Islam, R., & **Parvin, N**. (2023). Antibiotic Resistance: A Global Threat to Humanity. In Y. Koumpouros, A. Georgoulas, & G. Kremmyda (Eds.), *Transcending Humanitarian Engineering Strategies for Sustainable Futures* (pp. 82-105). IGI Global. https://doi.org/10.4018/978-1-6684-5619-4.ch005

List of Manuscript out of PhD Research

1. **Nargis Parvin**, Mohammad Shoeb, Abida Sultana and M. Shahed Reza. Detection and Quantitation of Tetracyclines in Beef and Broiler Chicken meat and liver using HPLC-PDA.

2. **Nargis Parvin**, Mohammad Shoeb and Abida Sultana. Analysis of Residual Amoxicillin in Beef and Broiler Chicken meat and liver using High Performance Liquid Chromatography with PDA Detector.

3. **Nargis Parvin**, Mohammad Shoeb, M. Shahed Reza and Abida Sultana. Detection and Quantification of Patulin, a mycotoxin in Beef and Broiler Chicken meat and liver using High Performance Liquid Chromatography with PDA Detector.

4. **Nargis Parvin**, Mohammad Shoeb and Abida Sultana. Investigation and Health Risk Assessment of Organochlorine Pesticides in Beef and Broiler Chicken meat and liver by Gas Chromatography equipped with ECD.

5. **Nargis Parvin**, Mohammad Shoeb, Mohammad Moniruzzaman, Badan Saha and Abida Sultana. Health Risk Assessment of Heavy Metals in Beef and Broiler Chicken meat and liver using ICP-MS.

6. **Nargis Parvin**, Mohammad Shoeb and Abida Sultana. Screneening of Fatty Acid Profile and Total Fat Content in Beef and Broiler Chicken meat and liver by GC-FID .

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Investigation of tetracycline residues in poultry meat samples from Dhaka city by high-performance liquid chromatography

Nargis Parvin, Mohammad Shoeb*, Nilufar Nahar, Rafiza Islam

Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

ABSTRACT

The objective of the study was to quantify tetracycline (TCs) i.e., oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC) residues in thirty poultry meat samples (n = 30) collected from the local market and super shop around the Dhaka University campus during May 2019 to January 2020. Three samples were collected from each of ten locations. All samples were extracted by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. Samples were analyzed by reversed-phase High-Performance Liquid Chromatograph equipped with Photo Diode Array Detector (LC-PDA) and a reported method was validated with good linear correlation coefficients of standards and matrix-matched calibration curves with r^2=1.00, 0.99, 0.99 and r^2= 0.99, 0.99,0.99 in the linearity range of 0-10 µg/kg for OTC, TC and CTC, respectively. The limit of detection (LOD) and limit of quantification (LOQ) for OTC, TC, and CTC were 1.05, 1.17, and 1.09 µg/kg and 3.15, 3.51 and 3.27 µg/kg, respectively. Accuracy that is expressed by the recovery percentages were calculated at two different concentrations (2.5 and 5 µg/kg) were 91 and 100%, 102 and 100%, and 106 and 100% for OTC, TC, and CTC, respectively. Intra-day (n=3) and inter-day (n=9, 3 days) precision data were under 10% for all sample matrixes. Standard deviations were calculated ±0.06, ±0.11 and ±0.03 and precision (expressed by RSD%) were found 5.57, 9.14 and 2.35%, respectively for OTC, TC and CTC. The HPLC-PDA method is affordable for screening of large number meat samples for residual antibiotics in biological matrices by any laboratories. The method is also cheaper in comparison with LC-MSMS. Analysis of real 30 poultry meat samples showed that the tetracyclines residues were below the quantification limit in all samples.

