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INVESTIGATION ON CHEMICAL CONTAMINANTS IN SELECTED FOOD STUFFS

THESIS FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

Submitted by

Md. Shahed Reza
Registration Number: 30
Session: 2015-2016 (Part Time)
Department of Chemistry
University of Dhaka

September, 2021

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By

Md. Shahed Reza
Registration Number: 30, Session: 2015-2016 (Part Time)
B.Sc(Hons.), MS in Chemistry, University of Dhaka

A Thesis Submitted

To

Department of Chemistry
Organic Research Laboratory
University of Dhaka
Dhaka-1000, Bangladesh

In Partial Fulfillment of the Requirements for the Award of Doctor of Philosophy (PhD) Degree in Chemistry

September, 2021

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CERTIFICATION

We certified that the thesis titled **Investigation on Chemical Contaminants in Selected Food Stuffs** is an original research work carried out by Md. Shahed Reza (Registration number: 30, Session: 2015-2016, Part Time) in the Department of Chemistry, University of Dhaka, Bangladesh, under our joint supervision and suggestions. We have examined and found the work is acceptable for the award of a degree of Doctor of Philosophy in Organic Chemistry.

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DECLARATION

Experimental work described in the thesis has been carried out by the author at the Department of Chemistry, University of Dhaka. A part of the work was done in Bangladesh Standards and Testing Institution (BSTI) and in National Food Safety Laboratory (NFSL), Institute of Public Health. This work has not been and will not be presented for any other degree.

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Dedicated
To
My Mother and Father

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Acknowledgement

Alhamdulillah by the grace of almighty Allah I am able to complete this research for the degree of PhD.

In all this years, during my PhD work, many people were directly and indirectly assisting me to enlighten my academic career. It was unimaginable for me in my doctoral work without the valuable support of these personalities.

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I am grateful to Food and Agricultural Organization of United Nations (FAO) for supporting me for collection of samples from city corporation markets of all the division of Bangladesh.

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Investigation on Chemical Contaminants in Selected Food Stuffs

Abstract

This study describes determination of pesticide residues and heavy metals in fruits and vegetables, heavy metals in turmeric powder, aflatoxins in wheat and maize, benzoic acid and sorbic acid in fruit drinks and tomato ketchup, sudan red in chili powder and antibiotic residues in pasteurized milk. Quick, easy, cheap, effective, and rugged method was used for pesticide and antibiotic residues. Gas chromatograph equipped with electron capture detector (GC-ECD), gas chromatograph-mass spectrometer (GC-MS) and liquid chromatograph-mass spectrometer (LC-MS/MS) were the major equipment for analysis of pesticide residues in fruit and vegetable samples. Microwave digester was used for sample preparation of fruits, vegetables and turmeric powder for analysis of heavy metals by atomic absorption spectrophotometer (AAS). Aflatoxins B_1, B_2, $G_1$and G_2 were determined in maize and wheat by high performance liquid chromatograph (HPLC) equipped with fluorescence detector and coring cell as post column derivatization system. Phosphate buffered saline was used for extraction of sample and clean-up by immunoafinity column for analysis of aflatoxins. Benzoic acid and sorbic acid in fruit drinks and tomato ketchup was determined by HPLC. Extraction of benzoic acid and sorbic acid from sample was performed using a mixture of ammonium acetate buffer solution and methanol, under pH 4.5. Sudan dyes I, II, III, IV have been determined in chili powder by HPLC. Sudan dyes I, II, III, IV were extracted by ethanol. Antibiotic residues were extracted by methanol water mixture and were clean-up by $MgSO_4$, PSA and C18. Antibiotic residues in pasteurized milk were analyzed by LC-MS/MS. Tomato and cabbage were used as representative matrix for method validation of pesticide residues. The LOD for pesticide residue was in the range of 0.02-0.81 µg/kg and LOQ was in the range of 0.08-2.71 µg/kg. Linear correlation coefficient (R^2) value ranged from 0.996 to 0.999. Recoveries were in the range of 81-97%. Potato was used as representative matrix for method validation of heavy metals. For arsenic, lead and cadmium LOD were 2.49, 2.39, 0.09 µg/kg and LOQ were 8.30, 7.96, and 0.29 µg/kg, respectively. Linear correlation coefficient (R^2) value for As, Pb and Cd were 0.998, 0.996 and 0.998, respectively. Recoveries for As, Pb and Cd were 98%, 95%, 96%, respectively. Turmeric powder was used as a representative matrix for determination of lead and chromium. For Pb and Cr LOD were 1.71, 2.17 µg/kg and LOQ were 5.69, 7.22 µg/kg, respectively. Linear correlation coefficient (R^2) value for Pb and Cr were 0.996 and 0.995. Recoveries for Pb and C were 98% and 96%, respectively. Wheat was used as representative matrix for method validation of aflatoxin. LOD of aflatoxin G_2, G_1, B_2 and B_1 were 0.006, 0.021, 0.020, 0.046 µg/kg and LOQ were 0.020, 0.069, 0.066, 0.153 µg/kg, respectively. Linear correlation coefficient (R^2) value were in the range of 0.998-0.999. Recoveries (%) were in the range of 85-96%. Apple fruit drink was used as representative matrix for method validation of benzoic acid and sorbic acid. LOD of benzoic acid and sorbic acid were 0.15 and 0.09 mg/kg and LOQ were 0.49 and 0.30 mg/kg, respectively. Linear correlation coefficient (R^2) value for benzoic acid and sorbic acid was 1. Recovery (%) of benzoic acid and sorbic

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acid with apple fruit drink was 99%. Chili powder was used as representative matrix for method validation of sudan red. LOD of sudan red-I, II, III and IV were 0.22, 0.50, 0.38 and 1.49 mg/kg and LOQ were 0.72, 1.66, 1.25 and 4.96 mg/kg, respectively. Linear correlation coefficient (R^2) value for sudan red-I, II, III and IV was 0.999. Recoveries (%) of sudan red-I, II, III and IV with chili powder were in the range 93-99%. Pasteurized milk was used as representative matrix for method validation of antibiotic residues. LOD of six antibiotics in pasteurized milk were in the range of 1.53-4.87 µg/kg and LOQ was in the range of 5.09-16.25 µg/kg. Linear correlation coefficient (R^2) value ranged from 0.995 to 0.999. Recoveries (%) of antibiotic were in the range of 84-101%. Fruits (n= 280) and vegetables (n= 455) samples were analyzed for pesticide residues. Chlorpyrifos was detected in 2 samples of cabbage which were within maximum residue limit (MRL) of 1.0 mg/kg set by Bangladesh Food Safety Authority (BFSA). Dimethoate was detected in 4 samples of green chili which were within MRL of 0.5 mg/kg set by BFSA. Carbofuran was detected in 2 sample of tomato and in 2 sample of eggplant. All these four samples were within MRL of 0.01 mg/kg set by European Commission (EC). Arsenic, lead and cadmium were analyzed for fruits (n= 280) and vegetables (n= 455) samples. Arsenic was detected in 13 potato samples, in 01 tomato samples, in 11 eggplant samples and in 1 carrot samples. Cadmium was detected in 6 potato samples. All these samples were within maximum limit of 0.1 mg/kg set by BFSA. Lead and chromium were analyzed in 17 turmeric powder samples. High amount of Pb and Cr were found in 8 turmeric powder samples. Eight samples exceeded maximum limit of Pb of 2.5 mg/kg set by Bangladesh Standards and Testing Institution (BSTI). Aflatoxin B_1, B_2, G_1 and G_2 were analyzed in 25 wheat and 25 maize samples. No targeted aflatoxin was detected in any sample of wheat and maize. Benzoic acid and sorbic acid were analyzed in 25 fruit drink and 27 tomato ketchup samples. Benzoic acid was detected in 17 fruit drink samples and in 21 tomato ketchup samples. Eleven fruit drink sample exceeded maximum limit of 120 mg/kg set by BSTI and 1 tomato ketchup sample exceeded maximum limit of 750 mg/kg set by BSTI. Sudan I, II, III and IV were analyzed in 20 chili powder samples. Sudan III was detected in 1 sample out of 20 samples. Six antibiotic residues were analyzed in 42 samples of pasteurized milk. No targeted antibiotic was detected in any sample. In this study analysis result showed that pesticide residues detected in 1.36 % sample of fruits and vegetables. Arsenic was detected in 3.54 % sample and cadmium was detected in 0.82% sample of fruits and vegetables. Lead and chromium was detected in 47.06% of the turmeric powder sample. Aflatoxin B_1, B_2, G_1 and G_2 was not detected in any wheat and maize sample. Benzoic acid was detected in 68% fruit drink sample and in 77.78% tomato ketchup sample. 64.71% of benzoic acid detected fruit drink sample exceeded maximum limit and 6.76% of benzoic acid detected tomato ketchup sample exceeded maximum limit. Sudan III was is detected in 5% chili powder sample. No targeted ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were detected in any sample of pasteurized milk.

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LIST OF ABBREVIATION

AAS	Atomic Absorption Spectrophotometer
BFSA	Bangladesh Food Safety Authority
DAE	Department of Agricultural Extension
EC	European Commission
EFSA	The European Food Safety Authority
EPA	The US Environmental Protection Agency
ESI	Electrospray ionization
EU	European Union
FAD	Fitness Against Doping
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
FLD	Fluorescence Detector
GC	Gas Chromatograph
GC-MS	Gas Chromatograph-Mass Spectrometer
GFA	Graphite Furnace Atomizer
HPLC	High Performance Liquid Chromatograph
IARC	International Agency for Research on Cancer
LC-MS/MS	Liquid Chromatograph-Mass Spectrometer(Triple Quadrupole)
LIT	Linear Ion Trap
MRM	Multiple Reaction Monitoring
MRL	Maximum Residue Limit
OCP	Organochlorine Pesticide
OPP	Organophosphorus Pesticide
PCBs	Polychlorinated Biphenyls
QqQ	Triple Quadrupole
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
UV	Ultraviolet
WHO	World Health Organization of the United Nations

1. Introduction

Food is the substance which gives energy and nutrition whether processed, semi-processed or raw. Food contains carbohydrates, protein, fat, vitamins, minerals and water **[1]**. Food is produced through farming. It includes animal and plant sources. Sufficient amounts of nutritious and safe and food is the prerequisite for sustaining life and to maintain good health. Unsafe food contains harmful chemical substances, bacteria, viruses, parasites and other microorganism. This unsafe food creates more than two hundred diseases ranging from diarrhoea to cancers **[2]**. Unsafe food can also create malnutrition to infants, young children and old aged people. Socioeconomic development is obstructed by food borne diseases. It is harmful for national economies and trade. Now a days food supply chains involved with various national borders. Good coordination among the producers, governments and consumers helps to ensure food safety. International organization like Codex Alimentarius Commission (CAC), World Health Organization (WHO), Food and Agriculture Organization (FAO) are actively working to maintain food safety through formulation of food standards, guidelines and other related activities. Codex standards and related texts contain requirements for food to ensure safe and nutritious food product for the consumer which is free from adulteration and contamination. The food safety is the most prioritized concern for producers, regulatory authorities and consumers. For the appearance of new food safety challenges the countries across the world upgrading and updating the food safety protocols to reduce risk. These programs must be overseen for effectiveness and reliability. Risk assessment shows that food safety risks frequently occurred by chemical, microbiological and environmental contaminants. Analytical methods became an essential part of food safety activity **[3]**. For an effective food safety control system, it is indispensible to maintain food safety from farm to fork. Bangladesh Food Safety Authority (BFSA) has been formed government of Bangladesh with a vision 'Safe food for all to protect life and health' **[4]**. Advanced analytical techniques are evolving to counteract the new food safety issues. Official standard analytical methods are used to monitor usual issues. Innovative analytical methods are being developing or modifying in response to new challenging issues of food contamination. Accurate and precise analytical results from fit for purpose and validated analytical methods are essential for regulators to make efficient scientific decisions.

1.1 Food Contaminants

Food contaminants are harmful chemical substances or microorganisms which enter in food. It can create various types of diseases to human. Agrochemicals, environmental contaminants, industrial processing contaminants, carcinogenic agent are some important food contaminants **[5]**. Food contaminants can enter in food during production, processing, storage or at the time of distribution. It can also enter the food from environment. The presence of these contaminants in food must be monitored cautiously because it can affect the quality of the food. Contaminants can also make the food unsafe for human consumption. Contamination of food may create a risk to human health. Food can become contaminated by various ways and processes. There are basically four types of food contaminations (a) chemical contamination (b) biological contamination (c) physical contamination (d) cross contamination **[6].**

1.1.1 Chemical Contamination

When food comes into contact with toxic chemicals, then chemical contamination occurs. Sometimes food itself produces toxic chemical. It can create chemical poisoning of food. Some emerging chemical contaminants are pesticides, herbicides, veterinary drug, heavy metals, naturally occurring toxins, preservatives and artificial food colouring agent **[7].**

1.1.2 Biological Contamination

Biological contamination arises from living micro-organisms like pathogenic bacteria or from the toxic substances produced by micro-organisms **[8]**. Biological contaminants are the primary cause of food-borne diseases. The important causes of biological contamination are food spoilage and food waste. Bacteria, viruses, parasites, protozoa, fungi and prions are the major microorganisms which can create food-borne illness. These are. Food-borne illnesses across the world are caused by bacteria or viruses. Most common bacteria and viruses are *Listeria, Salmonella*, *E. coli, Campylobacter* and Norovirus **[9]**.

1.1.3 Physical Contamination

When a physical object like human or animal hair, fingernails, broken glass, staples, packaging materials enters in food at the time of manufacturing, handling or distribution, physical contamination occurs. Physical objects in food can create biological contaminants as well. Extraneous matter from unclean fruit and vegetables, pests, rodent hair are also the examples of physical contaminants **[10]**.

1.1.4 Cross contamination

Accidental transfer of food contaminants from one surface to another is called cross-contamination. Cross-contamination generally occurs for inappropriate handling of food procedure. It is basically biological contamination but it can be also physical or chemical contamination. Microorganisms from sweat, sneezing, coughing, hands, hair, clothing, reusing cutting boards or utensils are the primary cause of cross contamination. Improper cleaning and sanitizing, improper food storage, improper waste disposal and pests can be source of cross-contamination **[11].**

1.2 Brief description of chemical contaminants

1.2.1 Pesticides

Pesticides are substances that are mainly used in agriculture in order to protect plants from pests or weeds. Pesticides also used to control malaria, dengue fever and schistosomiasis. Insecticides, fungicides, herbicides, rodenticides, and growth regulators of plants are some of the examples of pesticides **[12]**. Use of pesticides is an obligatory input to agricultural system. Random use of pesticides is the cause of contamination of all basic necessities of life that is air, water and food **[13]**. More than thousand active ingredients are being used in agricultural production. Organophosphorus pesticides (OPPs), pyrethroids and carbamates are extensively used pesticides in many crops due to their low persistency and high killing efficiency **[14]**. Agricultural producer change the active ingredients when a pesticide looses its efficacy due to resistant growing to a particular pest.

1.2.1.1 Organophosphorus pesticides

Organophosphorus pesticides (OPPs) are heavily used in agricultural production. It contains a phosphate ester side chain. The central phosphorous atom is double bonded to an oxygen or sulphur atom, and single bonded to two methoxy ($–OCH_3$) or ethoxy ($–OCH_3CH_3$) groups **[15].** The certain nerves of insects function by releasing acetylcholine (ACh) into the intracellular space where the nerve cell send signal to muscle cell**.** Acetylcholine stimulates the muscle cell for contraction. Acetylcholinesterase is an enzyme which stop this contraction that destroys the released ACh signal molecules. Organophosphorus compounds are highly toxic because they chemically bind to the acetylcholinesterase enzyme in such a way that it cannot destroy Ach. Then the insect dies with its muscles for prolonged contraction and its nervous system was in a state of sustained excitation **[16]**. Chemical structure of some OPPs are shown in Figure 1.

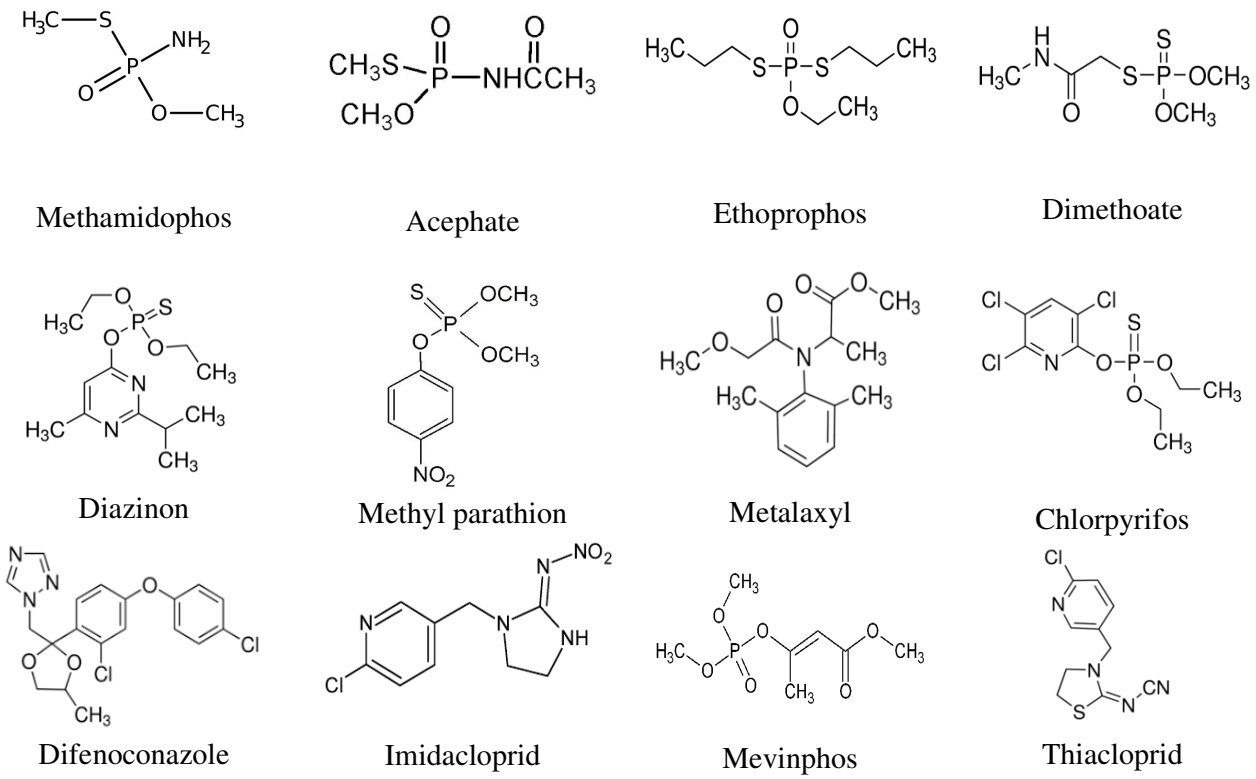


Figure 1: Chemical structure of some organophosphorus pesticide

1.2.1.2 Organochlorine pesticides

Organochlorine pesticides (OCPs) were used in agriculture to protect plants from the attack of pest. OCPs affect the nervous system of the pests. DDT was extensively used to prevent the spread of malaria, dengue, leishmaniasis and Japanese encephalitis. Another highly used OCP is lindane. OCPs are persistent organic pollutant which can accumulate in food chain **[17].** Aldrin, dieldrin, chlordane, DDT, endrin, heptachlor, lindane, benzenehexachloride, mixex, toxaphene are banned by Stockholm Convention **[18].** Chemical structure of some organochlorine pesticides are given in Figure 2.

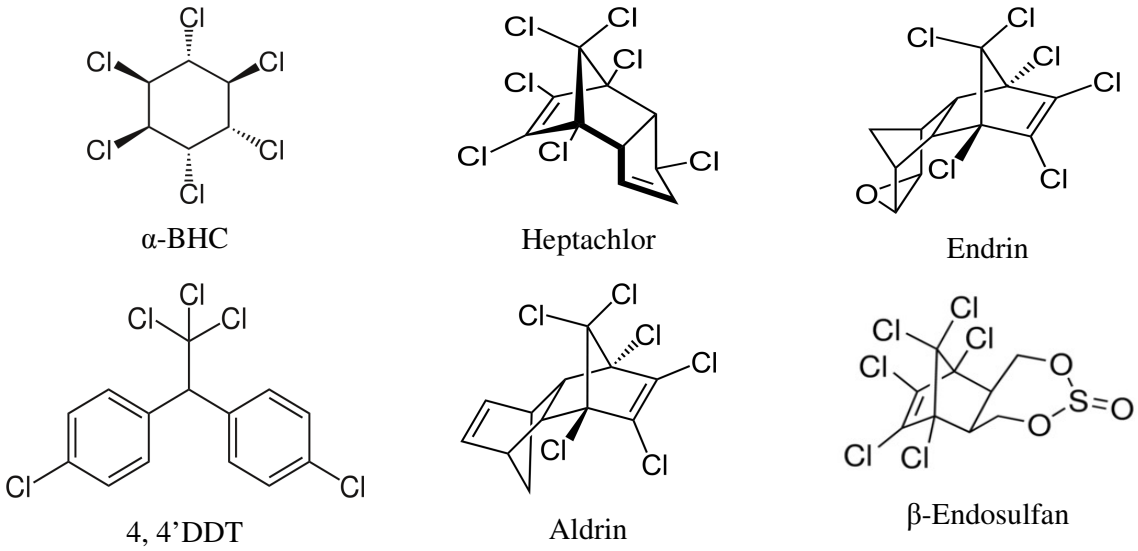


Figure 2: Chemical structure of some organochlorine pesticide

1.2.1.3 Carbamate pesticide

Carbamate compounds consist of esters of carbamic acid. It commonly used as insecticides. Carbamate pesticides have a common chemical formula RHN-COOR. Carbamates are extremely soluble in water, relatively polar and reactive. Aldicarb, carbaryl, carbofuran and captan are important carbamate **[19].** Chemical structure of some carbamate pesticides are given in Figure 3.