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*Corresponding authors:
shoeb71@yahoo.com

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1. Introduction

Antibiotics are chemical substances derived from a natural, semi-synthetic or synthetic way that affect antibacterial activity by killing or inhibiting the growth of bacterial pathogens (Nita, 2007). Antibiotics are classified according to the mechanism of action (inhibitors of protein synthesis, membrane function, anti-metabolites, cell wall synthesis, and nucleic acid synthesis), on basis of a range of effectiveness (bactericidal or bacteriostatic), range of working efficiency (narrow or broad spectrum) and according to chemical structure (Darwish et al., 2013). They are administered parenterally or intravenously, topically, and orally (Geidam et al., 2009). Oxytetracycline (OTC), tetracycline (TC), and chlortetracycline (CTC) are the most widely used chemicals in the group of broad-spectrum tetracycline antibiotics and chlortetracycline was the first tetracycline antibiotic to be discovered in 1948 (Nelson et al., 2011). The basic structure of a tetracycline consists of a hydro-naphthalene backbone containing four fused rings (Ng et al., 2003) (Fig. 1, Table 1).

Tetracyclines are given to animals for the prevention and treatment of particular diseases as well as enhanced growth and imposed for human consumption. Animals grow faster and healthier due to the administration of these antibiotics. Acquisition of resistance by microorganisms can occur because of the long-term administration of antimicrobial substances and the presence of residual antibiotics in the edible tissues of the animals, as well as in the environment i.e., soil and water. Therefore, the controlled use of antibiotics in veterinary medicine is an important matter in protecting the health of animals and consumers (Macarov et al., 2012; Chopra et al., 2001). But illegal, inappropriate, insensible, and uncontrolled usage of antibiotics in poultries causes residues in food of animal origin. Resistant bacteria populations increase and accumulate in various organs and tissues, particularly in the liver and kidneys as a result of insensible and unrestrained utilization of antibiotics (Doyle, 2006). Accordingly, if the withdrawal period is not successful, animals exposed to chemical substances may leave residues in their carcasses at the time of slaughter (Vragović et al., 2011). Nowadays, the existence of antibiotic residues in foods of animal origin has been a serious concern in the trading world. Antibiotics used in food animals can cause health hazards due to their secretion in edible animal tissues in trace amounts (Menkem et al., 2018). The presence of antibiotics may cause several adverse health effects to humans, like tissue damage, gastrointestinal disturbance, neurological disorders, and hypersensitivity (Ramatla et al., 2017). Bangladesh Food Safety Authority (BFSA, 2013) has set the maximum residue limit (MRL) values as 200, 600, 1200 µg/kg for tetracyclines in muscles, liver

and kidney of poultry. The maximum residue level of TC residues in animal products set by *Codex Alimentarius Commission* to be 200 µg/kg in muscle, 600 µg/kg in the liver, 1200 µg/kg in fat and kidney (FAO/WHO Expert Committee on Food Additives, 2004) while European Union regulated as 100, 300 and 600 µg/kg, respectively in these organs to protect human health in muscle for poultry meat (Council Regulation (EEC) No. 2377/90/EC, 1990). Several analytical techniques including capillary electrophoresis (CE) (Nozal et al., 2004; Hernandez et al., 2000; Hernandez et al., 2002), high-performance liquid chromatography (HPLC) (Biswas et al., 2007; Blanchflower et al., 1997; Charlet et al., 2003; Zhou et al., 2009; Koesukwiwat et al., 2007; Tong et al., 2009; Cinquina et al., 2003; Tavakoli et al., 2003) with UV, PDA and fluorescence detection (Patyra et al., 2014; Esponda et al., 2009; Schneider et al., 2007; Lu et al., 2004; Grandos et al., 2005), and liquid chromatography-electrospray tandem mass spectrometry (Mou et al., 2021; Jia et al., 2009; Lykkeberg et al., 2004; Guoa et al., 2016; Elkhabeer et al., 2020) are available for determination of residual TCs in different biological matrices. Bahmani et al. (2020) reported the presence of OTC and TC residues in four poultry meat samples ranging 67.5-425.3 and 91.2-252.3 µg/kg and Ibrahim et al. (2015) detected OTC residues in three tested poultry meat samples ranging 150-500 µg/kg using HPLC-UV method. As food safety is a burning issue in Bangladesh, the determination and quantification of tetracycline in edible tissues of poultry will help BFSA to ensure safe food for the public health. The present study describes a reported method for the validation, qualitative and quantitative determination of three tetracyclines namely tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) in poultry meat samples.

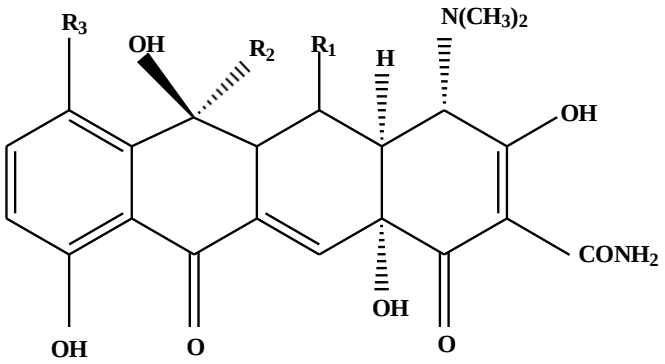

Fig. 1. Chemical structure of tetracycline

Table 1. Value of R_1, R_2 and R_3 of TCs structure

Tetracycline	R_1	R_2	R_3
TC	H	CH_3	H
OTC	OH	CH_3	H
CTC	H	CH_3	Cl

Note: R_1, R_2 and R_3 are functional groups of TCs

2. Materials and method

2.1. Sampling

A total of thirty poultry meat samples (n=30) were purchased from different super shops and local markets of Dhaka city and a control sample free from antibiotics was collected from the village. Three samples were collected from each of ten locations. Approximately, all samples were weighted 900-1000 g. The homogenous portion from muscles (thigh and breast) poultry samples obtained from each carcass were stored at -20 ℃ until analysis.

2.2. Chemicals and reagents

Oxytetracycline hydrochloride, tetracycline, and chlortetracycline hydrochloride (Sigma-Aldrich, Germany) were used to prepare standard solutions. HPLC grade acetonitrile (ACN) and methanol (MeOH), analytical grade C-18, primary secondary amine (PSA), oxalic acid (dehydrate), magnesium sulphate ($MgSO_4$), and sodium chloride (NaCl) were purchased from Sigma-Aldrich, Germany. Deionized water used for HPLC was obtained from the Mill-Q System (Denmark & USA).

2.3. Instruments

A reversed-phase High-Performance Liquid Chromatograph (HPLC; RF 1200, Prominence, Shimadzu) equipped with Photo Diode Array Detector (PDA; SPD-M20A Prominence) connected with a Rheodyne injector (20 µL sample loop) was used for the analysis of tetracyclines. A C-18 column (Luna; 250 × 4.60 mm; particle size 5µm) kept in an oven at ambient temperature was used for analysis. The analytical balance (Type ATY124, Shimadzu), homogenizer, sonicator (Hwashin tech. com.) centrifuge machine (Sigma, 2-16P), and vortex mixer (REAX 2000) were used for sample preparation.

2.4. Preparation of standard solution

Stock Solutions of each TCs standard (oxytetracycline, tetracycline, and chlortetracycline) were prepared by dissolving 10 g of compound in 10 mL MeOH to obtain a final concentration of 1.0 mg/mL. The stock solution was wrapped with aluminum foil paper to prevent photodegradation and stored at -20 ℃. The stock solution was diluted with MeOH to prepare required standard solutions and that were prepared daily when needed.

2.5. Fortification of samples

Poultry meat samples were fortified with a mixture of TCs standard at two spiking levels of 2.5 and 5 µg/kg.