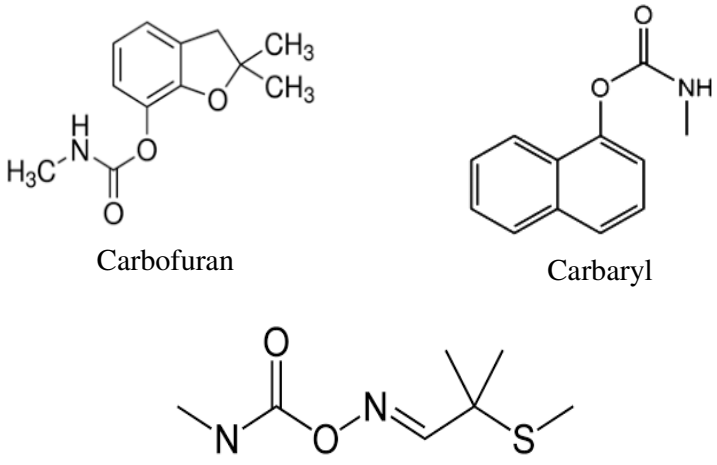


Figure 3: Chemical structure of some carbamate pesticide

1.2.2 Heavy metals

Heavy metal has largely scattered over the world. This heavy metal jeopardize the environment. It creates serious health hazards to human. Due to enormous industrial growth and economic trade, the environmental contaminants has increased enormously. Rapid urbanization, changes of uses of land and industrial revolution heavy metals are spreading in food chain **[20].** Arsenic (As), Lead (Pb), Cadmium (Cd) and Chromium (Cr) are most abundant toxic heavy metals in the environment and can easily enter in food system.

As is widespread in nature. As compounds dissolve in water and as a result it enters in our food chain. Arsenic might be found in a broad range of foods. As is found in both organic and inorganic form in food. Chronic arsenic toxicity causes skin lesions, nervous system damage, skin cancer and blood vessels diseases **[21]**. The International Agency for Research on Cancer (IARC) has classified arsenic as a carcinogenic agent in drinking water for human **[22].** World Health Organization (WHO) provisional guideline value of arsenic in drinking water is 10 μg/L **[23].**

Pb exposure is very fatal for children. At high level of lead exposure, it can attacks the brain and central nervous system. As a consequence it can creates coma, convulsions and even death. Acute lead toxicity may cause mental retardation and behavioural disorders of children **[24]**.

Cd toxicity can damage the gastrointestinal tract. Severe Cd toxicity affects the liver, heart and kidney. It is showed by animal studies. Kidney is the most sensitive organ with chronic toxicity to cadmium. Adverse effects of Cd is observed in human among them abnormal excretion of protein, glucose and amino acid in urine and renal tubular dysfunction are major **[25]**. IARC considered cadmium and cadmium compounds as carcinogen in human those who got occupation exposure **[26].**

Cr is the most prolific mineral in Earth's crust. In environment Cr is mostly stable in trivalent and hexavalent form. Cr (III) and Cr (VI) are originated from industries **[27].** Bronchogenic carcinoma is connected with Chromium (VI) in occupational exposure **[28].** Recently it was revealed that, Cr has strong connection with stomach cancer **[29].** Chromium exposure is associated with many of diseases such as epigenetic alterations, respiratory, reproductive problems and neurological disorders **[30].**

1.2.3 Natural toxins

Toxins are naturally occurring chemical or biological substances which are produced by various organisms. Mycotoxins are toxic secondary metabolites which produced by fungi. The primary concern of mycotoxin contamination is cereals and nuts **[31]**. There are many mycotoxins spread in the environment but currently few of them are regulated by different food safety authority. Aflatoxins, ochratoxin A, patulin, deoxynivalenol, zearalenone, fumonisins and T-2/HT-2 toxins are common mycotoxins which is under regulation **[32].** Aflatoxins B_1, B_2, G_1 and G_2 are produced by *Aspergillus flavus, A. parasiticus and A. nomius* **[33].** These mycotoxins consist of high molecular weight. It contain one or more oxygenated alicyclic rings. Chemical structure of some aflatoxins are given in Figure 4.

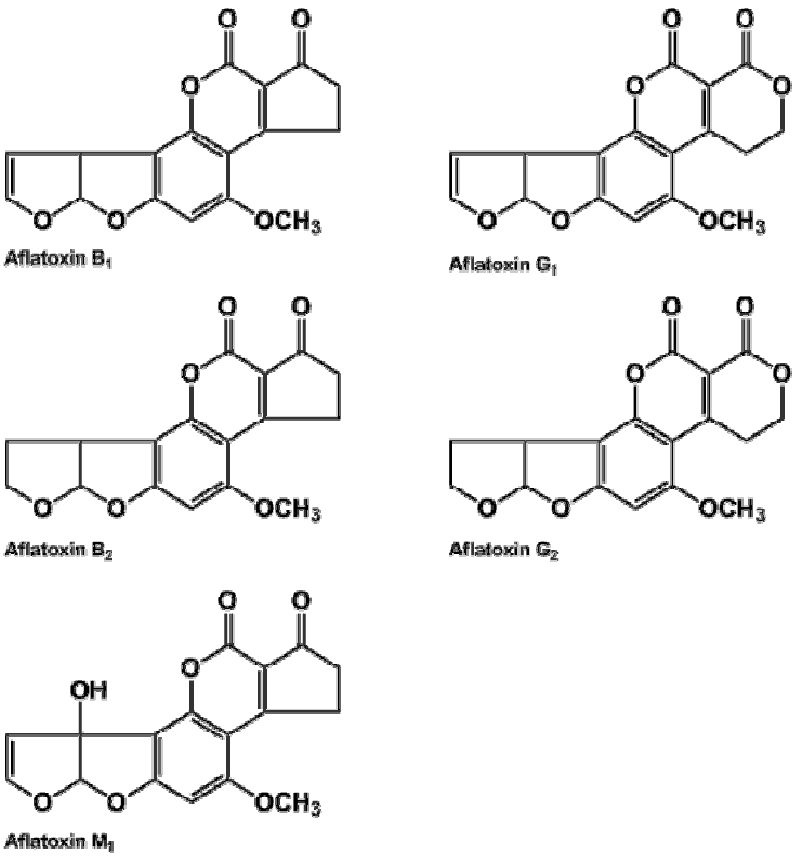


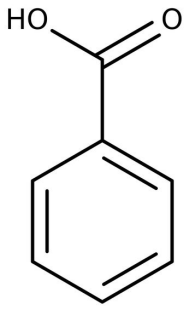
Figure 4: Chemical structure of aflatoxin B_1, B_2, G_1, G_2 and M_1

Food can be contaminated by aflatoxin when storage conditions are in favour for fungal growth. Earlier it was reported that aflatoxin contaminations was found in maize, peanuts, pistachio nuts, copra and cottonseeds **[34].** Aflatoxins have carcinogenic, mutagenic, teratogenic and immunosuppressive effect to most of the animal species **[35].** IARC has classified aflatoxins B_1,

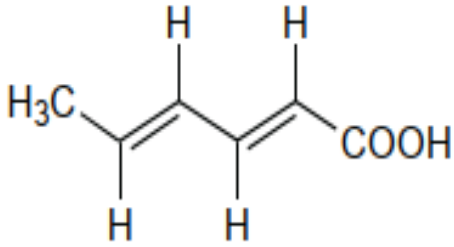
B_2, G_1 and G_2 and M_1 as carcinogenic agents [3**6].** European Commission (EC), US Food and Drug Administration (FDA), WHO and FAO consider aflatoxin as potential health hazard to human.

1.2.4 Preservatives

There are various types of food preservation technique available such as drying, pasteurization, thermal sterilization, aseptic packaging, freezing, chilling, irradiation, fermentation, chemical preservation etc. Chemical food preservative is a chemical agent which inhibit the microbial growth. Chemical preservative which exceeds the permitted levels can cause some adverse reactions such as acidosis, convulsions, asthma, and allergic reactions **[37]**. Excess amount of additives or wrong additive can be enter in food through formulation error. In fruit juices or fruit drinks carcinogenic compound benzene can be produced due to the presence of benzoic acid and ascorbic acid. This reaction can be stimulated by the exposure of light and heat **[38].** Chemical structure of benzoic acid and sorbic acid are given in Figure 5.



Benzoic acid



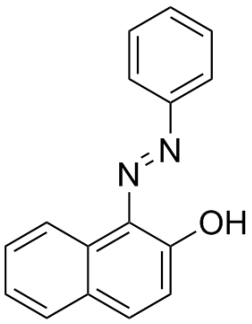
Sorbic acid

Figure 5: Chemical structure of benzoic acid and sorbic acid

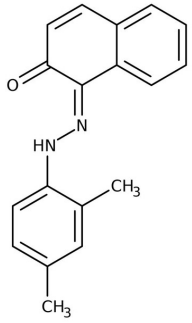
1.2.5 Artificial colouring agent

For food industries colour is the most considerable characteristic of food products. Many colouring agent are added to food products for enhancement of visual aesthetics and promotion of sales. Colour additives are widely used for uniformity of foods which already have some colours present in food. Sudan dyes are azo dyes. These dyes are traditionally used in waxes, drugs, plastics, oils, food, clothing, polishes, and are also used in histochemical analysis **[39]**. The IARC has classified these dyes as Class-III carcinogens. Sudan dyes are banned worldwide; however, many countries still utilize these dyes illegally in their food products **[40].** Although animal studies show that sudan dyes as carcinogenic substances, these artificial colouring agent was

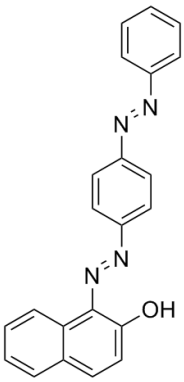
recently found in various food products in EU countries. These colouring agents are added to different food products including chili powder to intensify and prolong good appearance which is similar to the natural red colour. In the United Kingdom Sudan dyes have been found in more than six hundred food products such as worcestershire sauce, pizza, noodle soup and fish sauce. Sudan dyes are illegal to use in food products in EU countries**.** The European Food Safety Authority (EFSA) performed a toxicological research regarding various dyes found in food which is illegal to use . EFSA concluded their research with the strong evidence of genotoxicity and carcinogenicity caused by Sudan I **[41]**. Since sudan-I structurally resembles all other sudan dyes, the larger group have the same harmful effects **[42].** Chemical structure of Sudan I, II, III and IV are given in Figure 6.



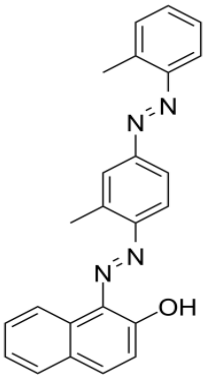
Sudan I



Sudan II



Sudan III



Sudan IV

Figure 6: Chemical structure of Sudan I, II, III and IV

1.2.6 Antibiotic

Antibiotics are substances which inhibit the growth of microorganisms or kill microorganisms. Antibiotics are used to treat or prevent infections caused by bacteria. Development of antibiotic is one of the outstanding innovation of modern drug **[43].** Antibiotic resistance (ABR) is now a global concern **[44].** Antibiotic-resistant bacteria may enter in human through food chain **[45].** Animal derived food and fishes are considered to be a strong source of antibiotic-resistant bacteria **[46].** Antibiotic are administered to live animals which can reside in animal tissues as residues. Chemical structure of some antibiotics are given in Figure 7.

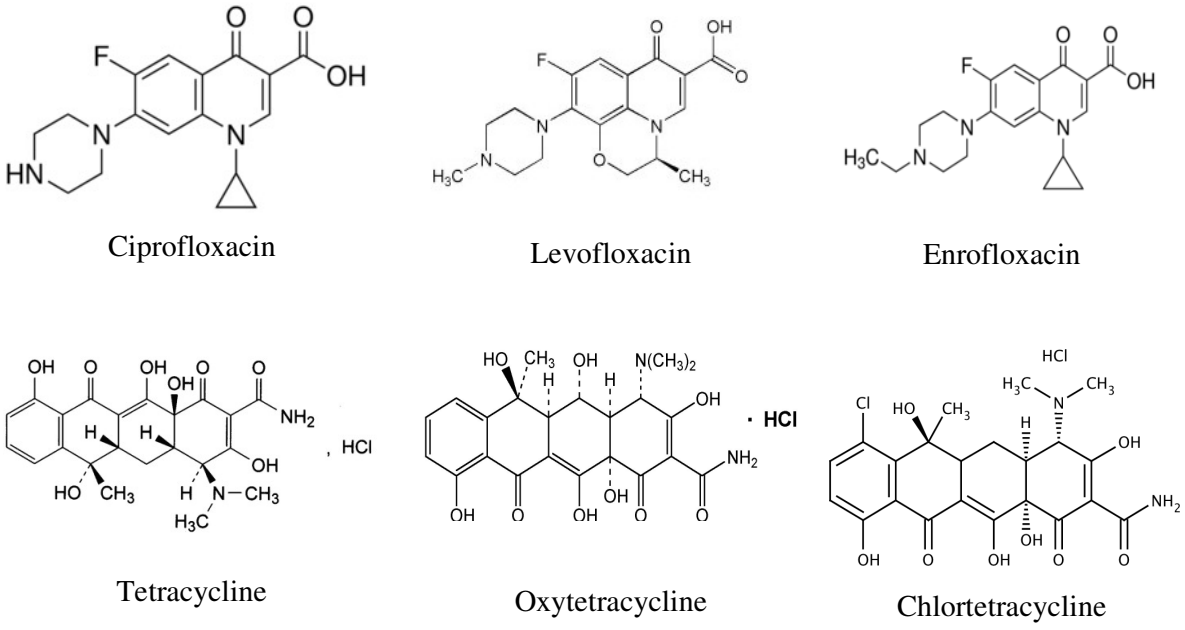


Figure 7: Chemical structure of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline

1.3 Brief description of equipments and methods used in this study

1.3.1 High performance liquid chromatograph (HPLC)

Chromatographic technique started from the mid-19th century. Chromatography was used primarily for the separation of plant pigments like chlorophyll. Modern chromatography like HPLC was discovered in 1930 to 1940. It is a useful technique for a wide range of separation. Modern definition by International Union of Pure and Applied Chemistry (IUPAC) "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction" **[47]**. HPLC is very efficient technique for precise separations of complex chemical mixtures into their individual compounds **[48].** Compounds are separated due to the molecules moves at different rates in the column.

1.3.2 Gas chromatograph (GC)

In GC separation occurs in the column, like capillary column. Two phases are involved one is stationary phase and another is mobile phase (carrier gas). Stationary phase resides in the column. Mobile phase moves over the stationary phase. Compounds are separated because the compound molecules move at diverse rates within the column. Intermolecular interactions attract compound molecules to the stationary phase that is hydrogen bonding **[49].**

Electron Capture Detector (ECD): Nickel-63 a radioactive element is placed inside the ECD. Nickel 63 emits beta particles which collide with nitrogen and ionize molecules. As a result free electron cloud is created in the ECD cell. The working principle of ECD is to maintain a constant current equal to the standing current through the electron cloud. This is occurred by applying a periodic pulse to the anode and cathode. If the current drops below the set standing current value, the number of pulses per second increases to maintain actual current value. When electronegative compounds enter into the ECD cell. The compound immediately combine with some of the fee electrons and temporarily reduce the number of electron remaining in the electron cloud **[50]**. When the number electron decreased in electron cloud, the pulse rate is increased to maintain a constant current equal to the standing current. ECD detector measure the pulse rate needed to maintain the standing current.

1.3.3 Atomic absorption spectrophotometer (AAS)

The atomic absorption spectrometry uses absorption of light of inherent wavelengths by atoms. All atoms are classified into two groups, atoms with low energies and atom with high energies. The state of low energies is called the ground state. State of high energies is called the excited state. At ground state absorbs external energies and it goes to the excited state. For example, sodium has two excited states, one is at 2.2 electron volt (eV) and another is at 3.6 eV **[51].** When 2.2 eV energy is given to the sodium atom at the ground state by external source, it moves up to the excited state (I) at 2.2 eV. When 3.6eV energy is given, it moves up to the excited state in (II) at 3.6eV. Energy is given as light. 2.2eV and 3.6eV correspond to 589.9 nm and 330.3nm wavelength , respectively. Sodium at the ground state, only light of these wavelengths are absorbed

1.3.4 Liquid chromatograph-mass spectrometer (LC-MS/MS)

The mass spectrometer have five major components. These components are sample inlet, ion source, mass analyzer, detector and data system**.** Components are shown in Figure 8.

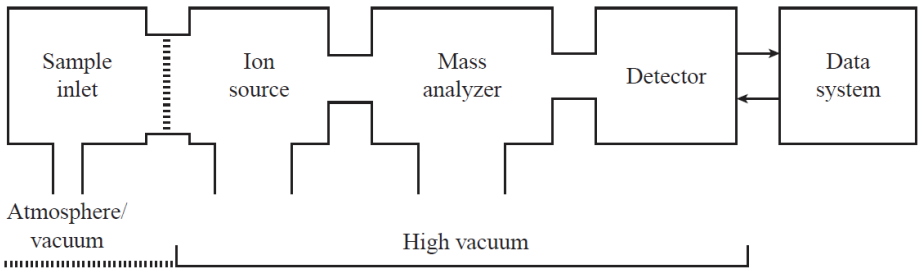


Figure 8: Components of mass spectrometer

The sample inlet is the primary component of the mass spectrometer. Sample enters into the inlet from the atmospheric pressure to the lower pressure of the mass spectrometer. Sample molecules are converted into gas phase ions in ion source unit**.** Electrospray ionization (ESI) is very convenient technique for analysis of high molecular weight, labile and nonvolatile compounds. In ESI, solution of sample molecules is sprayed out into a heated chamber through a fine capillary. Sample solution possesses a high voltage potential across its surface. Small charged droplets are throw out into the ionization chamber. Solvent molecules are evaporated by drying gas from the droplets. As a result the charge density of each droplet increases until the electrostatic repulsive forces exceed the surface tension of the droplet that is the Rayleigh limit. At this point the droplets break apart into smaller droplets (Figure 9).

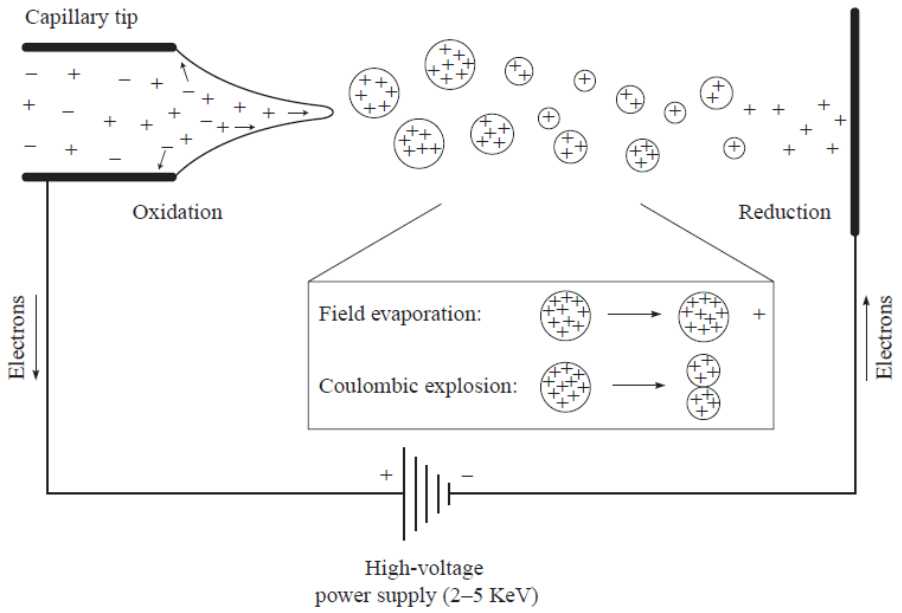


Figure 9: Mechanism of formation of small charged droplet

The ions are accelerated by an electromagnetic field then the mass analyzer separates the sample ions based on their mass-to-charge (*m/z*) ratio. The ions those have correct *m/z* values get the stable trajectories within the RF/DC quadrupole field. Ions with incorrect *m/z* values collide with the rods, or walls of the vacuum chamber and then neutralised. This can scan masses sequentially. Precursor ions are created in quadrupole 1 (Q1) and fragment ions are created in quadrupole 3 (Q3). Q2 is the collision cell where the mass fragmentation occurs. The ions are counted by the detector**.** The detector is usually an electron multiplier (Figure 10) **[52]**.

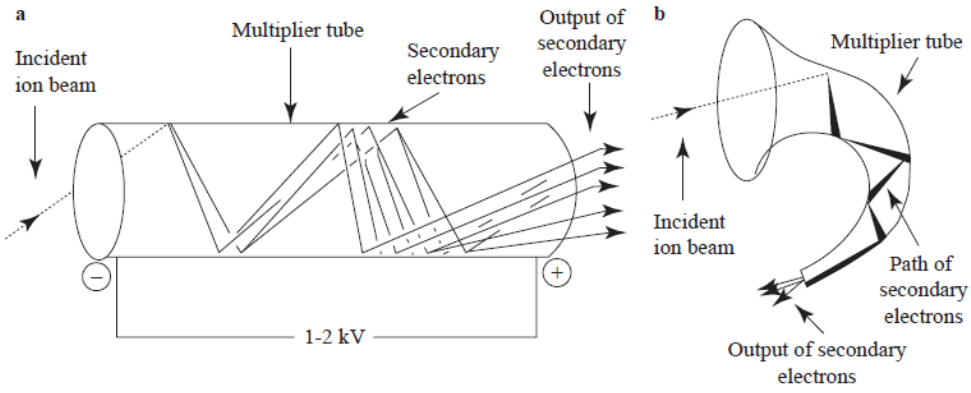


Figure 10: Diagram of electron multiplier

1.3.5 Gas chromatograph-mass spectrometer (GC-MS)

GC is very efficient equipment for separation of multi components. In MS identification of molecule is deduce from mass spectrum. GC-MS is a composite equipment which have all advantages of GC and MS. GC-MS usually has two vacuum pumps. High vacuum pump is called turbo molecular pump, evacuate carrier gas from GC and maintain the MS at higher vacuum. Low vacuum pump reduces the exhaust of the turbo molecular pump. The MS part comprises of ion source unit, mass separation unit and detection unit. Ion source ionizes sample molecules in vacuum. Mass separation unit separates ions according to their m/z ratio and detection unit detects ions. Electron ionization (EI) is a common ionization technique in GC-MS. Thermal electrons emitted from filament and hit the gaseous molecules then the molecules are ionized. This is called hard ionization. Molecular weight is derived from molecular ion and chemical structure is derived from fragment ions. Mass separation unit is usually quadrupole mass analyzer it allow only the ions to reach the detector which have right m/z value. The detector is usually an electron multiplier. Figure 11 shows the diagram of quadrupole mass analyzer **[53].**

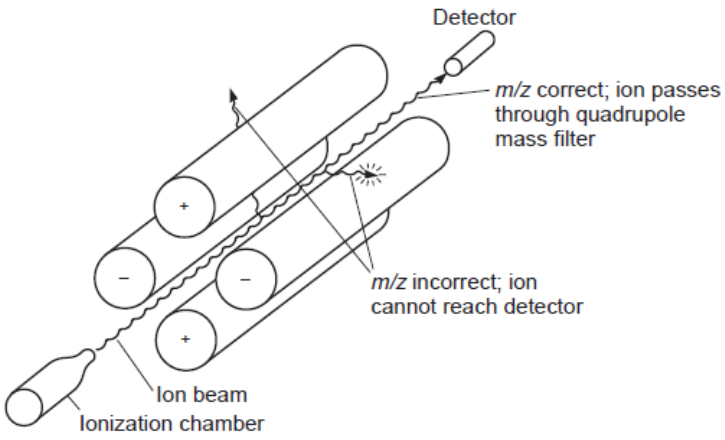


Figure 11: Diagram of quadrupole mass analyzer

1.3.6 QuEChERS method

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach consist of the principle of dispersive solid-phase extraction (d-SPE). Acetonitrile (ACN) was used in QuEChERS method for extraction of a 10 g homogenized sample. Partitioning of the water from the sample happened by using anhydrous $MgSO_4$ and NaCl. Samples are clean-up using d-SPE with anhydrous $MgSO_4$, primary secondary amine (PSA), C18 and graphitized carbon black (GCB) **[54].** QuEChERS is a very flexible method and it can be modified depending on the analytes, matrices and analytical equipments.

1.3.7 Immunoaffinity method

Immunoaffinity method is based on the use of antibodies specific to the molecule of interest. This facilitating its final identification and quantification **[55].** Immunoaffinity column (IAC) containing antibodies specific for aflatoxins B_1, B_2, G_1 and G_2 was used in this study. The aflatoxins are isolated, purified and concentrated on the column then removed from the antibodies with methanol. Post-column derivatization (PCD) is achieved with electrochemically generated bromine.

1.4 Objectives of this study

Chemical pesticides are heavily used in Bangladesh for high yielding of agricultural product. Most of the time farmers give over doses of pesticides in their agricultural field. Current study has been designed to determine the residue levels of some commonly used pesticides in fruits and vegetable samples collected from different region in Bangladesh. Environmental contaminants are widely distributed in nature and can easily enter in food chain. In this study As, Pb and Cd will be determined in fruits and vegetable samples. Pb and Cr are to be determined in turmeric powder. Aflatoxin is produced due fungal growth. Aflatoxins B_1, B_2, G_1 and G_2 will be determined in maize and wheat in this study. Food preservatives are profoundly used in Bangladesh. In this study benzoic acid and sorbic acid are to be determined in fruit and vegetable product. Sudan dyes are synthetic azo dyes which are not permitted by the authorities in different countries across the world for the purpose of food colouring. In the current study sudan dyes I, II, III, IV are to be determined in chili powder. Food products of animal origin containing residual antibiotic became a major concern human health. In this study, antibiotic residues in pasteurized milk are to be investigated. In this study sample preparation involves the modified QuEChERS approach for pesticide residue analysis of fruit and vegetable samples using GC-ECD, GC-MS and LC-MS/MS **[56]**. A hybrid tandem mass spectrometer equipped with electrospray (ESI)

ionization, triple quadrupole (QqQ) mass analyzer with linear ion trap (LIT) will be used for pesticide residue analysis. Also for antibiotic residue in pasteurized milk modified QuEChERS will be applied using LC-MS/MS. Heavy metals As, Pb and Cd in fruits and vegetables sample will be analyzed by AAS-GFA and also Pb and Cr in turmeric powder will be analyzed by AAS-GFA. Freeze drier and microwave digester will be used for sample preparation of fruit, vegetable and turmeric powder. Aflatoxins B_1, B_2, $G_1$and G_2 will be determined in maize and wheat by HPLC-FLD equipped with coring cell as post column derivatization system **[57].** Phosphate buffered saline is used for extraction of sample and clean-up by immunoafinity column for analysis of aflatoxins. Benzoic acid and sorbic acid in fruit drinks and tomato ketchup will be determined by HPLC-UV **[58]**. Extraction of benzoic acid and sorbic acid from sample will be performed using a mixture of ammonium acetate buffer solution and methanol, under specific pH condition. Sudan dyes I, II, III, IV are to be determined in Chili Powder by HPLC-UV **[59]**. Sudan dyes I, II, III, IV will be extracted from chili powder by ethanol. All analytical methods will be validated in line with international guideline, Eurachem **[60]**.

Therefore objectives of this study are to identify and quantify:

- Pesticide residues in fruits and vegetables
- Heavy metals (As, Pb and Cd) in fruits and vegetables.
- Heavy metals (Pb and Cr) in turmeric powder
- Aflatoxins (B_1, B_2, G_1 and G_2) in wheat and maize
- Preservatives-benzoic acid and sorbic acid in fruit drinks and tomato ketchup
- Colouring agent sudan I, II, III, IV in chili powder
- Antibiotic residues in pasteurized milk

2. Experimental

2.1 Materials

2.1.1 Chemicals, Reagent and Solvents:

All Chemicals, reagents and solvents used in thesis were procured from Sigma-Aldrich, Germany. Ammonium Formate (NH_3-fomate), Formic Acid (HCOOH) used were LC and LC-MS grades. All other chemicals and reagents solvents; Graphitize Carbon Black (GCB), Primary Secondary Amine (PSA), glacial acetic acid (AcOH), potassium hexacyanoferrate (II) $K_4[Fe(CN)_6].H_2O$, potassium bromide (KBr), magnesium sulfate ($MgSO_4$), potassium dihydrogen phosphate (KH_2PO_4), anhydrous disodium hydrogen phosphate (anhydr. Na_2HPO4), zinc sulfate ($ZnSO_4$), sodium chloride (NaCl), nitric acid (HNO_3) 65%, ammonium acetate (NH_4COCH_3), hydrogen peroxide (H_2O_2), magnesium nitrate [$Mg(NO_3)_2$] which used for the analysis were reagent grade.

2.1.2 Certified Reference Materials (CRMs)

(a) Mixed CRM standard of α-BHC (99.9% purity), γ-BHC (99.9% purity), β-BHC (96.2% purity), δ-BHC (99.9% purity), heptachlor (99.9% purity), aldrin (99.9% purity), heptachlor epoxide (99.9% purity), α-chlordane (99.9% purity), γ-chlordane (99.9% purity), α-endosulfan (99.2% purity), 4 ,4′ DDE (99.0% purity), dieldrin (99.9% purity), endrin (97.7% purity), β-endosulfan (99.9% purity), 4,4′ DDD (97.6% purity), 4, 4'DDT (98.0% purity), endrin aldehyde (99.9% purity), endosulfan sulphate (99.4% purity) and methoxychlor (99.9% purity) was purchased from Sigma-Aldrich, Germany.

(b) Mixed CRM standard of methamidophos (99% purity), acephate (99% purity), ethoprophos (99% purity), dimethoate (99% purity), diazinon (99% purity), methyl parathion (99% purity), metalaxyl (99% purity), fenitrothion (99% purity), malathion (99% purity), fenthion (99% purity), chlorpyrifos (99% purity), quinalphos (99% purity), methidathion (99% purity), fenamiphos (99% purity), ethion (99% purity) and propiconazole (99% purity) was purchased from Restek, USA.

(c) Mixed Certified Reference Materials (CRM) of 85 Organophosphorus Pesticides (OPPs); Mixed CRM standard of acephate (99% purity), acetamiprid (99% purity), buprofezin (99% purity), Carbaryl (99% purity), clothianidin (99% purity), cymoxanil (99% purity), dicrotophos (96% purity), dimethomorph (99% purity), dinotefuran (99% purity), formetanate HCl (85% purity), hexythiazox (99% purity), imazalil (99% purity), Imidacloprid (99% purity), linuron

(99% purity), metalaxyl (99% purity), methamidophos (99% purity), methomyl (99% purity), monocrotophos (98% purity), omethoate (99% purity), piperonyl butoxide (95% purity), prochloraz (99% purity), propamocarb (99% purity), propargite (99% purity), pyraclostrobin (99% purity), pyridaben (99% purity), pyrimethanil (99% purity), spinosad (97% purity), spiromesifen (99% purity), thiabendazole (99% purity), thiamethoxam (96% purity), trifloxystrobin (99% purity), aldicarb sulfoxide (99% purity), aldicarb (99% purity), benalaxyl (99% purity), bendiocarb (99% purity), bifenazate (99% purity), carbetamide (99% purity), carbofuran (99% purity), carboxin (99% purity), carfentrazone (99% purity) , diflubenzuron (99% purity), dioxacarb (99% purity), diuron (99% purity), fenamidone (99% purity), fenazaquin (99% purity), fenhexamid (99% purity), furalaxyl (99% purity), furathiocarb (99% purity), iprovalicarb (99% purity), isoprocarb (99% purity), mefenacet (99% purity), metconazole (99% purity), methiocarb (99% purity), oxamyl (99% purity), propham (99% purity), propoxur (99% purity), spiroxamine (98% purity), zoxamide (99% purity), 3-hydroxy carbofuran (99% purity), aminocarb (99% purity), bitertanol (99% purity), bupirimate (99% purity), clofentezine (99% purity), Difenoconazole (99% purity), epoxiconazole (99% purity), fenbuconazole (99% purity), fenuron (99% purity), flusilazole (99% purity), flutriafol (98% purity), fuberidazole (98% purity), isoproturon (99% purity), metobromuron (99% purity), Mevinphos (98% purity), nitenpyram (99% purity), paclobutrazol (99% purity), phoxim (99% purity), pymetrozine (99% purity), tebuconazole (99% purity), tebuthiuron (99% purity), temephos (94% purity), thiacloprid (99% purity), triadimefon (99% purity), triazophos (99% purity), tricyclazole (99% purity) and triflumizole (99% purity) were purchased from Restek, USA.

(d) CRM standard of heavy metals; arsenic (98.8% purity), lead (98.9% purity), cadmium (99% purity) and chromium (98.9%); CRMs of aflatoxins; B_1, B_2, G_1 and G_2 (99% purity); CRMs of food preservatives benzoic acid (100% purity) and sorbic acid (99.7% purity); CRMs of artificial colouring agent; sudan red I (95% purity), II (90% purity), III (92% purity) and IV (88% purity) and CRMs of antibiotics; ciprofloxacin (99.4% purity), levofloxacin (99.7% purity), enrofloxacin (99.8% purity), tetracycline (96.7% purity), oxytetracycline (96.1% purity) and chlortetracycline (93.3% purity) were purchased from Sigma Aldrich, Germany.

2.1.3 Equipment and Apparatus

Major equipment used for different studies are Liquid Chromatograph Mass Spectrometer-Mass Spectrometer with Electro spray ionization and Ion Trap Mass Analyzer (Tandem Spectrometer; LC-MS/MS; model:AB SCIEX 4000 QTRAP® system) and Liquid Chromatograph Mass Spectrometer-Mass Spectrometer with Electro spray ionization and Triple Quadrupole Mass Analyzer (LC-MS/MS-ESI-QQQ; model: Shimadzu 8060, Japan), gas chromatograph coupled with electron capture detector (GC-ECD, model: 2010, Shimadzu), Gas Chromatograph and Mass Spectrometer (GC-MS, Model: QP 2010 Ultra Shimadzu) equipped with electron ionization (EI), High Performance Liquid Chromatograph having Ultra Violet Detector (HPLC-UV; Model: Prominence, Shimadzu) & Fluorescence Detector (HPLC-FLD) and Atomic Absorption Spectrophotometer with Graphite Furnace Atomizer (AAS-GFA; Model: AA 7000, Shimadzu). Operational performance of all the equipments used in this study was checked by manufacturer's authorized and trained personnel.

Minor equipment and apparatus used were Microwave Digester (Milestone), Analytical Balance (Sartorius), Kitchen Homogeneizer (IKA, Korea), Centrifuge Machine (Hermle Z 216 MK) Vortex Mixture (Stuart SA 7), Ultrasonic bath, Solvent filtration system and Solid Phase Extraction unit (Supelco), Sample Grinder, Micropipettes (Eppendorf), Volumetric flask, Graduated glass pipettes, Tefelon made Centrifuge tubes, small centrifuge tube, sample vials for GC, GC-MS, LC and LC-MS/MS. All measuring equipments and glassware used in this study were calibrated by National Metrology Laboratory (NML). Pictures of major equipment used to carry out the research were given in Figure 12.

(a)

(b)

(c)

(d)

(e)

(f)

Figure12: (a) GC-ECD (Shimadzu 2010), (b) GC-MS (Shimadzu QP 2010 Ultra), (c) LC-MS/MS (AB SCIEX 4000 QTRAP®), (d) LC-MS/MS (Shimadzu 8060) (e) AAS (Shimadzu AA 7000), (f) HPLC-UV/FLD (Shimadzu Prominence)

2.2 Analysis of pesticide residues and heavy metals in fruits and vegetables

2.2.1 Sample collection of fruits and vegetables

Cabbage (*Brassica oleracea*), green chili (*Capsicum frutescens*), tomato (*Solanum lycopersicum*), carrot (*Daucus carota subsp. sativus*), cauliflower (*Brassica oleracea var. botrytis*), potato (*Solanum tuberosum*), green bean (*Phaseolus vulgaris*), long bean (*Vigna unguiculata ssp. sesquipedalis*), coriander leaf (*Coriandrum sativum*), eggplant (*Solanum melongena*), red amaranth (*Amaranthus cruentus*), lettuce (*Lactuca sativa*), capsicum (*Capsicum annuum*), banana (*Musa acuminata*), red apple (*Malus domestica*), green apple (*Malus*

domestica), dates (*Phoenix dactylifera*), orange (*Citrus X sinensis*), grape (*vitis vinifera*), pineapple (*Ananas comosus*), and mango (*Mangifera indica*) samples were collected from thirty five city corporation markets of seven divisions (Barisal, Chattogram, Dhaka, Khulna, Rajshahi, Rangpur, Sylhet) of Bangladesh. The name of the markets are --Notun Bazar Market, Chumatha Bazar Marker, Bottola Market, Ferry Ghat Bazar Market, and Police line Market of Barisal. Karnaphuli Market, Reazuddin Market, Kazir Dewri Market, Chawk Bazar Market, and Bohaddarhat Market of Chattogram. Kawran Bazar, Mohakhali Kacha Bazar, Mirpur-1 Kacha Bazar, Mohammadpur Krishi Market, and Gulshan-1 Kacha Bazar of Dhaka. Boikali Bazar, Banorgati Bazar, Rupsha Paikari Kacha Bazar, Jorakol Bazar and Boro Bazar of Khulna. Shaheb Bazar, Shiroil Kacha Bazar, Laksmipur Kacha Bazar, New Market Bazar and Rail Gate Bazar of Rajshahi. Rangpur Poura Market, Dhap Kacha Bazar, Satrasta Mahigonj Kacha Bazar, Kamal Kasna Kacha Bazar and C.O bazar market of Rangpur. City Super Market, Bondor Bazar, Modhuful Market, Hawkers Market, and Narinda Bazar of Sylhet. Eight (08) different fruits and thirteen (13) varieties of vegetables were collected from each of the mentioned 35 markets. Total 280 fruits sample and 455 vegetable samples were collected and kept into polyethylene zipper bags, properly labeled, and put into chill boxes with ice pads and carried to the laboratory and were stored in a freezer at -18 °C temperature condition until analysis was carried out. Picture of some fruits and vegetables were given in Figure 13. The sample IDs of fruits were given in Table 1 and 2. The sample IDs of vegetables were given in Table 3, 4 and 5.

(a) (b)

Figure 13: (a) Picture of some fruits sample and (b) Picture of some vegetables sample

Table 1: The sample IDs of fruits

Name of Market	Name of fruits and sample ID			
	Banana	**Red Apple**	**Green Apple**	**Dates**
Kawran Bazar, Dhaka	Ban 01	RA 01	GA 01	Dat 01
Mohakhali Kacha Bazar, Dhaka	Ban 02	RA 02	GA 02	Dat 02
Mirpur-1 Kacha Bazar, Dhaka	Ban 03	RA 03	GA 03	Dat 03
Mohammadpur Krishi Market, Dhaka	Ban 04	RA 04	GA 04	Dat 04
Gulshan-1 Kacha Bazar, Dhaka	Ban 05	RA 05	GA 05	Dat 05
Karnaphuli Market, Chattogram	Ban 06	RA 06	GA 06	Dat 06
Reazuddin Market, Chattogram	Ban 07	RA 07	GA 07	Dat 07
Kazir Dewri Market, Chattogram	Ban 08	RA 08	GA 08	Dat 08
Chawk Bazar Market, Chattogram	Ban 09	RA 09	GA 09	Dat 09
Bohaddarhat Market, Chattogram	Ban 10	RA 10	GA 10	Dat 10
Notun Bazar Market, Barisal	Ban 11	RA 11	GA 11	Dat 11
Chumatha Bazar Marker, Barisal	Ban 12	RA 12	GA 12	Dat 12
Bottola Market, Barisal	Ban 13	RA 13	GA 13	Dat 13
Ferry Ghat Bazar, Barisal	Ban 14	RA 14	GA 14	Dat 14
Police line Market, Barisal	Ban 15	RA 15	GA 15	Dat 15
Shaheb Bazar, Rajshahi	Ban 16	RA 16	GA 16	Dat 16
Shiroil Kacha Bazar, Rajshahi	Ban 17	RA 17	GA 17	Dat 17
Kacha Bazar, Rajshahi	Ban 18	RA 18	GA 18	Dat 18
New Market Bazar, Rajshahi	Ban 19	RA 19	GA 19	Dat 19
Rail Gate Bazar, Rajshahi	Ban 20	RA 20	GA 20	Dat 20
Rangpur Poura Market, Rangpur	Ban 21	RA 21	GA 21	Dat 21
Dhap Kacha Bazar, Rangpur	Ban 22	RA 22	GA 22	Dat 22
Satrasta Mahigonj Kacha Bazar, Rangpur	Ban 23	RA 23	GA 23	Dat 23
Kamal Kasna Kacha Bazar, Rangpur	Ban 24	RA 24	GA 24	Dat 24
C.O Bazar, Rangpur	Ban 25	RA 25	GA 25	Dat 25
Boikali Bazar, Khulna	Ban 26	RA 26	GA 26	Date 26
Banorgati Bazar, Khulna	Ban 27	RA 27	GA 27	Dat 27
Rupsha Paikari Kacha Bazar, Khulna	Ban 28	RA 28	GA 28	Dat 28
Jorakol Bazar, Khulna	Ban 29	RA 29	GA 29	Dat 29
Boro Bazar, Khulna	Ban 30	RA 30	GA 30	Dat 30
City Super Market, Sylhet	Ban 31	RA 31	GA 31	Dat 31
Bondor Bazar, Sylhet	Ban 32	RA 32	GA 32	Dat 32
Modhuful Market, Sylhet	Ban 33	RA 33	GA 33	Dat 33
Hawkers Market, Sylhet	Ban 34	RA 34	GA 34	Dat 34
Narinda Bazar, Sylhet	Ban 35	RA 35	GA 35	Dat 35