2.6. Extraction and clean up procedure

Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method was used for sample preparation. According to this method, poultry meat samples (10 g) were taken in a falcon tube. At first, 10 mL deionized water and 10 mL ACN were added to the samples. Then, meat samples were vortexed for 1 min, and 4 g magnesium sulphate ($MgSO_4$) and 1 g sodium chloride (NaCl) were added in each sample and again vortexed for 1 min. Finally, centrifuged at 4000 rpm for 10 min The clean-up procedures were performed by transferring 2 mL supernatant in a screw-capped test tube and adding 150 mg PSA (Primary Secondary Amine) and 250 mg C-18. Then vortexed for 1 min and centrifuged at 2000 rpm for 4 min.

2.7. HPLC analysis for TCs

The level of tetracyclines in extracted samples was determined using HPLC according to a reported method. Briefly, mobile phase an isocratic elution using 0.01 M oxalic acid buffer (a), ACN (b), MeOH (c) was applied by volume with 70:20:10 (v/v/v) for 15 min. The flow rate of the mobile phase was 1.0 mL/min and the column oven temperature was set up at 40 ℃. The injection volume of the standard or sample was 20 µL. TCs were detected at 360 and 375 nm using a Photodiode Array Detector (PDA).

2.8. Method validation

2.8.1. Linearity and sensitivity

This study was expanded to test the validity of a reported method. Linearity was assessed by calibration curve in the range of 0-10 µg/kg (0, 1.0, 2.5, 5.0 and 10µg/kg) for OTC, TC and CTC standard solutions at five points (Fig. 2) with triplicate

analysis (Table 3) and chromatogram of TCs standard were shown in Fig. 3.

The response of the PDA detector was linear and highly correlated with the amounts of TCs injected, where the enumerated correlation coefficient (r^2) ranged from 0.99 to 1.00 and each TC standard had its linear equation. The sensitivity of the method is ordinarily explained by the slope of the analytical calibration curve. The sensitivity of this reported method was found to be 1.05, 1.17 and 1.09 µg/kg for OTC, TC and CTC, respectively (Table 3). The calibration curves of standard compounds were drawn using peak area versus concentration following the linear least squares regression procedure. The accuracy is revealed as the relative standard deviation (RSD%) of the slope of the curves.