Table 2: The sample IDs of fruits

Name of Market	Name of fruits and sample ID			
	Orange	**Grape**	**Pineapple**	**Mango**
Kawran Bazar, Dhaka	Org 01	Grp 01	Pin 01	Man 01
Mohakhali Kacha Bazar, Dhaka	Org 02	Grp 02	Pin 02	Man 02
Mirpur-1 Kacha Bazar, Dhaka	Org 03	Grp 03	Pin 03	Man 03
Mohammadpur Krishi Market, Dhaka	Org 04	Grp 04	Pin 04	Man 04
Gulshan-1 Kacha Bazar, Dhaka	Org 05	Grp 05	Pin 05	Man 05
Karnaphuli Market, Chattogram	Org 06	Grp 06	Pin 06	Man 06
Reazuddin Market, Chattogram	Org 07	Grp 07	Pin 07	Man 07
Kazir Dewri Market, Chattogram	Org 08	Grp 08	Pin 08	Man 08
Chawk Bazar Market, Chattogram	Org 09	Grp 09	Pin 09	Man 09
Bohaddarhat Market, Chattogram	Org 10	Grp 10	Pin 10	Man 10
Notun Bazar Market, Barisal	Org 11	Grp 11	Pin 11	Man 11
Chumatha Bazar Marker, Barisal	Org 12	Grp 12	Pin 12	Man 12
Bottola Market, Barisal	Org 13	Grp 13	Pin 13	Man 13
Ferry Ghat Bazar, Barisal	Org 14	Grp 14	Pin 14	Man 14
Police line Market, Barisal	Org 15	Grp 15	Pin 15	Man 15
Shaheb Bazar, Rajshahi	Org 16	Grp 16	Pin 16	Man 16
Shiroil Kacha Bazar, Rajshahi	Org 17	Grp 17	Pin 17	Man 17
Kacha Bazar, Rajshahi	Org 18	Grp 18	Pin 18	Man 18
New Market Bazar, Rajshahi	Org 19	Grp 19	Pin 19	Man 19
Rail Gate Bazar, Rajshahi	Org 20	Grp 20	Pin 20	Man 20
Rangpur Poura Market, Rangpur	Org 21	Grp 21	Pin 21	Man 21
Dhap Kacha Bazar, Rangpur	Org 22	Grp 22	Pin 22	Man 22
Satrasta Mahigonj Kacha Bazar, Rangpur	Org 23	Grp 23	Pin 23	Man 23
Kamal Kasna Kacha Bazar, Rangpur	Org 24	Grp 24	Pin 24	Man 24
C.O Bazar, Rangpur	Org 25	Grp 25	Pin 25	Man 25
Boikali Bazar, Khulna	Org 26	Grp 26	Pin 26	Man 26
Banorgati Bazar, Khulna	Org 27	Grp 27	Pin 27	Man 27
Rupsha Paikari Kacha Bazar, Khulna	Org 28	Grp 28	Pin 28	Man 28
Jorakol Bazar, Khulna	Org 29	Grp 29	Pin 29	Man 29
Boro Bazar, Khulna	Org 30	Grp 30	Pin 30	Man 30
City Super Market, Sylhet	Org 31	Grp 31	Pin 31	Man 31
Bondor Bazar, Sylhet	Org 32	Grp 32	Pin 32	Man 32
Modhuful Market, Sylhet	Org 33	Grp 33	Pin 33	Man 33
Hawkers Market, Sylhet	Org 34	Grp 34	Pin 34	Man 34
Narinda Bazar, Sylhet	Org 35	Grp 35	Pin 35	Man 35

Table 3: The sample IDs of vegetables

Name of Market	Name of vegetables and sample ID				
	Cabbage	**Green Chili**	**Tomato**	**Carrot**	**Cauliflower**
Kawran Bazar, Dhaka	Cab 01	GC 01	T 01	C 01	CF 01
Mohakhali Kacha Bazar, Dhaka	Cab 02	GC 02	T 02	C 02	CF 02
Mirpur-1 Kacha Bazar, Dhaka	Cab 03	GC 03	T 03	C 03	CF 03
Mohammadpur Krishi Market, Dhaka	Cab 04	GC 04	T 04	C 04	CF 04
Gulshan-1 Kacha Bazar, Dhaka	Cab 05	GC 05	T 05	C 05	CF 05
Karnaphuli Market, Chattogram	Cab 06	GC 06	T 06	C 06	CF 06
Reazuddin Market, Chattogram	Cab 07	GC 07	T 07	C 07	CF 07
Kazir Dewri Market, Chattogram	Cab 08	GC 08	T 08	C 08	CF 08
Chawk Bazar Market, Chattogram	Cab 09	GC 09	T 09	C 09	CF 09
Bohaddarhat Market, Chattogram	Cab 10	GC 10	T 10	C 10	CF 10
Notun Bazar Market, Barisal	Cab 11	GC 11	T 11	C 11	CF 11
Chumatha Bazar Marker, Barisal	Cab 12	GC 12	T 12	C 12	CF 12
Bottola Market, Barisal	Cab 13	GC 13	T 13	C 13	CF 13
Ferry Ghat Bazar, Barisal	Cab 14	GC 14	T 14	C 14	CF 14
Police line Market, Barisal	Cab 15	GC 15	T 15	C 15	CF 15
Shaheb Bazar, Rajshahi	Cab 16	GC 16	T 16	C 16	CF 16
Shiroil Kacha Bazar, Rajshahi	Cab 17	GC 17	T 17	C 17	CF 17
Kacha Bazar, Rajshahi	Cab 18	GC 18	T 18	C 18	CF 18
New Market Bazar, Rajshahi	Cab 19	GC 19	T 19	C 19	CF 19
Rail Gate Bazar, Rajshahi	Cab 20	GC 20	T 20	C 20	CF 20
Rangpur Poura Market, Rangpur	Cab 21	GC 21	T 21	C 21	CF 21
Dhap Kacha Bazar, Rangpur	Cab 22	GC 22	T 22	C 22	CF 22
Satrasta Mahigonj Kacha Bazar, Rangpur	Cab 23	GC 23	T 23	C 23	CF 23
Kamal Kasna Kacha Bazar, Rangpur	Cab 24	GC 24	T 24	C 24	CF 24
C.O Bazar, Rangpur	Cab 25	GC 25	T 25	C 25	CF 25
Boikali Bazar, Khulna	Cab 26	GC 26	T 26	C 26	CF 26
Banorgati Bazar, Khulna	Cab 27	GC 27	T 27	C 27	CF 27
Rupsha Paikari Kacha Bazar, Khulna	Cab 28	GC 28	T 28	C 28	CF 28
Jorakol Bazar, Khulna	Cab 29	GC 29	T 29	C 29	CF 29
Boro Bazar, Khulna	Cab 30	GC 30	T 30	C 30	CF 30
City Super Market, Sylhet	Cab 31	GC 31	T 31	C 31	CF 31
Bondor Bazar, Sylhet	Cab 32	GC 32	T 32	C 32	CF 32
Modhuful Market, Sylhet	Cab 33	GC 33	T 33	C 33	CF 33
Hawkers Market, Sylhet	Cab 34	GC 34	T 34	C 34	CF 34
Narinda Bazar, Sylhet	Cab 35	GC 35	T 35	C 35	CF 35

Table 4: The sample IDs of vegetables

Name of Market	Name of vegetables and sample ID				
	Potato	**Green Been**	**Long Been**	**Coriander Leaf**	**Eggplant**
Kawran Bazar, Dhaka	P 01	GB 01	LB 01	CL 01	B 01
Mohakhali Kacha Bazar, Dhaka	P 02	GB 02	LB 02	CL 02	B 02
Mirpur-1 Kacha Bazar, Dhaka	P 03	GB 03	LB 03	CL 03	B 03
Mohammadpur Krishi Market, Dhaka	P 04	GB 04	LB 04	CL 04	B 04
Gulshan-1 Kacha Bazar, Dhaka	P 05	GB 05	LB 05	CL 05	B 05
Karnaphuli Market, Chattogram	P 06	GB 06	LB 06	CL 06	B 06
Reazuddin Market, Chattogram	P 07	GB 07	LB 07	CL 07	B 07
Kazir Dewri Market, Chattogram	P 08	GB 08	LB 08	CL 08	B 08
Chawk Bazar Market, Chattogram	P 09	GB 09	LB 09	CL 09	B 09
Bohaddarhat Market, Chattogram	P 10	GB 10	LB 10	CL 10	B 10
Notun Bazar Market, Barisal	P 11	GB 11	LB 11	CL 11	B 11
Chumatha Bazar Marker, Barisal	P 12	GB 12	LB 12	CL 12	B 12
Bottola Market, Barisal	P 13	GB 13	LB 13	CL 13	B 13
Ferry Ghat Bazar, Barisal	P 14	GB 14	LB 14	CL 14	B 14
Police line Market, Barisal	P 15	GB 15	LB 15	CL 15	B 15
Shaheb Bazar, Rajshahi	P 16	GB 16	LB 16	CL 16	B 16
Shiroil Kacha Bazar, Rajshahi	P 17	GB 17	LB 17	CL 17	B 17
Kacha Bazar, Rajshahi	P 18	GB 18	LB 18	CL 18	B 18
New Market Bazar, Rajshahi	P 19	GB 19	LB 19	CL 19	B 19
Rail Gate Bazar, Rajshahi	P 20	GB 20	LB 20	CL 20	B 20
Rangpur Poura Market, Rangpur	P 21	GB 21	LB 21	CL 21	B 21
Dhap Kacha Bazar, Rangpur	P 22	GB 22	LB 22	CL 22	B 22
Satrasta Mahigonj Kacha Bazar, Rangpur	P 23	GB 23	LB 23	CL 23	B 23
Kamal Kasna Kacha Bazar, Rangpur	P 24	GB 24	LB 24	CL 24	B 24
C.O Bazar, Rangpur	P 25	GB 25	LB 25	CL 25	B 25
Boikali Bazar, Khulna	P 26	GB 26	LB 26	CL 26	B 26
Banorgati Bazar, Khulna	P 27	GB 27	LB 27	CL 27	B 27
Rupsha Paikari Kacha Bazar, Khulna	P 28	GB 28	LB 28	CL 28	B 28
Jorakol Bazar, Khulna	P 29	GB 29	LB 29	CL 29	B 29
Boro Bazar, Khulna	P 30	GB 30	LB 30	CL 30	B 30
City Super Market, Sylhet	P 31	GB 31	LB 31	CL 31	B 31
Bondor Bazar, Sylhet	P 32	GB 32	LB 32	CL 32	B 32
Modhuful Market, Sylhet	P 33	GB 33	LB 33	CL 33	B 33
Hawkers Market, Sylhet	P 34	GB 34	LB 34	CL 34	B 34
Narinda Bazar, Sylhet	P 35	GB 35	LB 35	CL 35	B 35

Table 5: The sample IDs of vegetables

Name of Market	Name of vegetables and sample ID		
	Red Amaranth	**Lettuce**	**Capsicum**
Kawran Bazar, Dhaka	AM 01	Let 01	Cap 01
Mohakhali Kacha Bazar, Dhaka	AM 02	Let 02	Cap 02
Mirpur-1 Kacha Bazar, Dhaka	AM 03	Let 03	Cap 03
Mohammadpur Krishi Market, Dhaka	AM 04	Let 04	Cap 04
Gulshan-1 Kacha Bazar, Dhaka	AM 05	Let 05	Cap 05
Karnaphuli Market, Chattogram	AM 06	Let 06	Cap 06
Reazuddin Market, Chattogram	AM 07	Let 07	Cap 07
Kazir Dewri Market, Chattogram	AM 08	Let 08	Cap 08
Chawk Bazar Market, Chattogram	AM 09	Let 09	Cap 09
Bohaddarhat Market, Chattogram	AM 10	Let 10	Cap 10
Notun Bazar Market, Barisal	AM 11	Let 11	Cap 11
Chumatha Bazar Marker, Barisal	AM 12	Let 12	Cap 12
Bottola Market, Barisal	AM 13	Let 13	Cap 13
Ferry Ghat Bazar, Barisal	AM 14	Let 14	Cap 14
Police line Market, Barisal	AM 15	Let 15	Cap 15
Shaheb Bazar, Rajshahi	AM 16	Let 16	Cap 16
Shiroil Kacha Bazar, Rajshahi	AM 17	Let 17	Cap 17
Kacha Bazar, Rajshahi	AM 18	Let 18	Cap 18
New Market Bazar, Rajshahi	AM 19	Let 19	Cap 19
Rail Gate Bazar, Rajshahi	AM 20	Let 20	Cap 20
Rangpur Poura Market, Rangpur	AM 21	Let 21	Cap 21
Dhap Kacha Bazar, Rangpur	AM 22	Let 22	Cap 22
Satrasta Mahigonj Kacha Bazar, Rangpur	AM 23	Let 23	Cap 23
Kamal Kasna Kacha Bazar, Rangpur	AM 24	Let 24	Cap 24
C.O Bazar, Rangpur	AM 25	Let 25	Cap 25
Boikali Bazar, Khulna	AM 26	Let 26	Cap 26
Banorgati Bazar, Khulna	AM 27	Let 27	Cap 27
Rupsha Paikari Kacha Bazar, Khulna	AM 28	Let 28	Cap 28
Jorakol Bazar, Khulna	AM 29	Let 29	Cap 29
Boro Bazar, Khulna	AM 30	Let 30	Cap 30
City Super Market, Sylhet	AM 31	Let 31	Cap 31
Bondor Bazar, Sylhet	AM 32	Let 32	Cap 32
Modhuful Market, Sylhet	AM 33	Let 33	Cap 33
Hawkers Market, Sylhet	AM 34	Let 34	Cap 34
Narinda Bazar, Sylhet	AM 35	Let 35	Cap 35

2.2.2 Extraction and clean-up of pesticide from fruits and vegetables sample (QuEChERS method)

The QuEChERS method was used to extract organochlorine and organophosphorus pesticides from different matrices. Fruits/Vegetable samples were taken out from the freezer and thawed at room temperature, cut into small pieces, and homogenized by a kitchen blender. The homogenized sample (10 g) was taken in a 50 mL Teflon tube (centrifuge tube), 10 mL ACN was added and shaken vigorously for 2 minutes. $MgSO_4$ (7.5 g) and NaCl (1g) were added to it, again shaken vigorously. Then the content of the Teflon tube was centrifuged at 5000 rpm for 5 minutes, 2 mL of the extract was transferred into a small centrifuge tube containing PSA (100 mg), anhydr. $MgSO_4$ (150 mg) and GCB (30 mg), centrifuged at 10000 rpm for 5 minutes. The clear extract was filtered through a 0.22µm PTFE syringe filter and ready for analysis by GC-ECD, GC-MS and LC-MS/MS. Extraction procedure of pesticide from fruits and vegetables sample was given in **Scheme-1**.

Fruits and vegetables sample were homogeneised with a blender

↓

Sample (10 g) was added to Teflon made centrifuge tube (50 mL)

↓

Samples were spiked at desired concentration

↓

ACN was added and shaken vigorously for 2 minutes

↓

$MgSO_4$ and NaCl were added and shaken vigorously

↓

Centrifuge at 5000 rpm for 5 minutes

↓

The extract (2 mL) was transferred to a small centrifuge tube

↓

Vigorously shaken for 2 minutes

↓

Centrifuged at 10000 rpm for 5 minutes

↓

Extract was filtered through a 0.22 µm PTFE syringe filter

Scheme-1: Extraction procedure of pesticide from fruits and vegetables sample

2.2.3 Determination of 19 organochlorine pesticides in fruits and vegetable samples by GC-ECD

2.2.3.1 Preparation standard solution of 19 organochlorine pesticides

Mixed CRM standard of α-BHC, γ-BHC, β-BHC, δ-BHC, heptachlor, aldrin, heptachlor epoxide, α-chlordane, γ-chlordane, α-endosulfan, 4,4′-DDE, dieldrin, endrin, β-endosulfan, 4,4′-DDD, 4,4′-DDT, endrin aldehyde, endosulfan sulfate, and methoxychlor was dissolved in toluene at concentration 2000 mg/L. First stock solution of mixed CRM standards were prepared at the concentration of 1.0 mg/L by taking 0.5 mL of mixed CRM standard (2000 mg/L) in a 100 mL volumetric flask and diluted with toluene. Calibration standards were prepared at concentrations of 10, 20, and 30 µg/L by dilution with toluene. Calibration curves were made and some representative calibration curves were given in Figure-25.

2.2.3.2 GC-ECD operating conditions for analysis of organochlorine pesticides

Injection Port
Injection Mode: Split
Temperature: 250.0 C
Carrier Gas: N_2
Split Ratio: 10.0
Injection Volume: 1µL

Detector ECD
Temperature: 330.0 C
Makeup Gas: N_2

Column Oven
Initial Temperature: 180.0 C
Column Oven Temperature Program:

Rate(C/min)	Temperature(C)	Hold Time (min)
----	180.0	0.0
5.00	220.0	12.0
5.00	260.0	0.0

Total Program Time: 14.44 min

Column Information:
Dimension: 0.25 um × 30.0 m × 0.25 mm ID

Analysis results were given in Table 17 and 18

2.2.3.3 Method validation of 19 OCPs

The tomato sample T 01 was used as a control sample where no targeted 19 OCPs were present in the matrix. For selectivity, blank control sample was run in GC-ECD. The control sample was spiked at concentration 20 µg/L. Then CRM standard (10 µg/L) of 19 different OCPs and spiked control sample were run in GC-ECD with same analytical condition. The chromatograms were given in Figure 20, 21 and 22. For LOD and LOQ ten replicate control tomato sample were spiked at concentration 10 µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control tomato sample. LOD and LOQ were given in Table 13. Calibration

standards were prepared at concentrations of 10, 20, and 30 µg/L of 19 OCPs. The working range was 10-30 µg/L. Calibration curves were made and some representative calibration curves were given in Figure-25. Linear correlation coefficient (R^2) of 19 OCPs were given in Table 14. For accuracy (recovery experiment) control tomato sample was spiked with 19 OCP CRM standard at concentration 20 µg/L. The chromatograms were given in Figure 26 and 27. Recovery was given in Table 15. Precision was calculated from ten replicates of spiked tomato sample with CRM standard of 19 OCPs at concentration 10 µg/kg. Relative standard deviation (RSD%) of 19 OCPs was given in Table 16.

2.2.4 Determination of 16 organophosphorus pesticides in fruits and vegetable samples by GC-MS

2.2.4.1 Preparation of standard solution of 16 organophosphorus pesticides

Mixed CRM standard of methamidophos, ethoprophos, dimethoate, diazinon, methyl parathion, metalaxyl, fenitrothion, malathion, fenthion, chlorpyrifos, quinalphos, methidathion, fenamiphos, ethion and propiconazole was at concentration 100 mg/L in acetonitrile. Stock solution was prepared at concentration of 1.0 mg/L by taking 1 mL of mixed CRM standard (100 mg/L) in 100 mL volumetric flask and diluted with acetonitrile. Calibration standard was prepared at concentration of 5,10, 20 and 50 µg/L by serial dilution with acetonitrile. Calibration curves were made and some representative calibration curves were given in Figure-37

2.2.4.2 GC-MS operating conditions for 16 organophosphorus pesticides

GC Parameter
Column Oven Temp.: 90.0 °C
Injection Temp.: 250.00 °C
Injection Volume: 1µL
Split Ratio: 10.0

MS Parameter
Ion Source Temp: 200.00 °C
Interface Temp.: 250.00 °Cs
Operational Mode: SIM

Oven Programme

Rate	Temperature(°C)	Hold Time(min)
----	90.0	1.0
25.00	180.0	1.0
3.00	270.0	0.0
20.00	310.0	3.00

Column: Rxi® - 5ms (30m x 0.25mm, 0.25µm)

Analysis results were given in Table 24 and 25

2.2.4.3 Method validation of 16 OPPs

Cabbage sample, Cab 01 was used as control sample where no targeted 16 OPPs were present in the sample matrix. For selectivity blank control sample of cabbage was run in GC-MS. The control sample was spiked at concentration 5 µg/L. Then CRM standard of 16 OPPs and spiked control cabblge sample were run with the same operating condition of GC-MS. The chromatograms were given in Figure 30, 31 and 32. For LOD and LOQ ten replicate control cabbage sample were spiked at concentration 5 µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control cabbage sample. LOD and LOQ were given in Table 20. Calibration standards were prepared at concentrations of 5, 10, 20, and 50 µg/L of 16 OPPs. The working range was 5-50 µg/L. Calibration curves were made and some representative calibration curves were given in Figure-37. Linear correlation coefficient (R^2) of 16 OPPs were given in Table 21. Control cabbage sample was spiked with 16 OPP CRM standards at concentration 10 µg/ L for recovery experiment. The chromatograms were given in Figure 38 and 39. Recovery was given in Table 22. Precision was calculated from ten replicates of spiked cabbage sample with CRM standard of 16 OPPs at concentration 20 µg/kg. Relative standard deviation (RSD%) of 16 OPPs were given in Table 23.

2.2.5 Determination of 85 organophosphorus pesticide residues in fruits and vegetable samples by LC-MS/MS

2.2.5.1 Preparation of standard solution of 85 organophosphorus pesticides

CRM standard of 85 organophosphorus pesticides was at concentration 100 µg/mL in acetonitrile in three ampules; Mix-1 (31 pesticides), Mix-2 (27 pesticides), and Mix-3 (27 pesticides)

Calibration standards:

Stock solutions of Mix-1, Mix-2, and Mix- 3 were prepared at a concentration of 1.0 mg/L in three different volumetric flasks. Calibration standards of Mix-1, Mix-2, and Mix-3 were was prepared at the concentration of 3, 6, & 12 µg/L, 6.25, 12.5 & 25 µg/L and 3.75, 7.5 & 15 µg/L, respectively, by serial dilution with ACN. Calibration curves were made and some representative calibration curves were given in Figure-53

2.2.5.2 LC-MS/MS operating condition for 85 organophosphorus pesticides

LC-Parameter

Column: Ultra Aqueous C18 column, 100 mm x 2.1 mm, 3 µm
Column Temperature (°C): 50 °C
Auto-sampler Temperature (°C): 5 °C
Injection Volume (µL): 10
Flow Rate (mL/min): 0.5
Mobile Phase Gradient:
Solvent A: Water with 4 mM NH_4-formate and 0.1% formic acid
Solvent B: Methanol with 4 mMNH_4-formate and 0.1% formic acid

LC-gradient program

Time (min)	%A	%B
0.00	90	10
1.50	90	10
4.00	40	60
8.00	30	70
11.00	0	100
12.00	0	100
12.01	90	10
15.00	90	10

MS Parameter

Maximum Pressure:	255 bar	Ionization Mode:	ESI+
Source Temperature:	350 °C	Collision Gas:	Nitrogen at 10 psi (68.9 kPa)
Ion Spray Voltage:	5.5 kV	Mode:	MRM

Analysis results were given in Table 31 and 32

2.2.5.3 Method validation of 85 OPPs

Control sample for determination of 85 OPPs in fruits and vegetable, the tomato sample T 01 was used, where no targeted 85 OPPs were present in the control sample matrix. For selectivity blank control sample of tomato was run in LC-MS/MS. The control sample was spiked with OPP mix-1, mix-2 and mix-3 at concentration 6, 12.5 and 7.5 µg/L, respectively. Then CRM standard of 31 OPPs in mix-1, 27 OPPs in mix-2 and 27 OPPs in mix-3 and spiked control sample were run with the same analytical condition of LC-MS/MS. The chromatograms were given in Figure 46-49. For LOD and LOQ ten replicates of control tomato sample were spiked at concentration 3, 6.25 and 3.75 µg/L for mix-1, mix-2 and mix-3. LOD and LOQ were calculated from the standard deviation of that ten replicate control tomato sample. LOD and LOQ were given in table 27. Calibration standards of Mix-1, Mix-2, and Mix-3 were prepared at the concentration of 3, 6, & 12 µg/L, 6.25, 12.5 & 25 µg/L and 3.75, 7.5 & 15 µg/L, respectively. The working ranges were 3-12 µg/L, 6.25-25 µg/L and 3.75-15 µg/L for Mix-1, Mix-2, and Mix-3, respectively. Calibration curves were made and some representative calibration curves were given in Figure-53. Linear correlation coefficient (R^2) of 85 OPPs were given in table 28. Control tomato sample was spiked with three mixtures at concentration 6, 12.5 and 7.5 µg/L, respectively for recovery experiment. The chromatograms were given in Figure 54. Recovery was given in Table 29. Precision was calculated from ten replicates of spiked tomato sample with CRM standard of 85 OPPs at concentration 3, 6.25 and 3.75 µg/L. Relative standard deviation (RSD%) of 85 OPPs was given in Table 30.

2.2.6 Determination of As, Pb and Cd in vegetables and fruit by atomic absorption spectrophotometer (AAS) equipped with graphite furnace atomizer (GFA)

2.2.6.1 Extraction of As, Pb and Cd from fruits and vegetable samples

As, Pb and Cd were extracted from fruits and vegetables sample following a procedure is given below in **Scheme-2**

Freeze-dried sample (0.5g) was taken in microwave digestion tube

↓

10 mL 65% HNO_3 and 2 mL 30% H_2O_2 were added

↓

Sample was digested in the microwave digester and cool at room temperature after digestion

↓

Sample was transfered into a 10 mL volumetric flask

↓

500 µL of 2 % Mg $(NO_3)_2$ & 500µL 1% Pd were added in 10 mL volumetric flask
De-ionised water was added to make up to the mark

↓

Sample in volumetric flask were taken for analysis by AAS-GFA

Scheme-2: Extraction procedure of As, Pb and Cd from fruits and vegetable samples

2.2.6.2 Preparation of standard solution of As, Pb and Cd

All analytical standards were at concentration of 1000 mg/L. Primary stock solution of As and Pb were prepared at concentration 10 mg/L by dilution with 0.5% HNO_3. Calibration standard of As and Pb was prepared at concentration of 5, 10, 15 and 20 µg/L by dilution with 0.5% HNO_3. Primary stock solution of Cd was prepared at concentration 4 mg/L by dilution with 0.5% HNO_3. Calibration standards of Cd were made at concentration of 0.2, 0.4, 0.6 and 0.8 µg/L by dilution with 0.5% HNO_3. Calibration curves of As, Pb and Cd were made, the calibration curves were given in Figure-66.

2.2.6.3 AAS-GFA operating condition for As, Pb and Cd

Wavelength was set 193.7 nm, 283.3 nm and 228.8 nm for As, Pb and Cd , respectively. Lamp current was set 12 mA, 10 mA and 8 mA for As, Pb and Cd , respectively. Slit width set for all three element was 0.7 nm. Hollow cathode lamp background was corrected by deuterium lamp. Graphite furnace temperature program of As, Pb and Cd were set following the manufacturer operating manual. Microwave digester operating temperature T1 was 200 °C and T2 was 110 °C. Pressure was 45 bar and power was maximum. In 15 minute temperature was rise up to 200 °C and the temperature was hold for another 15 minute, then temperature was descend to 110 °C in 15 minute. Total run time was 45 minute. Analysis results were given in Table 35-37

2.2.6.4 Method validation of As, Pb and Cd

Potato sample P 01 was taken as control sample where no targeted As, Pb and Cd were present in the sample matrix. For selectivity blank control sample of potato was run in AAS-GFA. The control sample was spiked with CRM standard of As and Pb at concentration 10 µg/L and with CRM standard of Cd at concentration 0.4 µg/L. Then CRM standard of As, Pb and Cd and spiked control sample were run with the same analytical condition of AAS-GFA. The absorption spectrums were given in Figure 59-65. For LOD and LOQ ten replicate control potato sample were spiked with As and Pb at concentration 5 µg/L and with Cd at concentration 0.2µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control potato sample. LOD and LOQ were given in table 33. Calibration standards were prepared at concentrations of 5, 10, 15, and 20 µg/L for As and Pb. For Cd calibration standards were prepared at concentrations of 0.2, 0.4, 0.6, and 0.8 µg/L The working range for As and Pb were 5-20 µg/L and for Cd was 0.2-0.8 µg/L. Calibration curves were made and calibration curves were given in Figure-66. For accuracy (recovery experiment) control potato sample was spiked with As and Pb at concentration 10 µg/L and with Cd at concentration 0.4 µg/L. The absorption spectrums were given in Figure 67-72. Precision was calculated from ten replicates of spiked potato control sample which were spiked with CRM of As and Pb at concentration 5 µg/L and with CRM of Cd at concentration 0.2 µg/L. Relative standard deviation (RSD%) of As, Pb and Cd were given in Table 34.

2.3 Determination of Pb and Cr in turmeric powder by atomic absorption spectrophotometer (AAS) equipped with graphite furnace atomizer (GFA)

2.3.1 Sample Collection

Turmeric (*Curcuma longa*) powder samples (n=17) were collected from five city corporation markets of Dhaka, namely Kawran Bazar, Mohakhali Kacha Bazar, Mirpur-1 Kacha Bazar, Mohammadpur Krishi Market, and Gulshan-1 Kacha Bazar. Three (03) samples were collected from each of four markets and five samples were collected from Gulshan-1 Kacha Bazar. The samples were properly labeled and carried to the laboratory. Samples were stored in room temperature condition. Picture of some turmeric powder sample was given in Figure 14. Sample IDs and place of collection of turmeric powder were given in Table 6.

Figure 14: Picture of some turmeric powder sample

Table 6: Sample IDs of turmeric powder

Turmeric Powder		
Sample ID	**Brand**	**Name of Market**
TP 01	Pran 500 g pack	Kawran Bazar
TP 02	Pran 200 g pack	Kawran Bazar
TP 03	Pran 100 g pack	Kawran Bazar
TP 04	Pran 1000 g pack	Mohakhali kacha bazar
TP 05	Pran 250 g Jar	Mohakhali kacha bazar
TP 06	Pran 200 g Jar	Mohakhali kacha bazar
TP 07	Pran 400 g pack	Mirpur-1 kacha bazar
TP 08	Pran 200 g pack	Mirpur-1 kacha bazar
TP 09	Radhuni 50 g pack	Mirpur-1 kacha bazar
TP 10	Radhuni 100 g pack	Mohammadpur Krishi Bazar
TP 11	Radhuni 200 g pack	Mohammadpur Krishi Bazar
TP 12	Loose turmeric powder	Mohammadpur Krishi Bazar
TP 13	Radhuni 400 g pack	Gulshan-1 kacha Bazar
TP 14	Radhuni 1000 g pack	Gulshan-1 kacha Bazar
TP 15	ACI 200 g pack	Gulshan-1 kacha Bazar
TP 16	Shan 200 g pack	Gulshan-1 kacha Bazar
TP 17	ACI 100 g pack	Gulshan-1 kacha Bazar

2.3.2 Extraction of Pb and Cr from turmeric powder sample

Pb and Cr were extracted from turmeric powder sample following the below procedure in **Scheme-3**

Weighted 0.5 g sample was taken in microwave digestion tube

↓

10 mL 65% HNO_3 and 2 mL 30% H_2O_2 was added

↓

Sample was digested in the microwave digester and cool at room temperature after digestion

↓

Sample was transfered into a 10 mL volumetric flask

↓

500 µL of 2 % Mg $(NO_3)_2$ & 500µL 1% Pd were added in 10 mL volumetric flask
De-ionised water was added to up to the mark

↓

Sample in volumetric flask were taken for analysis by AAS-GFA

Scheme-3: Sample preparation procedure of fruits and vegetable for analysis of Pb and Cr

2.3.3 Standard solution preparation of Pb and Cr

All analytical standards were at a concentration of 1000 mg/L. A primary stock solution of Pb and Cr were prepared at a concentration 10 mg/L by dilution with 0.5% nitric acid (HNO_3). Calibration standards of Pb and Cr were prepared at concentrations of 5, 10, 15, and 20 µg/L by dilution with 0.5% HNO_3. Calibration curves of Pb and Cr were made, the calibration curves were given in Figure-78.

2.3.4 AAS-GFA operating conditions for Pb and Cr

Wavelength was set 283.3 nm and 357.9 nm for Pb and Cr , respectively. Lamp current was set at 10 mA for Pb and Cr. The slit width was set 0.7 nm and 0.5 nm for Pb and Cr , respectively. The hollow cathode lamp background was corrected by a deuterium lamp. Graphite furnace temperature programs of Pb and Cr were set following the manufacturer operating manual. Microwave digester operating temperature T1 was 200 °C and T2 was 110 °C. The pressure was 45 bar and the power was maximum. In 15 minutes the temperature was rising up to 200 °C and the temperature was hold for another 15 minutes, then the temperature descended to 110 °C in 15 minutes. The total run time was 45 minutes. Analysis results were given in Table 40

2.3.5 Method validation of Pb and Cr for determination of turmeric powder

Turmeric powder sample TP 09 was taken as control sample where no targeted Pb and Cr were present in the sample matrix. For selectivity blank control sample of turmeric powder was run in AAS-GFA. The control sample was spiked with CRM of Pb and Cr at concentration 10 µg/L. Then CRM standard of Pb and Cr and spiked control turmeric powder sample were run with the same analytical condition of AAS-GFA. The absorption spectrums were given in Figure 73-77. For LOD and LOQ ten replicate control turmeric powder sample were spiked with Pb and Cr at concentration 5 µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control turmeric powder sample. LOD and LOQ were given in table 38. Calibration standards were prepared at concentrations of 5, 10, 15, and 20 µg/L for Pb and Cr. The working range for Pb and Cr were 5-20 µg/L. Calibration curves were made and calibration curves were given in Figure-78. For accuracy (recovery experiment) control turmeric powder sample was spiked with Pb and Cr at concentration 10 µg/L. The absorption spectrums were given in Figure 79-82. Precision was calculated from ten replicates of spiked turmeric powder control sample which were spiked with CRM standard of Pb and Cr at concentration 5 µg/. Relative standard deviation (RSD%) of Pb and Cr were given in Table 39.

2.4 Determination of aflatoxin B_1, B_2, G_1 and G_2 in wheat and maize by HPLC-FLD

2.4.1 Sample Collection

Wheat (*Triticum*) samples (n=25) were collected from silos of Narayangonj, Chattogram, Santahar, Khulna, and Asugonj and maize (*Zea mays*) samples (n=25) were collected from Bogra and Dinajpur food storage. Five (05) wheat samples were collected from each of the silos and taken into a polyethylene zipper bag, properly labeled and put into cool boxes with an ice pad for carrying to the laboratory. Thirteen (13) maize samples were collected from Bogra food storage and twelve (12) maize samples were collected from Dinajpur food storage. Samples were taken into polyethylene zipper bags, properly labeled and put into cool boxes with an ice pad for carrying to the laboratory. Samples were carried to the laboratory and stored at 4 °C temperature condition in the refrigerator. Picture of some wheat and maize sample were given in Figure 15. Sample IDs and place of collection of wheat and maize were given in Table 7

(a) **(b)**

Figure 15: (a) Picture of some wheat sample and (b) Picture of some maize sample

Table 7: Sample IDs and place of collection of wheat and maize

Wheat		Maize	
Sample ID	**Place of Collection**	**Sample ID**	**Place of Collection**
Wt 01	Narayangonj silo	Mz 01	Bogra Food Storage
Wt 02	Narayangonj silo	Mz 02	Bogra Food Storage
Wt 03	Narayangonj silo	Mz 03	Bogra Food Storage
Wt 04	Narayangonj silo	Mz 04	Bogra Food Storage
Wt 05	Narayangonj silo	Mz 05	Bogra Food Storage
Wt 06	Chattogram silo	Mz 06	Bogra Food Storage
Wt 07	Chattogram silo	Mz 07	Bogra Food Storage
Wt 08	Chattogram silo	Mz 08	Bogra Food Storage
Wt 09	Chattogram silo	Mz 09	Bogra Food Storage
Wt 10	Chattogram silo	Mz 10	Bogra Food Storage
Wt 11	Santahar silo	Mz 11	Bogra Food Storage
Wt 12	Santahar silo	Mz 12	Bogra Food Storage
Wt 13	Santahar silo	Mz 13	Bogra Food Storage
Wt 14	Santahar silo	Mz 14	Dinajpur Food Storage
Wt 15	Santahar silo	Mz 15	Dinajpur Food Storage
Wt 16	Khulna silo	Mz 16	Dinajpur Food Storage
Wt 17	Khulna silo	Mz 17	Dinajpur Food Storage
Wt 18	Khulna silo	Mz 18	Dinajpur Food Storage
Wt 19	Khulna silo	Mz 19	Dinajpur Food Storage
Wt 20	Khulna silo	Mz 20	Dinajpur Food Storage
Wt 21	Asugonj silo	Mz 21	Dinajpur Food Storage
Wt 22	Asugonj silo	Mz 22	Dinajpur Food Storage
Wt 23	Asugonj silo	Mz 23	Dinajpur Food Storage
Wt 24	Asugonj silo	Mz 24	Dinajpur Food Storage
Wt 25	Asugonj silo	Mz 25	Dinajpur Food Storage

2.4.2 Extraction of aflatoxin B_1, B_2, G_1 and G_2 from wheat and maize samples

Preparation of phosphate buffered saline (PBS): PBS was prepared by adding 0.20g potassium chloride, 0.20g potassium dihydrogen phosphate and 1.16g anhydrous disodium hydrogen phosphate to 900 mL ultra-pure water. After dissolution the pH was adjusted to 7.4 (with 0.1 M HCl or 0.1M NaOH as appropriate) and 1.0 L solution was prepared with ultra-pure water. Aflatoxin B_1, B_2, G_1 and G_2 were extracted from wheat and maize sample following the below procedure in **Scheme-4**.

Homogenized 25g of the test sample was weighted into a blender jar

↓

NaCl (5g) and 125 mL of extraction solvent (MeOH:Water, 70:30) were added and homogenized with a mixer for 2 min at high speed

↓

The filtrate (15 mL) was pipette into a conical flask of appropriate size with glass stopper then 30 mL of PBS was added (Total Volume 45 mL)

↓

In case of residual turbidity the sample were filter through a 0.45µm sample filter

↓

The second filtrate (15 mL) was pipette in a beaker and pass it through the conditioned Immunoaffinity (IA) column with gentle vacuum

↓

IA Column was washed with 10 mL water

↓

Finally eluted with 2 mL methanol (1+1) from Immunoaffinity (IA) column and collected in a 2 mL volumetric flask. Diluted to the mark with water.

Scheme-4: Extraction procedure of aflatoxin B_1, B_2, G_1 and G_2 from wheat and maize sample

2.4.3 Standard solution preparation of aflatoxins G_2, G_1, B_2 and B_1

CRM standard of mixed aflatoxins were at concentration of G_2: 0.504 µg/mL, G_1: 2.020 µg/mL, B_2: 0.503 µg /mL and B_1: 2.030 µg/mL. Calibration standards were prepared as follows:

Concentration of Aflatoxin (µg/L)			
G_2	G_1	B_2	B_1
0.252	1.010	0.252	1.015
0.504	2.020	0.503	2.030
0.756	3.030	0.755	3.045
1.260	5.050	1.258	5.057
2.520	10.100	2.515	10.150

Calibration curves of aflatoxin were made (Figure-88)

2.4.4 HPLC-FLD operating condition for aflatoxins

HPLC equipped with a fluorescence detector was used for the analysis of aflatoxin. The column was a reversed-phase C18 and dimensions were 250 mm (length), ´ 4.6mm (internal diameter) and 5 µm (particle size). The flow rate was 1.0 mL/min, the column oven temperature was 40 ºC. The fluorescence detector excitation wavelength was 360 nm and the emission wavelength was 425 nm. The injection volume was 10 µL. The composition of mobile phase A was 1 liter water containing 216.4 mg KBr and 159.1 µL HNO_3 and mobile phase B was methanol. The percentage

ratio of mobile phase A and B was 55% and 45%, respectively. Analysis results were given in Table 44

2.4.5 Method validation of aflatoxins

Wheat sample, Wt 01 was taken as control sample where no targeted aflatoxin G_2, G_1, B_2 and B_1 were present in the sample matrix. For selectivity blank control sample of wheat was run in HPLC-FLD. The control sample was spiked with CRM standards of aflatoxin G_2, G_1, B_2 and B_1 at concentration 0.756, 5.05, 0.755, 3.045 µg/L, respectively. Then CRM standard of aflatoxin G_2, G_1, B_2 and B_1 and spiked control wheat sample were run with the same operating condition of HPLC-FLD. The chromatograms were given in Figure 83, 84 and 84. For LOD and LOQ ten replicate control wheat were spiked with CRM standard of aflatoxin G_2, G_1, B_2 and B_1 at concentration 0.252, 1.010, 0.252 and 1.015 µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control wheat sample. LOD and LOQ were given in table 41. Five level calibration standards were prepared. Working range for aflatoxin G_2, G_1, B_2 and B_1 was 0.252-2.520 µg/L, 1.010-10.100 µg/L, 0.252-2.520 µg/L and 1.015-10.150 µg/L, respectively Calibration curves were made and calibration curves were given in Figure-88. For accuracy (recovery experiment) control wheat sample was spiked with CRM standards of aflatoxin G_2, G_1, B_2 and B_1 at concentration 0.756, 5.050, 0.755, 3.045 µg/L, respectively. The chromatograms were given in Figure 89 and 90. Recovery was given in table 42. Precision was calculated from ten replicates of spiked wheat control sample which was spiked with CRM standard of aflatoxin G_2, G_1, B_2 and B_1 at concentration 1.26, 5.05, 1.256 and 5.057 µg/L, respectively. Relative standard deviation (RSD%) of aflatoxin G_2, G_1 , B_2 and B_1 were given in Table 43.