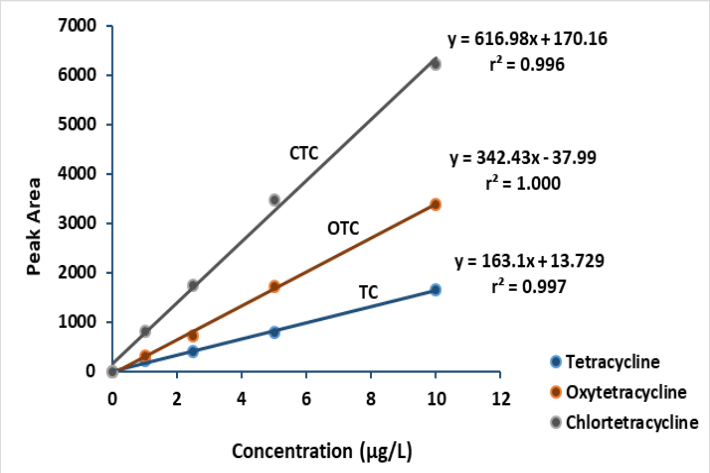

Fig. 2. Calibration curve for OTC, TC, and CTC standards

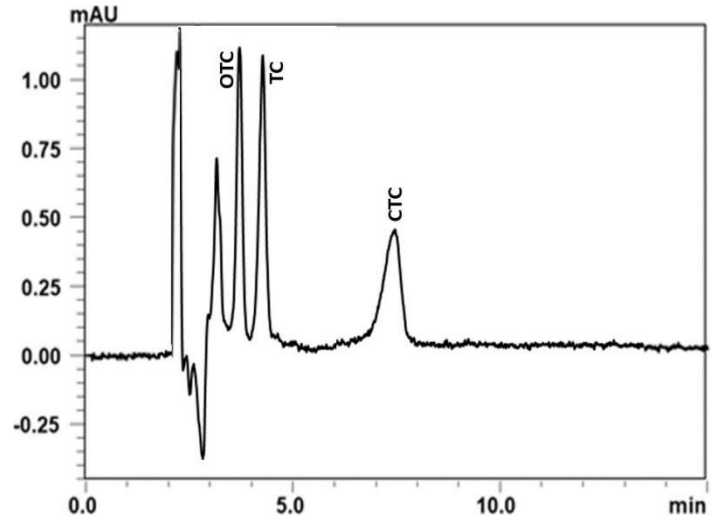

Fig. 3. Chromatogram of TC standards

2.8.2. Accuracy and precision

Accuracy is a measurement of the methodical deviation of the results from the true value. It is shown as the percentage of recovery while precision is the variability of the results narrated by the relative standard deviation (RSD%) of a set of replicate results. RSD (%) is related to the error of within-laboratory of a method. Recovery and precision (RSD %) were measured for five replicate analyses for both intra-day (n = 3) and inter-day (n=9, 3 days) experiments. Recovery was determined by comparison of response for samples spiked into sample matrix prior to sample preparation with response for spiked samples after all sample preparation steps. Therefore, any superficial recovery loss resulting from matrix suppression was eliminated from the recovery calculation. Poultry meat samples were fortified at two different concentrations (2.5 and 5 µg/kg), respectively in three replicates for measuring recoveries. The all-up precision ranged from 2.35 – 9.14 (RSD %) (Table 3) for poultry meat (muscles from breast & thigh). Although the acquired recovery values of all analytes in different samples within the Association of Official Analytical Chemists (AOAC approved on 19 Dec 2002 and used by ISO 17025) acceptable range for trace analysis; 60–120% of the values of OTC, TC, and CTC of poultry meat samples. The relative standard deviation (RSD) was lower than 10% than compliance Codex Alimentarius commission. For both inter-day and intra-day experiments, the RSD was under 10%. A method is considered as validated when RSD values are optimal.

2.8.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) is calculated by three times of signal to noise ratio. LOD is the lowest concentration of an analyte in a sample. The limit of quantitation (LOQ) is the lowest amount that can be quantified within allowable accuracy and precision at the signal-to-noise ratio of 10. Both LOD and LOQ were assessed and the acquired data were given in Table 3. It was observed that the LOD of the proposed method was 1.05, 1.17, and 1.09 µg/kg for OTC, TC, and CTC respectively. The similar values of LOQ were 3.15, 3.51, and 3.27 µg/kg.