2.5 Quantitative measurement of benzoic acid and sorbic acid in fruit drinks and tomato ketchup by HPLC

2.5.1 Sample Collection

For determination of benzoic acid and sorbic acid, the fruit drink (n=25) and tomato ketchup (n=27) samples were collected from five city corporation markets of Dhaka, namely Kawran Bazar, Mohakhali Kacha Bazar, Mirpur-1 Kacha Bazar, Mohammadpur Krishi Market, and Gulshan-1 Kacha Bazar. Five (05) fruit drink samples were collected from each of the five markets. Five (05) tomato ketchup samples were collected from each of the four markets and seven samples were collected from Mohammadpur Krishi Market. The sample was properly labeled and put into cool boxes with ice pad for carrying to the laboratory. Samples were carried to the laboratory and stored at 4 °C temperature condition in the refrigerator. Picture of some fruit drink and tomato ketchup sample were given in Figure 16. Sample IDs and place of collection of fruit drink and tomato ketchup were given in Table 8 and 9

(a) (b)

Figure 16: (a) Picture of some fruit drink sample and (b) Picture of some tomato ketchup sample

Table 8: Sample IDs and place of collection of fruit drink

Fruit Drink		
Sample ID	**Brand**	**Name of Market**
FD 01	Pran Fruitix	Kawran Bazar
FD 02	Pran Mango Fruit Drinks	Kawran Bazar
FD 03	Pran Fruit fun Mango Fruit Drink	Kawran Bazar
FD 04	Pran Cocktail Fruit Drink	Kawran Bazar
FD 05	Pran Junior Mixed Fruit Drink	Kawran Bazar
FD 06	Pran Pome Granate Fruit Drink	Mohakhali kacha bazar
FD 07	Pran Latina Apple	Mohakhali kacha bazar
FD 08	Shejan Classic Mango Drink	Mohakhali kacha bazar
FD 09	Shejan premium Mango Drink	Mohakhali kacha bazar
FD 10	Shejan Smart Mango Drink	Mohakhali kacha bazar
FD 11	Shejan Mango Drink PET Bottle	Mirpur-1 kacha bazar
FD 12	Sajeeb Junior Mango Drink	Mirpur-1 kacha bazar
FD 13	Puro Junior Mango Drink	Mirpur-1 kacha bazar
FD 14	Frotina Junior Mango Drink	Mirpur-1 kacha bazar
FD 15	Sajeeb Mango Drink	Mirpur-1 kacha bazar
FD 16	Starship Mango Drink	Mohammadpur Krishi Bazar
FD 17	Frootina Mango Drink	Mohammadpur Krishi Bazar
FD 18	Pran Litchi	Mohammadpur Krishi Bazar
FD 19	Puro Mango Drink	Mohammadpur Krishi Bazar
FD 20	Garden Fresh Mango Fruit Drink 500 mL	Mohammadpur Krishi Bazar
FD 21	Garden Fresh Mango Fruit Drink 200 mL	Gulshan-1 kacha Bazar
FD 22	Kishwan Orange Drink	Gulshan-1 kacha Bazar
FD 23	Frutika Orange Drink	Gulshan-1 kacha Bazar
FD 24	Frutika Red Grape	Gulshan-1 kacha Bazar
FD 25	RoohAfza	Gulshan-1 kacha Bazar

Table 9: Sample IDs and place of collection of tomato ketchup

Tomato Ketchup		
Sample ID	**Brand**	**Name of Market**
TK 01	Ahmed Tomato Ketchup	Kawran Bazar
TK 02	Ahmed Hot Tomato Ketchup	Kawran Bazar
TK 03	BD Tomato Ketchup small pack	Kawran Bazar
TK 04	Ahmed Tomato Ketchup in 4.5 PET Bottle	Kawran Bazar
TK 05	Kissan Tomato Ketchup small bottle	Kawran Bazar
TK 06	Ruchi Tomato Ketchup	Mohakhali kacha bazar
TK 07	Pran the Chef hot Tomato Ketchup	Mohakhali kacha bazar
TK 08	Arisen Tomato Ketchup small Bottle	Mohakhali kacha bazar
TK 09	Arisen Tomato Ketchup big Bottle	Mohakhali kacha bazar
TK 10	Kissan Tomato Ketchup small pack	Mohakhali kacha bazar
TK 11	Ruchi hot Tomato Ketchup	Mirpur-1 kacha bazar
TK 12	Kissan Tomato Ketchup big bottle	Mirpur-1 kacha bazar
TK 13	Kissan Tomato Ketchup	Mirpur-1 kacha bazar
TK 14	Pran Hot Tomato Ketchup Small pack	Mirpur-1 kacha bazar
TK 15	Best Tomato Ketchup	Mirpur-1 kacha bazar
TK 16	Pran Tomato Ketchup	Mohammadpur Krishi Bazar
TK 17	Pran Hot Tomato Ketchup big pack	Mohammadpur Krishi Bazar
TK 18	Pran Tomato Ketchup 250 gm	Mohammadpur Krishi Bazar
TK 19	Pran Tomato Ketchup 350 gm	Mohammadpur Krishi Bazar
TK 20	Heinz tomato ketchup small bottle	Mohammadpur Krishi Bazar
TK 21	Heinz tomato ketchup small pack	Mohammadpur Krishi Bazar
TK 22	Sajeeb tomato ketchup	Mohammadpur Krishi Bazar
TK 23	Kazifarms kitchen tomato ketchup	Gulshan-1 kacha Bazar
TK 24	Best Tomato Ketchup small pack	Gulshan-1 kacha Bazar
TK 25	BD Tomato Ketchup	Gulshan-1 kacha Bazar
TK 26	Shezan Tomato ketchup	Gulshan-1 kacha Bazar
TK 27	Yakin Tomato Ketchup	Gulshan-1 kacha Bazar

2.5.2 Extraction of benzoic acid and sorbic acid from fruit drink and tomato ketchup sample

Benzoic acid and sorbic acid were extraction of from fruit drink and tomato ketchup following the below procedure:

Samples were homogenized carefully. 10 mL of fruit drink and tomato ketchup sample was diluted by approximately 75mL of extraction solution in a 100 mL volumetric flask. The flask was put in the ultrasonic bath, mixed contents for at least 10 min. Then 1 mL clean up solution (I) and 1 mL of clean-up solution (II) was added for clarification. The solution was mixed carefully after each addition and diluted to the mark with the extraction solution at 20 °C. The solution was

filtered through a filter paper, the first mL of filtrate was discarded. Finally the solution was filtered through a micro syringe filter.

2.5.3 Reagent Preparation

Ammonium acetate/Acetic acid buffer solution: 1000 volume parts of ammonium acetate solution was mixed with 1.2 volume parts of acetic acid

Mobile phase preparation: 50 volume parts of ammonium acetate /acetic acid buffer solution was mixed with 40 volume parts of methanol and pH was adjusted to 4.5 to 4.6 with acetic acid. Mobile phase was filtered with vacuum filter and sonicated with ultrasonic bath.

Extraction solution: 60 volume parts of ammonium acetate/acetic acid buffer solution mixed with 40 volume parts of methanol.

Clean-up solution I: 150g of potassium hexacyanoferrate (II) was dissolved in water in a 1000 mL volumetric flask. Diluted to the mark with water and mixed the solution.
Clean up solution II: 300g of zinc sulfate was dissolved in a 1000 mL volumetric flask. Solution was diluted to the mark with water and mixed well.

2.5.4 Preparation of standard solution of benzoic acid and sorbic acid

Benzoic acid and sorbic acid stock solution was prepared at concentration of 1000 mg/L by taking 100 mg of benzoic acid and sorbic acid CRM standard in 40 ml of methanol and make up to the mark with water in a 100 mL volumetric flask separately. Mixed benzoic acid and sorbic acid calibration standards were prepared at concentration of 5, 10, 20, 50 and 100 mg/L by dilution with extraction solution (60 volume parts of ammonium acetate/acetic acid buffer solution with 40 volume parts of methanol). Calibration curves were made and calibration curves were given in Figure 98

2.5.5 HPLC-UV operating condition for benzoic acid and sorbic acid

HPLC equipped with a UV detector was used for the analysis of benzoic acid and sorbic acid. The column was reversed-phase C18 and dimensions were 250 mm (length), 4.6 mm (internal diameter), 5 µm (particle size). The flow rate was 1.0 mL/min, the column oven temperature was ambient. The measurement wavelength of UV detection was 235 nm. The injection volume was 10 µL. The composition of the mobile phase (isocratic) was 50 volume parts of ammonium acetate /acetic acid buffer solution was mix with 40 volume parts of methanol and adjusted to a pH of 4.5 with acetic acid. Analysis results were given in Table-48.

2.5.6 Method validation of benzoic acid and sorbic acid

Apple fruit drink sample, FD 07 was taken as control sample where no targeted benzoic acid and sorbic acid were present in the sample matrix. For selectivity blank control sample of apple fruit drink was run in HPLC-UV. The control sample was spiked with CRM standards of benzoic acid and sorbic acid at concentration 5 mg/L. Then CRM standard of benzoic acid and sorbic acid and spiked control apple fruit drink sample were run with the same operating condition of HPLC-UV. The chromatograms were given in Figure 93, 94 and 95. For LOD and LOQ ten replicate control apple fruit drink were spiked with CRM standard of benzoic acid and sorbic acid at concentration 5 mg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control apple fruit drink sample. LOD and LOQ were given in table 45. Calibration standards were prepared at concentration 5, 10, 20, 50 and 100 mg/L. Working range for benzoic acid and sorbic acid was 5-100 mg/L. Calibration curves were made and calibration curves were given in Figure-98. For accuracy (recovery experiment) control apple fruit drink sample was spiked with CRM standards of benzoic acid and sorbic acid at concentration 50 mg/L. The chromatograms were given in Figure 99 and 100. Recovery was given in Table 46. Precision was calculated from ten replicates of spiked apple fruit drink control sample at concentration 50 mg/L. Relative standard deviation (RSD%) of benzoic acid and sorbic acid were given in Table 47.

2.6 Quantitative determination of sudan red I, II, III, IV in chili powder by HPLC

2.6.1 Sample Collection

Chili (*Capsicum annuum*) powder samples (n=20) were collected from five city corporation markets of Dhaka, namely, Kawran Bazar, Mohakhali Kacha Bazar, Mirpur-1 Kacha Bazar, MohammadpurKrishi Market, and Gulshan-1 Kacha Bazar. Four (04) chili powder samples were collected from each of the five markets. Packaged samples were properly labeled and loose samples were taken into polyethylene zipper bags and properly labeled for carrying to the laboratory. Samples were carried to the laboratory and stored at room temperature condition. Picture of some chili powder were given in Figure 17. Sample IDs and place of collection of chili powder were given in Table 10

Figure 17: Picture of some chili powder sample

Table 10: Sample IDs and place of collection of chili powder

Chili powder		
Sample ID	**Brand**	**Market**
CP-01	Pran Chili powder	Kawran Bazar
CP-02	Radhuni Chili powder	Kawran Bazar
CP-03	Sajeeb Chili powder	Kawran Bazar
CP-04	Fresh Chili powder	Kawran Bazar
CP-05	ACI pure Chili powder	Mohakhali kacha bazar
CP-06	Rani Chili powder	Mohakhali kacha bazar
CP-07	Ahmed Chili powder	Mohakhali kacha bazar
CP-08	Modern Chili powder	Mohakhali kacha bazar
CP-09	BD Chili powder	Mirpur-1 kacha bazar
CP-10	Zisan Chili powder	Mirpur-1 kacha bazar
CP-11	Loose chili powder	Mirpur-1 kacha bazar
CP-12	Loose chili powder	Mirpur-1 kacha bazar
CP-13	Loose chili powder	Mohammadpur Krishi Bazar
CP-14	Loose chili powder	Mohammadpur Krishi Bazar
CP-15	Radhuni chili powder	Mohammadpur Krishi Bazar
CP-16	Pran Chili powder	Mohammadpur Krishi Bazar
CP-17	ACI pure Chili powder	Gulshan-1 kacha Bazar
CP-18	Radhuni chili powder	Gulshan-1 kacha Bazar
CP-19	Loose chili powder	Gulshan-1 kacha Bazar
CP-20	Loose chili powder	Gulshan-1 kacha Bazar

2.6.2 Extraction of sudan red I, II, III and IV from chili powder samples

Sudan red I, II, III and IV were extracted from chili powder samples following the below procedure in **Scheme-5**.

Weighted 0.5g sample was taken in a 15mL falcon tube

↓

5mL Ethanol was added

↓

The sample was vortex and shaken in an orbital shaker for 10 min

↓

Sample was centrifuged at 6000 rpm for 5min

↓

Supernatant was transferred to a clean 10mL volumetric flask

↓

Residue was re-extracted with 5mL ethanol

↓

Extract was vortex and shaken in an orbital shaker for 10min

↓

Sample was centrifuged at 6000 rpm for 5 min

↓

Supernatant was taken into the same volumetric flask, make up to mark with ethanol

↓

Filter sample through a 0.2 µm nylon sample filter

Scheme-5: Sudan red I, II, III and IV extraction procedure from chili powder

2.6.3 Standard solution preparation of Sudan Red I, II, III and IV

Stock solution of Sudan Red I, II, III and IV were prepared at concentration of 1000 mg/L by taking 0.05g of each CRM standard separately in a 50 mL volumetric flask and was dissolved in ethanol. Calibration standards of mixed Sudan Red I, II, III and IV were prepared at concentration 0.05, 0.1, 0.3, 0.5, 3 and 5 mg/L by dilution with ethanol. Calibration curves were made and calibration curves were given in Figure 110.

2.6.4 HPLC-UV operating condition for Sudan Red I, II, III and IV

HPLC equipped with UV fixed wave length detector was used for analysis of sudan red I, II, III and IV. Column was reverse phase C18 and dimensions were 150 mm (length), 4.6mm (internal diameter) and 5 µm (particle size). Flow rate was 1.0 mL/min, column oven temperature was 40 ºC. Measurement wavelength of uv detection was 480 nm. Injection volume was 10 µL. Composition of mobile phase A was 0.1% formic acid and mobile phase B was acetonitrile. The

percentage ratio of mobile phase A and B was 10% and 90%, respectively. Analysis results were given in Table 52.

2.6.5 Method validation of sudan red I, II, III and IV

Chili powder sample, CP 02 was taken as control sample where no targeted sudan I, II, III and IV were present in the sample matrix. For selectivity blank control sample of chilli powder was run in HPLC-UV. The control sample was spiked with CRM standards of sudan I, II, III and IV at concentration 3 mg/L. Then CRM standard of sudan I, II, III and IV and spiked control sample were run with the same operating condition of HPLC-UV. The chromatograms were given in Figure 105, 106 and 107. For LOD and LOQ ten replicate control chilli powder were spiked with CRM standard of sudan I, II, III and IV at concentration 1.0 mg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control chili powder sample. LOD and LOQ were given in Table 49. Calibration standards were prepared at concentration 0.05, 0.1, 0.5, 3 and 5 mg/L. Working range sudan I, II, III and IV was 0.05-5.0 mg/L. Calibration curves were made and calibration curves were given in Figure-110. For accuracy (recovery experiment) control chilli powder sample was spiked with CRM standards of sudan I, II, III and IV at concentration 3 mg/L. The chromatograms were given in Figure 111 and 112. Recovery was given in table 50. Precision was calculated from ten replicates of spiked chili powder control sample at concentration 1 mg/L. Relative standard deviation (RSD%) of sudan I, II, III and IV were given in Table 51.

2.7 Determination of Antibiotic Residues in Pasteurized milk by LC-MS/MS

2.7.1 Sample Collection

Pasteurized milk sample (n=42) was collected from three local marketof Dhaka city namely Shwapno at Gulshan-2, Agora at Japan Garden City Mohammadpur and Mina Bazar at Dhanmondi. Fourteen (14) samples were collected from each of the three markets. Sample were properly labeled and put into cool boxes with ice pad for carrying to laboratory. Samples were carried to laboratory and stored at -18 ℃ in a freezer. Picture of some pasteurized milk sample were given in Figure 18. Sample IDs, brand, batch number and place of collection were given in Table 11.

Figure 18: Picture of some pasteurized milk sample

Table 11: Sample IDs, brand, batch and place of collection of pasteurized milk

Sample ID	Brand	Batch Number	Name of Market
PM 01	Aarong Dairy	PA50902B	Shwapno at Gulshan-2
PM 02	Farm Fresh Milk	226-Kha/19, 3- Ba (Na)	Shwapno at Gulshan-2
PM 03	Dairy Fresh	06.01.Dha	Shwapno at Gulshan-2
PM 04	Milk vita	190806 (1)	Shwapno at Gulshan-2
PM 05	Aarong Dairy	PA10601A	Shwapno at Gulshan-2
PM 06	Aarong Dairy	PA20601A	Shwapno at Gulshan-2
PM 07	Dairy Fresh	22.01	Shwapno at Gulshan-2
PM 08	PURA	18201	Shwapno at Gulshan-2
PM 09	Ultra	005	Shwapno at Gulshan-2
PM 10	Igloo	015-1	Shwapno at Gulshan-2
PM 11	Milk Vita	191228 (A:15)	Shwapno at Gulshan-2
PM 12	Igloo	360-2	Shwapno at Gulshan-2
PM 13	Ayran	220	Shwapno at Gulshan-2
PM 14	Farm Fresh Milk	355-ka/19	Shwapno at Gulshan-2
PM 15	Pran Milk	A. 4/A	Agora at Japan Garden City, Mohammadpur
PM 16	Aarong Dairy	PA 11201A	Agora at Japan Garden City, Mohammadpur
PM 17	Aarong Dairy	PA5 01 02B	Agora at Japan Garden City, Mohammadpur
PM 18	Aarong Dairy	PA5 01 03B	Agora at Japan Garden City, Mohammadpur
PM 19	ULTRA	338	Agora at Japan Garden City, Mohammadpur
PM 20	SAFE	01	Agora at Japan Garden City, Mohammadpur
PM 21	PURA	21301	Agora at Japan Garden City, Mohammadpur
PM 22	Max Pure		Agora at Japan Garden City, Mohammadpur
PM 23	Milk Vita	2000818(A).03	Agora at Japan Garden City, Mohammadpur
PM 24	Farm Fresh	225-Kha/20	Agora at Japan Garden City, Mohammadpur
PM 25	Aarong Dairy	PA 50802B	Agora at Japan Garden City, Mohammadpur
PM 26	Igloo	231-2	Agora at Japan Garden City, Mohammadpur
PM 27	Pran Milk	A. Ka 2.3/B	Agora at Japan Garden City, Mohammadpur
PM 28	Mou	081401	Agora at Japan Garden City, Mohammadpur
PM 29	Ultra Milks	483	Mina Bazar at Dhanmondi
PM 30	Ayran	199	Mina Bazar at Dhanmondi
PM 31	Farm Fresh	315-Kha/20	Mina Bazar at Dhanmondi
PM 32	BAQARAH	A	Mina Bazar at Dhanmondi
PM 33	Milk Vita	210109 (A:02)	Mina Bazar at Dhanmondi
PM 34	Aarong Dairy	PA50101B	Mina Bazar at Dhanmondi
PM 35	Aarong Dairy Premium	PR50112B	Mina Bazar at Dhanmondi
PM 36	Fancy	001	Mina Bazar at Dhanmondi
PM 37	Ultra	159	Mina Bazar at Dhanmondi
PM 38	Aarong Dairy	PT50813B	Mina Bazar at Dhanmondi
PM 39	Milk Fresh	20	Mina Bazar at Dhanmondi
PM 40	Milk Fresh	22	Mina Bazar at Dhanmondi
PM 41	Milk Vita	210828 (03)	Mina Bazar at Dhanmondi
PM 42	PURA	191001	Mina Bazar at Dhanmondi

2.7.2 Sample Preparation

Sample preparation of pasteurized milk for antibiotic residue was performed as follows in **Scheme-6**

Pasteurized milk (2 mL) was weighted in a 50 mL Falcon tube then18 mL Diluent (Methanol: water = 9:1) was added then shaken and vortex for 15 min

↓

QuEChERS salt (6g $MgSO_4$ + 1.5 AcONa) was added and vortex for 5 min and centrifuged at 5000 rpm for 10 min

↓

3 mL Supernatant was transfer into 15 mL Falcon tube and 5 mL n-Hexane was added

↓

Shaken and Vortex for 5 min, two layers were created and upper layer was discarded

↓

1mL from lower layer was taken into QuEChERS dSPE tube containing 150 mg $MgSO_4$, 50 mg PSA and 50 mg C18

↓

Shaken and Vortex for 5 min and centrifuged at 7000 rpm for 10 min. The upper portion was taken in a LC vial for analysis by LC-MS/MS

Scheme-6: Sample preparation of pasteurized milk for analysis of antibiotic residue

2.7.3 Standard solution preparation of Antibiotics:

First stock solution of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline, and chlortetracycline was prepared at a concentration of 1000 mg/L in methanol-water mixture (9:1), the second stock was prepared at a concentration of 10 mg/L by dilution and the mixed third stock was prepared at concentration 250 µg/L. Mixed calibration standards was prepared in a 1.0 mL LC vial at concentration of 5, 10, 20, 50 and 100 µg/L by dilution of third stock. Calibration curves were made and calibration curves were given in Figure-125.

2.7.4 LC-MS/MS operating condition for antibiotic residues

LC-Parameter

Column: GISS C18 column, 100 mm x 2.1 mm, 3 µm
Column Temperature (°C): 40 °C
Auto-sampler Temperature (°C): 4 °C
Injection Volume (µL): 2
Flow Rate (mL/min): 0.2
Mobile Phase Gradient:
Solvent A: 1% formic acid with water
Solvent B: Acetonitrile

LC-gradient program

Time (min)	% A	% B
1.00	80	20
6.00	60	40
8.00	0	100
10.00	0	100
12.50	99	1
15.00	99	1

MS Parameter

Interface	ESI
Interface temperature	300°C
Desolvation temperature	526°C
DL temperature	250°C
CID gas flow	270 kPa
Acquisition Mode	Multiple Reaction Monitoring (MRM)
Polarity	Positive

Analysis results were given in Table-56

2.7.5 Method validation of antibiotics

Pasteurized milk sample, PM 01 was taken as control sample where no targeted antibiotics were present in the sample matrix. For selectivity blank control sample of pasteurized milk sample was run in LC-MS/MS. The control sample was spiked with CRM standards of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration 50 µg/L. Then CRM standard of antibiotics and spiked control pasteurized milk sample were run with the same operating condition of LC-MS/MS. The chromatograms were given in Figure 114, 115 and 116. For LOD and LOQ ten replicate pasteurized milk control sample were spiked with antibiotic at concentration 5 µg/L. LOD and LOQ were calculated from the standard deviation of that ten replicate control pasteurized milk sample. LOD and LOQ were given in table 53. Calibration standards were prepared at concentration 5, 10, 20, 50 and 100 µg/L. Working range of antibiotics were 5-100 µg/L. Calibration curves were made and calibration curves were given in Figure-125. For accuracy (recovery experiment) control pasteurized milk sample was spiked with CRM standards at concentration 50 µg/L. The chromatograms were given in Figure 126. Recovery were given in Table 54. Precision was calculated from ten replicates of spiked pasteurized milk control sample at concentration 50 µg/L. Relative standard deviation (RSD%) of antibiotic were given in Table 55.

3. Results and Discussion

In this study at first the analytical methods were validated. The validation was performed as described in this study in line with international guideline Eurachem **[61].** After validation of methods the samples were analyzed using that validated method. The following method validation performance characteristics were performed in this study.

(a) Selectivity
(b) Limit of Detection (LOD)
(c) Limit of Quantification(LOQ)
(d) Working Range and Linearity
(e) Accuracy (Recovery)
(f) Precision (Repeatability)

Selectivity

Selectivity relates to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior.

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

LOD and LOQ was calculated using the following formula **[62]**

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

Where,
S_0 is the estimated standard deviation of single results at or near zero concentration
$S_0'$is the standard deviation used for calculating LOD and LOQ.
n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.
n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD was calculated as 3×So′ and LOQ was calculated as 10×So′

Working Range and Linearity

The working range is an interval, in which a method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ. The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed.