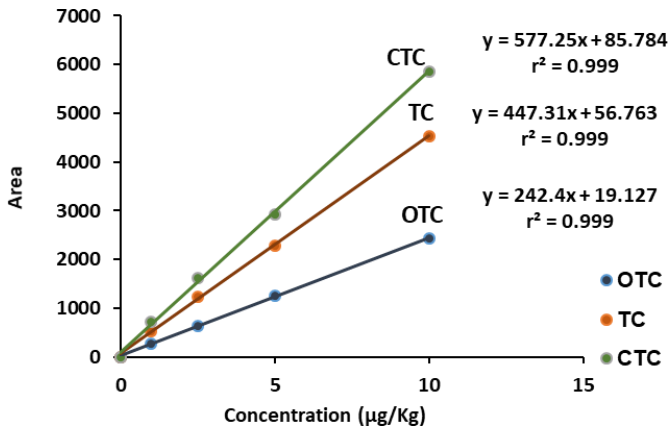

Fig. 4. Matrix-matched calibration curve for OTC, TC, and CTC

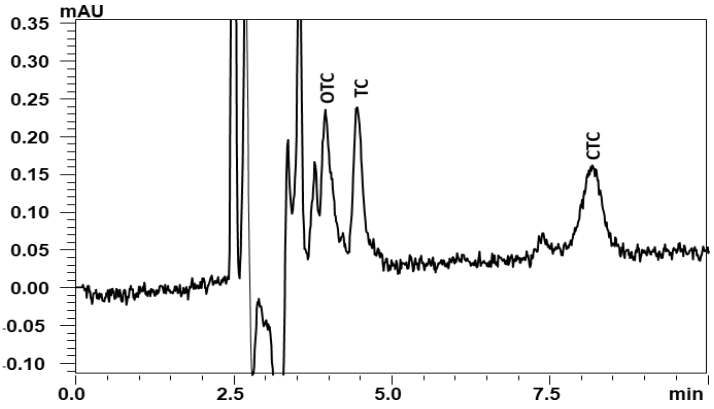

Fig. 5. Chromatogram of TC standards at spiking level 5 µg/kg

3. Results and discussion

The residual tetracycline (OTC, TC, and CTC) in poultry meat samples were analyzed by reversed-phase HPLC with a Photo Diode Array detector (PDA). TCs standard solution was separated on the C_{18} column at two wavelengths, i.e., 360 and 375 nm. The average retention time was 3.8, 4.30, and 7.8 min, respectively for OTC, TC, and CTC. Linear calibration curves were produced for each standard OTC, TC, and CTC in the range of 0-10 µg/L, and the correlation coefficient, (r^2) ranging from 0.99-1.00 was found for the standard.

The matrix match calibration curves were also carried out and a similar correlation coefficient was found which indicated that the matrix did not affect the analysis of OTC, TC, and CTC in poultry meat samples (Fig. 5). The value of the correlation coefficient obtained for each calibration curve showed that the correlation between peak area and concentration was excellent.

The LOD and LOQ values for OTC, TC, and CTC were 1.05, 1.17, and 1.09 µg/kg and 3.15, 3.51 & 3.27 µg/kg, respectively. Triplicate (n=3) recovery experiments for OTC, TC, and CTC were done by fortification of the control matrix (poultry from the village) at two fortified levels 2.5 µg/kg and 5 µg/kg. The average percent recoveries were 91 and 100%, 102 and 100%, and 106 and 100% for OTC, TC, and CTC, respectively with the repeatability (RSD %) of 5.57, 9.14, and 2.35%, respectively. Thirty poultry meat samples were analyzed. However, residual tetracyclines in all samples were found below the detection limit (Fig. 6, Fig. 7).

Food safety is of great significance and a global issue to consumers all over the world (Shahbazi et al., 2015). Nowadays, antibiotic drugs are generally used to distribute three purposes in poultry in the livestock, firstly animals are administered with high doses of antibiotics for relatively shorter periods as therapeutic use, secondly, prophylactic use which includes disclosure of animals with medium dose of antimicrobials for long durations, thirdly the growth enhancement that is sub-therapeutic doses of antibiotics. As an example, it was said that 10 or 100 times less than therapeutic doses are given for a very long time or throughout the entire life of the animals (Marshall et al., 2011; Chowdhury et al., 2009). Several types of antimicrobial drugs were reported to be commonly used at the farmers' level belonging to most of the tetracycline and sulfonamides group (Milagro et al., 2008). Different groups of drugs have different withdrawal periods and their residual action in the animal body acts at different times (Khatun et al., 2018).

Table 2. Slope, Intercept and Correlation coefficient (r^2) of both TCs standard and matrix-match TCs

Antibiotics	Slope standard TCs	Intercept standard TCs	Correlation coefficient (r^2)	Slope (matrix-match)	Intercept (matrix-match)	Correlation coefficient (r^2) (matrix-match)
OTC	342.43	-37.99	1.00	242.40	19.13	0.99
TC	161.10	13.73	0.99	447.31	56.76	0.99
CTC	616.98	170.16	0.99	577.25	85.78	0.99

Note: r^2= correlation coefficient; OTC=Oxytetracycline; TC= Tetracycline; CTC= Chlortetracycline