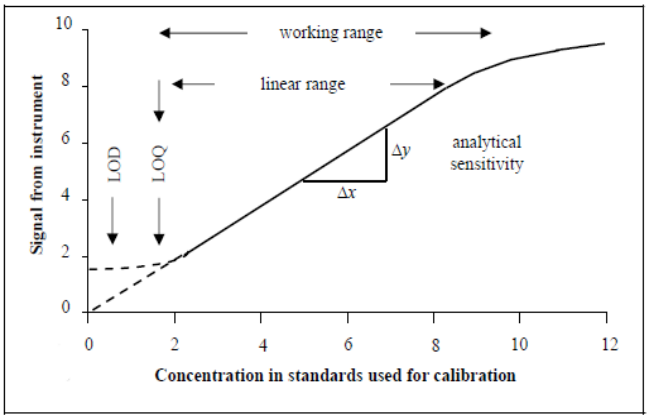


Figure 19: Analytical sensitivity, working range and linear range

The Figure-19 shows a response curve obtained with an instrumental method. The working range, linear range, analytical sensitivity, LOD and LOQ are identified **[63].**

Accuracy (Recovery)

Accuracy is the closeness of a single result to a reference value. Method validation need to investigate the accuracy of results by considering both systematic and random effects on single results.

Accuracy can be expressed as a relative recovery **[64]**

$$R(\%) = \frac{x' - \bar{x}}{x_{spike}} \times 100$$

x′ = is the mean value of the spiked sample, $\bar{x}$ is the mean value of unspike sample and x_{spike} is the added concentration.

Precision (Repeatability)

Replication is essential for obtaining reliable estimation of precision. Replicate analysis are designed to take into consideration of all the variations in analytical conditions which is expected during routine use of the method. Precision is expressed as a relative standard deviation since it is approximately constant over the range of interest **[65].**

Relative Standard Deviation is calculated as RSD % = $\frac{SD}{Average} \times 100$

3.1 Analysis of pesticide residues and heavy metals in fruits and vegetables

Fruit (n=280) and vegetable (n=455) samples were collected from 35 city corporation markets of seven divisions of Bangladesh. Among them, fruits were 8 types and vegetables were 13 types. Nineteen organochlorine pesticides were targeted for analysis by GC-ECD, sixteen organophosphorus pesticides by GC-MS and eighty five organophosphorus pesticides by LC-MS/MS in fruits and vegetable samples. Arsenic, lead, and cadmium were analyzed by AAS-GFA in the fruits and vegetable samples.

3.1.1 Determination of 19 organochlorine pesticides in fruits and vegetables samples by GC-ECD

For this analysis, at first, the QuEChERS method was validated using tomato as a representative control matrix. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of organochlorine pesticides (OCPs) in different fruits and vegetables analyses. Targeted compounds in the analytes were identified in comparison with the retention time of CRM standards with the retention time of components to found present in samples.

3.1.1.1 Method Validation Performance Characteristics

3.1.1.1.1 Selectivity

Blank of control tomato matrix T 01, mixed CRM standard of α-BHC, γ-BHC, β-BHC, δ-BHC, heptachlor, aldrin, heptachlor epoxide, α-chlordane, γ-chlordane, α-endosulfan, 4 ,4′ DDE, dieldrin, endrin, β-endosulfan, 4,4′ DDD, 4, 4'DDT, endrin aldehyde, endosulfan sulphate and methoxychlor and spiked control tomato matrix were analyzed by the same conditions of GC-ECD. The below chromatograms in Figure 20, 21 and 22 are the supporting evidence which give sufficient reliability for selectivity. The chromatograms (Figure 20, 21 and 22) are given below showed that compounds were well resolved and there is no significant interference of matrix with targeted OCPs. Table 12 shows the retention time of 19 OCPs.

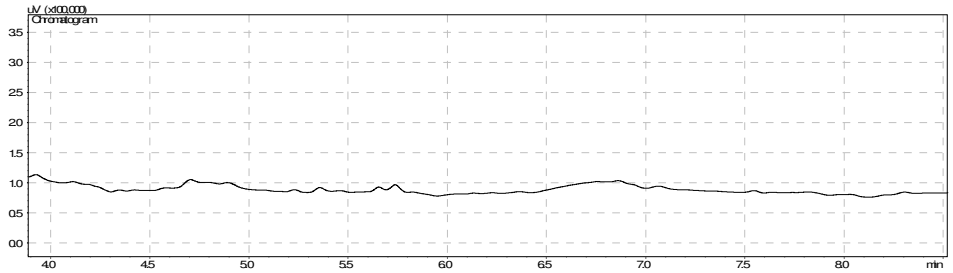


Figure 20: Chromatogram of blank control tomato matrix

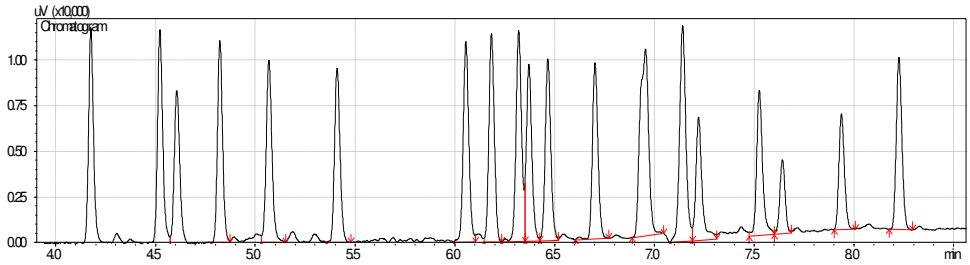


Figure 21: Chromatogram of CRM standard (10 µg/L) of 19 different OCPs

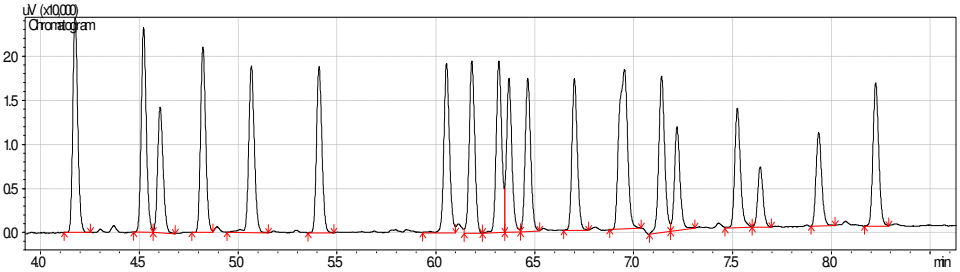


Figure 22: Chromatogram of tomato control sample spiked with CRM standard (20 µg/L) of 19 OCPs

Table 12: Retention time of organochlorine pesticides

Serial Nunber	Name of organochlorine pesticide	Retention time (min)
1	α-BHC	4.17
2	γ-BHC	4.52
3	β-BHC	4.61
4	δ-BHC	4.82
5	Heptachlor	5.07
6	Aldrin	5.41
7	Heptachlor epoxide	6.05
8	α-Chlordane	6.18
9	γ-Chlordane	6.32
10	α-Endosulfan	6.37
11	4 ,4′ DDE	6.47
12	Dieldrin	6.70
13	Endrin	6.95
14	β-Endosulfan	7.14
15	4,4′ DDD	7.22
16	4, 4'DDT	7.53
17	Endrin aldehyde	7.64
18	Endosulfan sulphate	7.94
19	Methoxychlor	8.23

3.1.1.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked tomato sample with CRM standard of 19 OCPs at concentration 10 µg/kg (Table 13).

Table 13: LOD and LOQ of organochlorine pesticides

No.	Name of Organochlorine Pesticide	LOD (µg/kg)	LOQ (µg/kg)
1	α-BHC	0.21	0.70
2	γ-BHC	0.22	0.75
3	β-BHC	0.14	0.48
4	δ-BHC	0.20	0.67
5	Heptachlor	0.24	0.80
6	Aldrin	0.21	0.72
7	Heptachlor epoxide	0.33	1.12
8	α-Chlordane	0.20	0.69
9	γ-Chlordane	0.21	0.71
10	α-Endosulfan	0.34	1.13
11	4 ,4′ DDE	0.25	0.83
12	Dieldrin	0.24	0.81
13	Endrin	0.38	1.25
14	β-Endosulfan	0.30	0.98
15	4,4′ DDD	0.31	1.02
16	4, 4'DDT	0.33	1.11
17	Endrin aldehyde	0.63	2.11
18	Endosulfan sulphate	0.46	1.54
19	Methoxychlor	0.21	0.70

3.1.1.1.3 Working Range and Linearity

The working range for 19 OCPs was 10-30 µg/L. Chromatograms of calibration standard were given below at concentration 10 µg/L and 30 µg/L in Figure 23 and 24. Linear correlation coefficient (R^2) of 19 OCPs was given in Table 14. Some representative calibration curves were given in Figure 25. All other calibration curves are attached in the annexure in Figures 136-141.

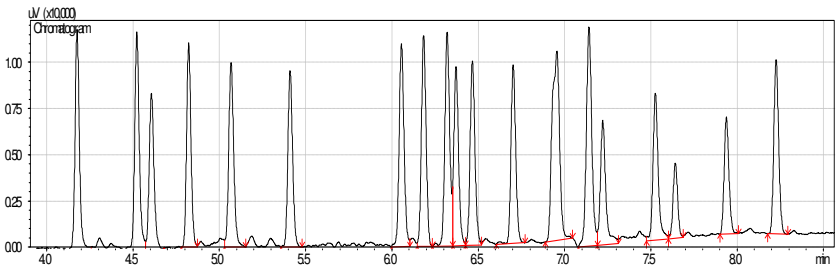


Figure 23: CRM standard (10 µg/L) of 19 organochlorine pesticides

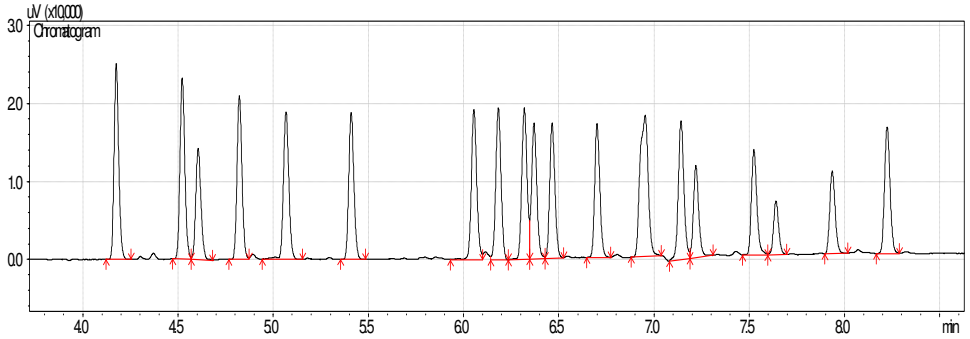


Figure 24: CRM standard (30 µg/L)of 19 organochlorine pesticides

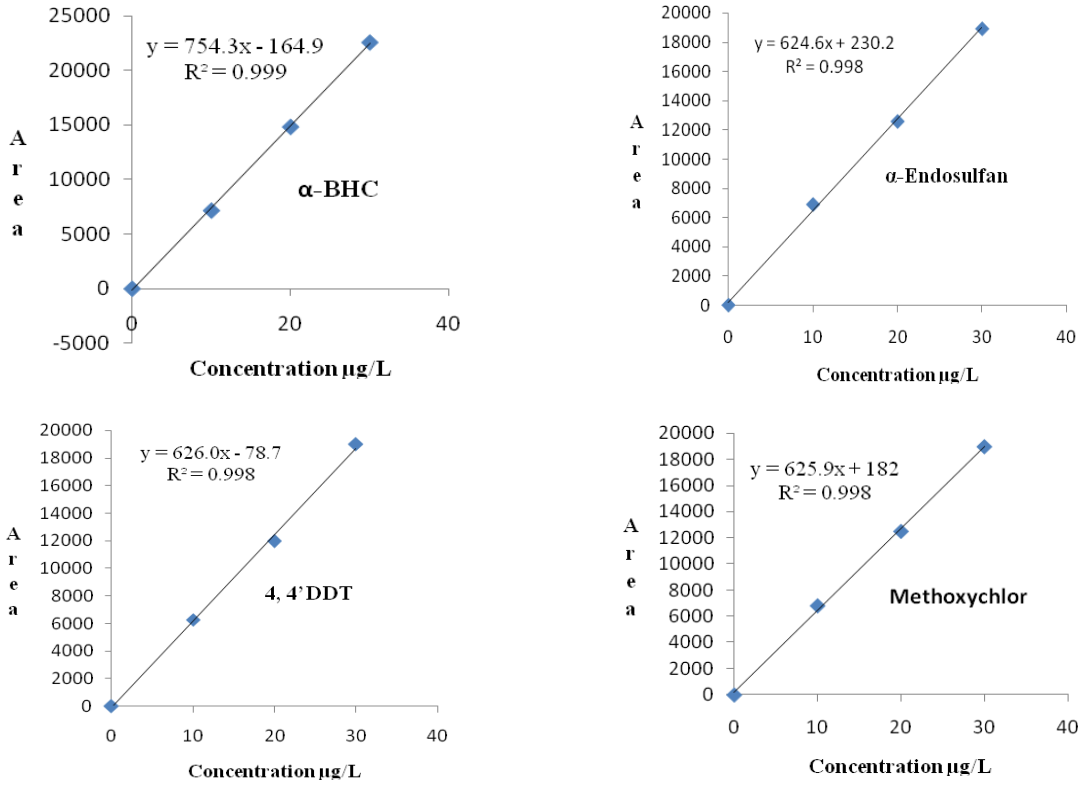


Figure 25: Calibration curve of α BHC, α-Endosulfan, 4, 4′ DDT and Methoxychlor

Table 14: Linear Correlation Coefficient (R^2) of OCPs

Name of Organochlorine Pesticide	Linear Correlation Coefficient (R^2)
α-BHC	0.999
γ-BHC	0.998
β-BHC	0.999
δ-BHC	0.996
Heptachlor	0.998
Aldrin	0.997
Heptachlor Epoxide	0.999
α-Chlordane	0.999
γ-Chlordane	0.998
α-Endosulfan	0.998
4 ,4′ DDE	0.999
Dieldrin	0.997
Endrin	0.999
β-Endosulfan	0.998
4,4′ DDD	0.998
4, 4'DDT	0.998
Endrin Aldehyde	0.996
Endosulfan sulphate	0.997
Methoxychlor	0.998

3.1.1.1.4 Accuracy (Recovery)

Tomato sample was spiked with CRM standard (20 µg/ L) for recovery experiment. Chromatogram of CRM standard (20 µg/L) of 19 different OCPs and spiked tomato control matrix with CRM standard (20 µg/L) of 19 OCPs were given in Figure-26 and 27. The recovery results were given in Table 15.

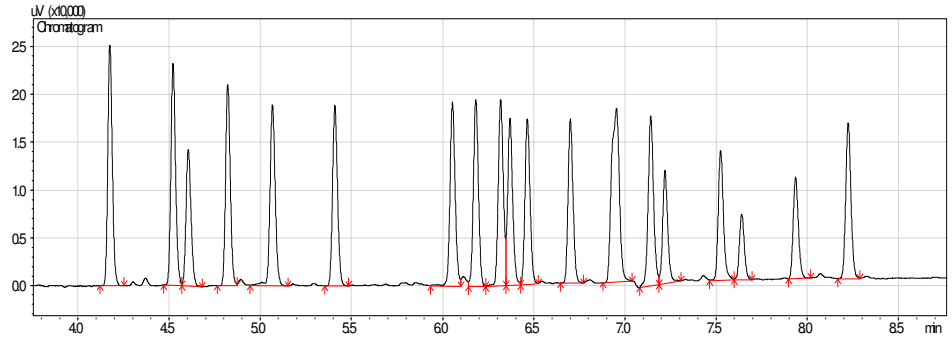


Figure 26: Chromatogram of CRM standard (20 µg/L) of 19 different OCPs

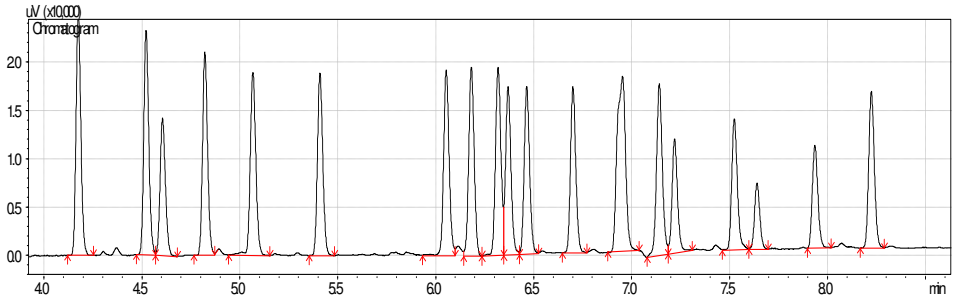


Figure 27: Chromatogram of spiked tomato control matrix with CRM standard (20 µg/L) of 19 OCPs

Table 15: Recovery of organochlorine pesticide

Name of organochlorine pesticide	Recovery % with 20 µg/L spike in tomato
α-BHC	90±2.46
γ-BHC	93±1.75
β-BHC	91±1.84
δ-BHC	92±2.17
Heptachlor	89±3.32
Aldrin	93±2.89
Heptachlor epoxide	83±1.67
α-Chlordane	93±2.37
γ-Chlordane	86±1.05
α-Endosulfan	89±2.61
4 ,4′ DDE	91±2.53
Dieldrin	92±3.52
Endrin	94±1.71
β-Endosulfan	93±3.30
4,4′ DDD	94±2.50
4, 4'DDT	91±2.13
Endrin aldehyde	93±2.88
Endosulfan sulphate	91±3.74
Methoxychlor	97±2.67

3.1.1.1.5 Precision (Repeatability)

Precision was calculated from ten replicates of spiked tomato sample with CRM standard of 19 OCPs at concentration 10 µg/kg. Relative Standard Deviation (RSD%) of 19 OCPs was given in Table 16.

Table 16: Relative standard deviation (RSD %) of organochlorine pesticides

Name of organochlorine pesticide	RSD%
α-BHC	1.64
γ-BHC	1.65
β-BHC	1.12
δ-BHC	1.49
Heptachlor	1.84
Aldrin	1.69
Heptachlor Epoxide	2.64
α-Chlordane	1.56
γ-Chlordane	1.55
α-Endosulfan	2.46
4 ,4′ DDE	1.82
Dieldrin	1.75
Endrin	2.78
β-Endosulfan	2.18
4,4′ DDD	2.39
4, 4'DDT	2.42
Endrin Aldehyde	4.96
Endosulfan sulphate	3.25
Methoxychlor	1.48

3.1.1.2 Sample Analysis

Fruits (n=280) and vegetable (n=455) samples were analyzed for 19 OCPs. Representative chromatogram of one fruit and one vegetable samples were given in Figure 28 and 29. Similar chromatogram was found for all other fruits and vegetables sample. Analysis results of fruits and vegetable samples are given in Table 17 and 18.

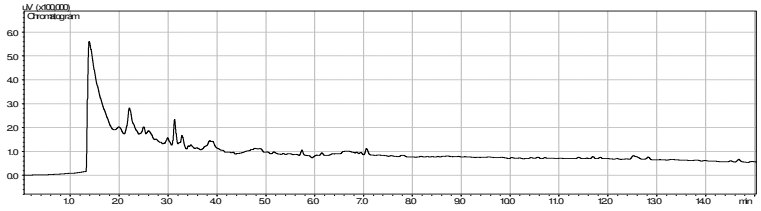


Figure 28: Chromatogram of tomato-02 sample for OCPs

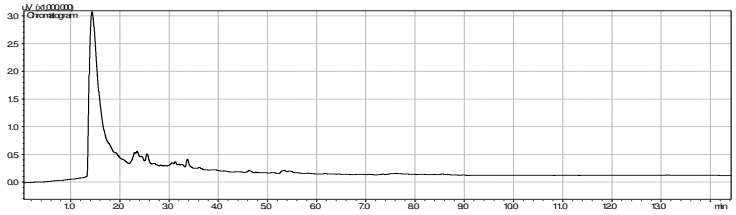


Figure 29: Chromatogram of mango-01 sample for OCPs

Table 17: Amount of organochlorine pesticide in fruit samples

Sl No.	Sample Name	Number of sample	Results (mg/kg)
01	Banana	35	Not Detected
02	Red apple	35	Not Detected
03	Green apple	35	Not Detected
04	Dates	35	Not Detected
05	Orange	35	Not Detected
06	Grape	35	Not Detected
07	Pineapple	35	Not Detected
08	Mango	35	Not Detected

Table 18: Amount of organochlorine pesticide in vegetable samples

Sl No.	Sample Name	Number of sample	Results (mg/kg)
01	Cabbage	35	Not Detected
02	Green Chili	35	Not Detected
03	Tomato	35	Not Detected
04	Carrot	35	Not Detected
05	Cauliflower	35	Not Detected
06	Potato	35	Not Detected
07	Green Bean	35	Not Detected
08	Long Bean	35	Not Detected
09	Coriander Leaf	35	Not Detected
10	Eggplant	35	Not Detected
11	Red Amaranth	35	Not Detected
12	Lettuce	35	Not Detected
13	Capsicum	35	Not Detected

3.1.1.3 Discussion

Nineteen organochlorine pesticides in fruits (n=280) and vegetables (n=455) sample were analyzed of by GC-ECD. LOD and LOQ were in the range of 0.14-0.63 µg/kg and 0.48-2.11 µg/kg, respectively. Calibration range was 10-30 µg/L and linear correlation coefficient (R^2) value was in the range of 0.996-0.999. Recovery (%) for control tomato matrix was in the range of 83-97%. Relative standard deviation (RSD%) for repeatability was in the range of 1.12-4.96.