Table 3. Fortified level, mean recovery, average, SD, RSD, LOD, and LOQ of TCs

Antibiotics	Fortified level (µg/kg)	Mean Recovery (%) Inter-day (n=3 Days)	Average±SD	RSD (%)	LOD (µg/kg) (3:1)	LOQ (µg/kg) (10:1)
OTC	2.5 & 5	91 &100	1.05 ± 0.06	5.57	1.05	3.15
TC		102 & 100	1.17 ± 0.11	9.14	1.17	3.51
CTC		106 & 100	1.09 ± 0.03	2.35	1.09	3.27

SD=standard deviation; RSD=relative standard deviation; LOD=limit of detection; LOQ=limit of quantitation

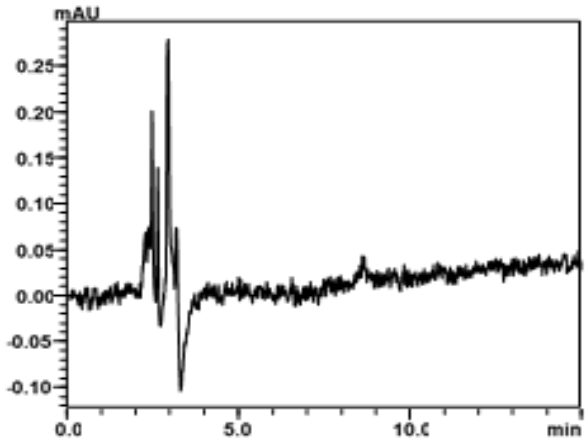

Fig. 6. Reagent Blank

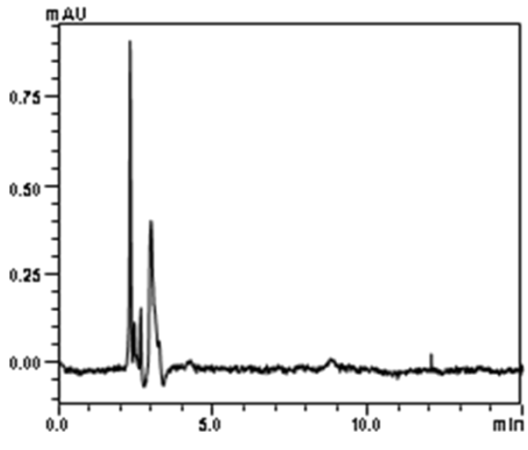

Fig. 7. Poultry meat sample without containing any TCs residue

The presence of residual tetracycline in poultry meat and organs higher than the maximum residue limit was reported in the literature due to improper use of antibiotics by producers which is a great concern to the consumers and harmful for public health globally (Bahmani et al., 2020; Arslanbas et al., 2018; Shahbazi et al., 2015; Ibrahim et al., 2015). However, the present study revealed that poultry meat samples are free of residual tetracyclines and safe for consumption which indicated that poultry farmers maintain the proper doses and withdrawal period of the antibiotics. The residual antibiotics may exit from treated animals by excretion and enter into the environment as a result, they were not detected in the present study (Aga et al., 2003). Therefore, further research on a large scale and other food matrices are necessary to ensure safe food for consumers in Bangladesh.

4. Conclusion

Tetracycline antibiotics have been used globally for the treatment of bacterial infections in livestock. Some antibiotics like chloramphenicol and nitrofuran have been banned in many countries for livestock (Mou et al., 2021). A few researches were performed by Thin Layer Chromatography for the detection of residual antibiotics in Bangladesh. Sarker et al. (2018) reported the percentage of OTC residues 74, 47 and 36% in liver, thigh muscles and breast muscles. But adequate research in Bangladesh has been done by HPLC method due to insufficient facilities in all research laboratories, though that is an advanced and more sensitive method than TLC. The HPLC-PDA is an efficient, sensitive, and reliable method for the analysis of tetracyclines in poultry meat samples. The analyzed samples were free of residual tetracyclines and safe for consumers.

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Conflict of interest

The authors declare no conflict of interest.

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