Organochlorines are a group of chlorinated compounds. Persistent organic pollutants (POPs) are harmful for human health. Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, lindane, benzenehexachloride, mixex, toxaphene are banned by Stockholm Convention **[66].** Bangladesh is a signatory country of Stockholm Convention. Organochlorine pesticides are stable compounds. It stay in the environment for a long time. It was reported that in Ghana that organochlorine pesticide β-HCH, γ-HCH, heptachlor, γ-chlordane, p p′ -DDT were found in watermelon, β-HCH, γ-HCH, δ-HCH, γ-chlordane, p,p′-DDE, endrin, β-endosulfan, p, p′-DDT, p, p′-DDD and methoxychlor were found in chili peppers, β-HCH, γ-HCH, δ-HCH, γ-Chlordane, p, p′-DDE, dieldrin, endrin, β-endosulfan, p, p′-DDT, and methoxychlor were found in onion **[67]**. Although OCPs are banned in Bangladesh, it was reported earlier that DDT and its metabolites, DDE and DDD were detected in poultry meat sample in the range of 0.039-0.769 mg/kg **[68].** Organochlorine pesticides are banned worldwide but there is a perception that it can be used illegally in agricultural production. In Bangladesh, there is no baseline data available for organochlorine pesticides in fruits and vegetables. For these reasons in this present study organochlorine pesticides were included for fruits and vegetables. Nineteen organochlorine pesticides were analyzed in 280 fruits sample and in 455 vegetables sample. No targeted organochlorine pesticide was detected in any sample of fruits and vegetables. It can be presume that targeted organochlorine pesticides are no longer used in agricultural production in Bangladesh and we can see the resemblance in this study by observing the analysis results.

3.1.2 Determination of 16 different organophosphorus pesticides in fruits and vegetables by GC-MS

Sixteen oganophosphorous pesticides in fruit (n=280) and vegetable (n=455) samples were analyzed by GC-MS. The QuEChERS method was validated using cabbage as a representative control matrix. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of organophosphorus pesticides (OPPs) in different fruits and vegetables analyses. Compounds were separated by gas chromatograph and then detected and quantified by quadrupole mass specteometer. Targeted compounds in the analytes were identified in comparison with the retention time and mass spectrum of CRM standards of 16 OPPs with the retention time and mass spectrum of components to found present in samples.

3.1.2.1 Method Validation Performance Characteristics

3.1.2.1.1 Selectivity

Blank of control cabbage sample Cab 01, mixed CRM standard of methamidophos, acephate, ethoprophos, dimethoate, diazinon, methyl parathion, metalaxyl, fenitrothion, malathion, fenthion, chlorpyrifos, quinalphos, methidathion, fenamiphos, ethion and propiconazole and spiked control cabbage sample were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. The below chromatograms in Figure 30, 31 and 32 are the supporting evidence which give sufficient reliability for selectivity. The chromatograms (Figure 30, 31 and 32) are given below showed that compounds were well resolved and there is no significant interference of matrix with targeted OPPs. Table 19 shows the time window, retention time, target ion and fragment ions of 16 organophosphorus pesticides. Representative mass spectrum, total ion and target ion chromatogram of methamidophos and ethion were given in Figure 33 and 34. Mass spectrum, total ion and target ion chromatogram of all other OPPs were given in the annexure in Figure 142-155.

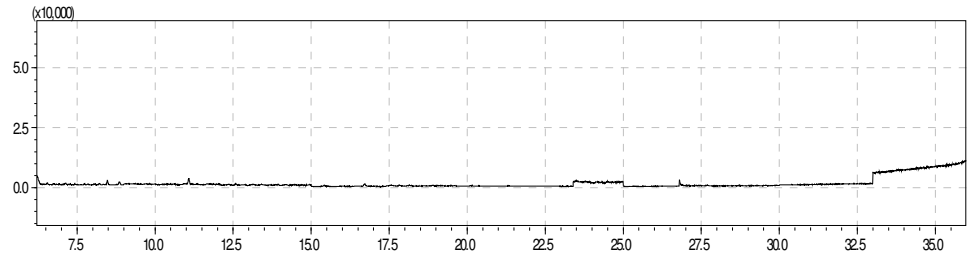


Figure-30: SIM chromatogram of blank cabbage control matrix

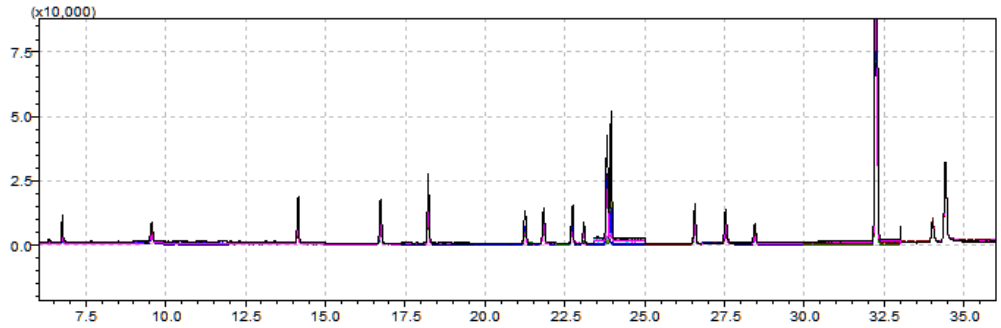


Figure-31: SIM chromatogram of CRM standard (5 µg/L) of 16 organophosphorus pesticides

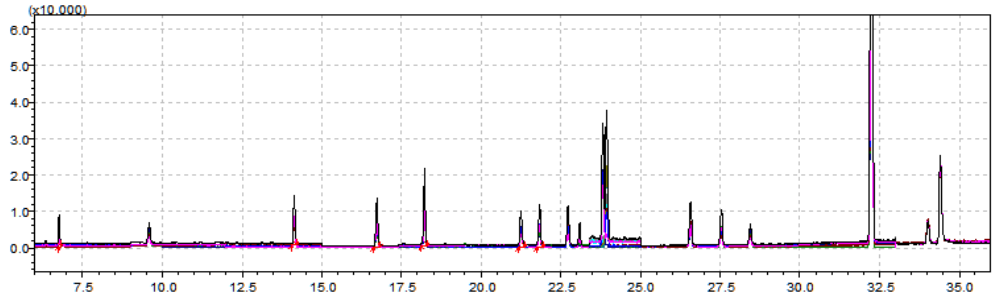


Figure-32: SIM chromatogram of 5 µg/L spiked cabbage control sample with 16 CRM standards

Table 19: Time window, retention time, target ion and fragment ion of 16 organophosphorus pesticides

Compound Name	Time window (min)	Retention time (min)	Target ion	Fragment ions
Methamidophos	6.00-9.00	6.76	94	64, 79, **94**, 110, 141
Acephate	9.00-12.00	9.70	136	79, 94, **136,** 142, 183
Ethoprophos	12.0-15.00	14.14	158	97, 139, **158**, 200, 242
Dimethoate	15.00-17.40	16.73	87	**87,** 93, 125, 229
Diazinon	17.40-19.50	18.21	137	**137**, 152, 179, 199, 304
Methyl Parathion	19.50-21.50	21.24	109	79, **109**, 125, 233, 263
Metalaxyl	21.50-22.20	21.8	206	130, 160, **206**, 249, 279
Fenitrothion	22.20-22.80	22.7	125	79, 109, **125**, 260, 277
Malathion	22.80-23.40	23.10	127	93, **127,** 173, 285, 330
Fenthion	23.40-25.0	23.83	125	79, 109, **125**, 169, 278
Chlorpyrifos	23.40-25.0	23.95	197	97, **197**, 258, 314, 349
Quinalphos	25.0-26.80	26.5	146	90, 118, **146,** 157, 298
Methidathion	26.80-27.80	27.54	145	85, 93, 125, **145**, 302
Fenamiphos	27.80-30.0	28.45	154	80, **154,** 217, 260, 303
Ethion	30.0-33.0	32.22	231	97, 125, 153, **231,** 384
Propiconazole	33.0-36.0	34.4	173	69, **173**, 191, 259, 340

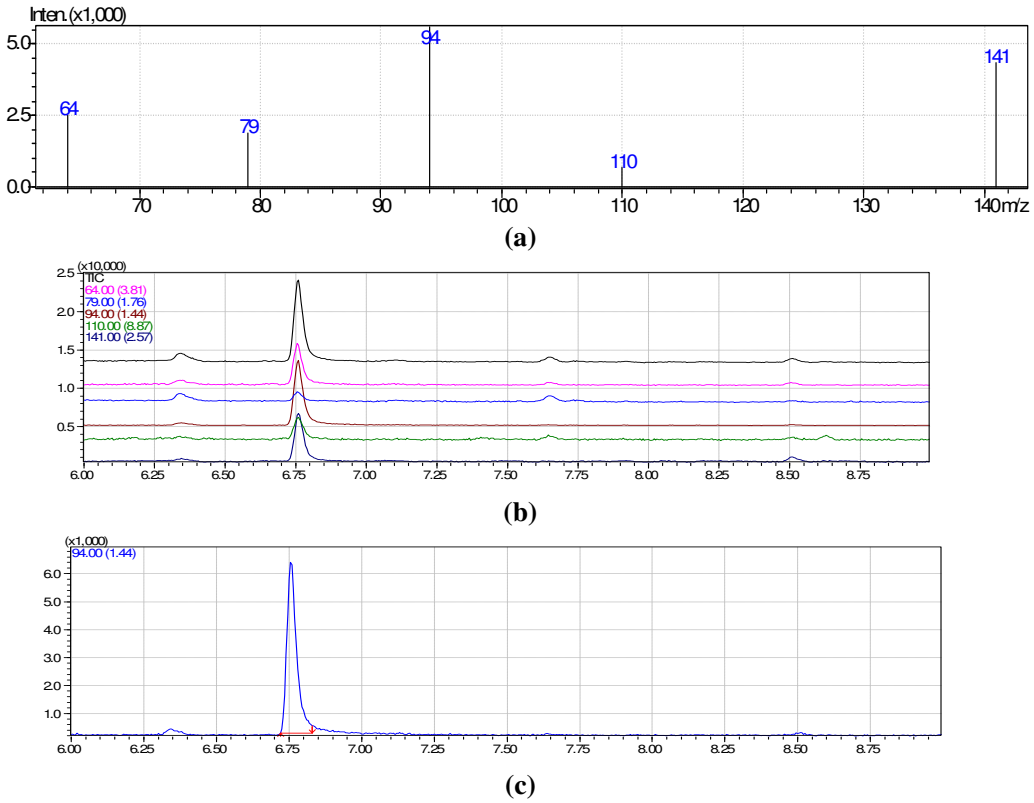


Figure-33: (a) Mass spectrum, (b) total ion and (c) target ion (m/z 94.00) chromatogram of methamidophos

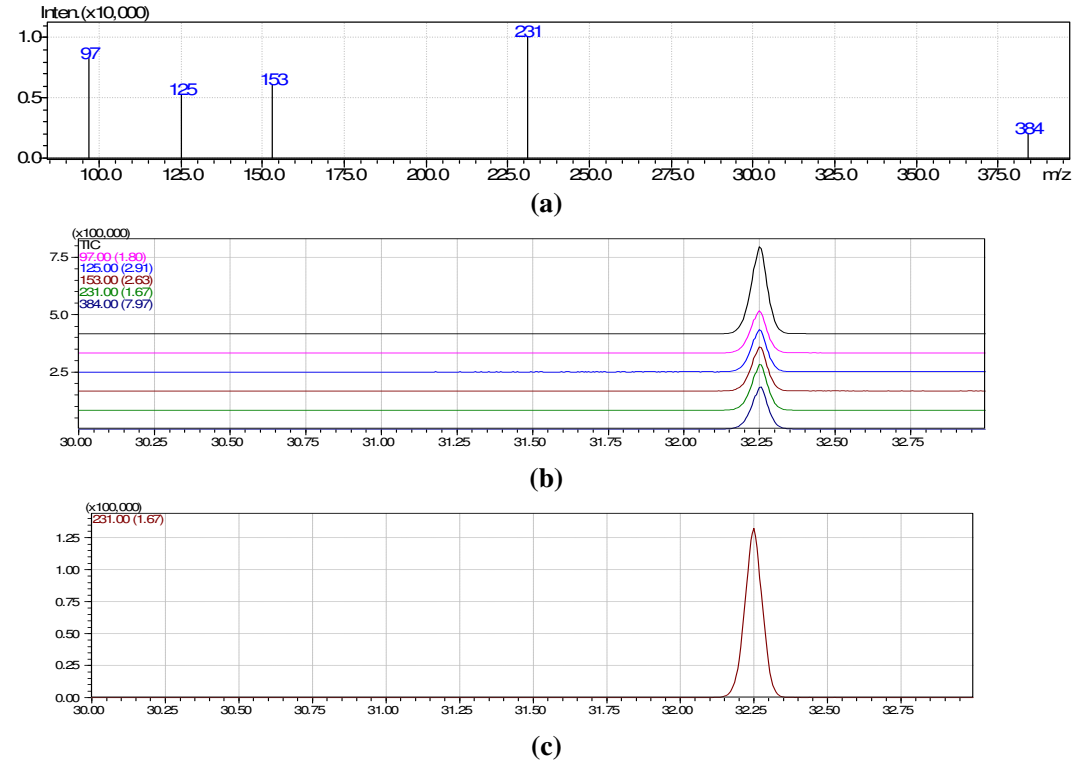


Figure-34: (a) Mass spectrum, (b) total ion and (c) target ion(m/z 231.00) chromatogram of ethion

3.1.2.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked cabbage sample with CRM standard of 16 organophosphorus pesticides at concentration 10 µg/kg (Table 20).

Table 20: LOD and LOQ of 16 organophosphorus pesticides

Sl No.	Compound Name	LOD (µg/kg)	LOQ (µg/kg)
01	Methamidophos	0.28	0.93
02	Acephate	0.44	1.48
03	Ethoprophos	0.35	1.17
04	Dimethoate	0.33	1.10
05	Diazinon	0.34	1.14
06	Methyl parathion	0.48	1.61
07	Metalaxyl	0.36	1.21
08	Fenitrothion	0.81	2.71
09	Malathion	0.48	1.58
10	Fenthion	0.32	1.06
11	Chlorpyrifos	0.30	1.01
12	Quinalphos	0.43	1.44
13	Methidathion	0.33	1.10
14	Fenamiphos	0.15	0.50
15	Ethion	0.24	0.80
16	Propiconazole	0.38	1.27

3.1.2.1.3 Working Range and Linearity

The working range for 16 organophosphorus pesticides was 5-50 µg/L. Chromatograms of calibration standards were given below at concentration 5 µg/L and 50 µg/L in Figure 35 and 36. Representative calibration curves of some OPPs were given in Figure 37. All other calibration curves were attached in the annexure in Figure 156-160. Linear correlation coefficient (R^2) of 16 organophosphorus pesticides were given in Table 21.

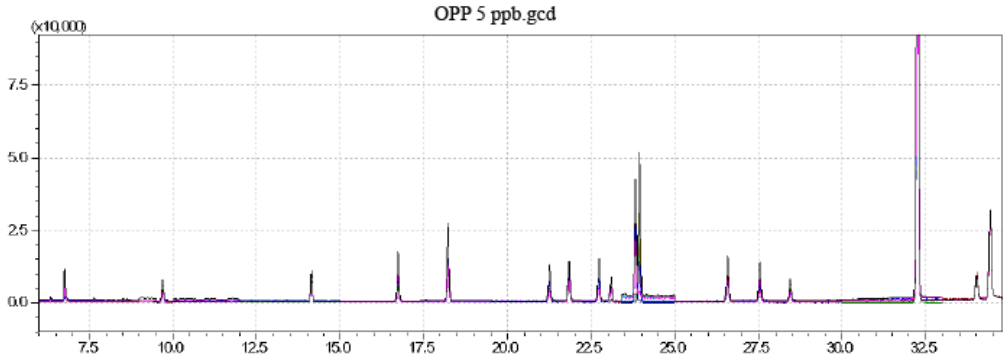


Figure-35: SIM chromatogram of 5 µg/L calibration standard

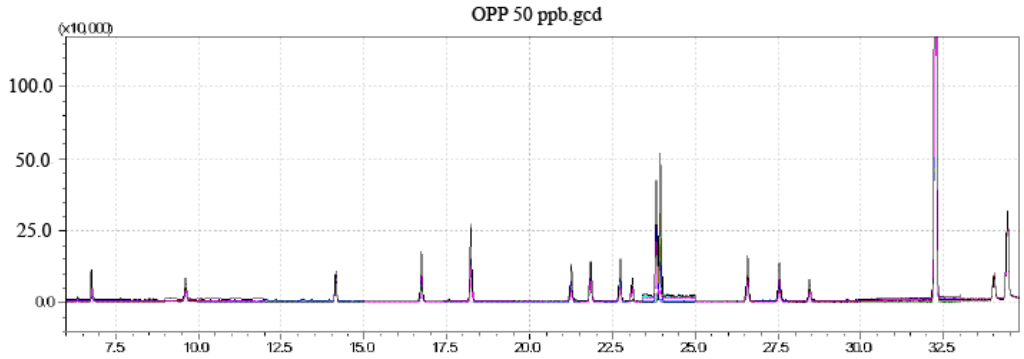


Figure-36: SIM chromatogram of 50 µg/L calibration standard

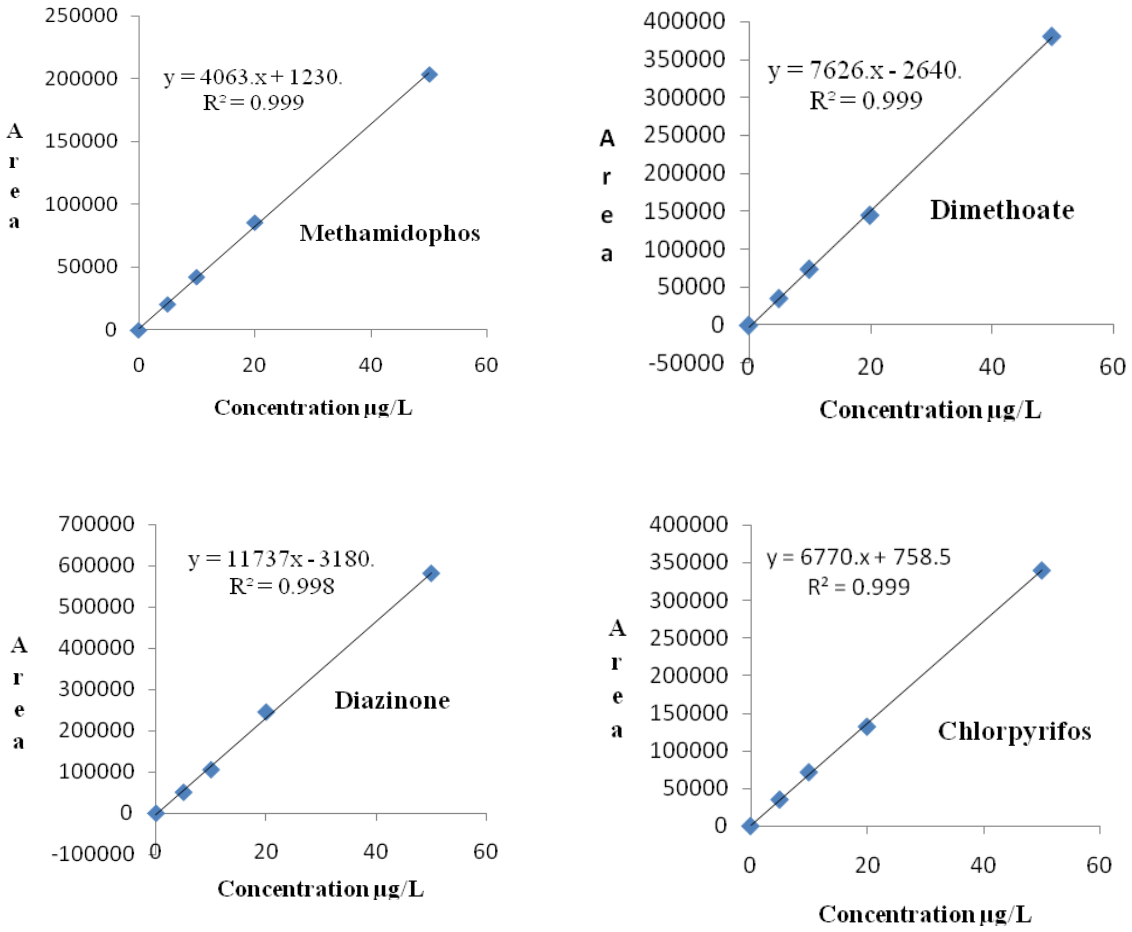


Figure-37: Calibration curve of Methamidophos, Dimethoate, Diazinone and Chlorpyrifos

Table 21: Linear correlation coefficient (R^2) of 16 organophosphorus pesticides

Serial Number	Name of Pesticide	Linear correlation coefficient (R^2)
01	Methamidophos	0.999
02	Acephate	0.997
03	Ethoprophos	0.999
04	Dimethoate	0.999
05	Diazinon	0.998
06	Methyl parathion	0.999
07	Metalaxyl	0.999
08	Fenitrothion	0.998
09	Malathion	0.999
10	Fenthion	0.999
11	Chlorpyrifos	0.999
12	Quinalphos	0.999
13	Methidathion	0.999
14	Fenamiphos	0.998
15	Ethion	0.999
16	Propiconazole	0.998

3.1.2.1.4 Accuracy (Recovery)

Control cabbage sample was spiked with CRM standard 16 OPPs at concentration 10 µg/ L for recovery experiment. Chromatogram of CRM standard (10 µg/L) of 16 different OPPs and spiked cabbage control matrix with CRM standard (10 µg/L) of 16 OPPs were given in Figure 38 and 39. The recovery results were given in Table 22.

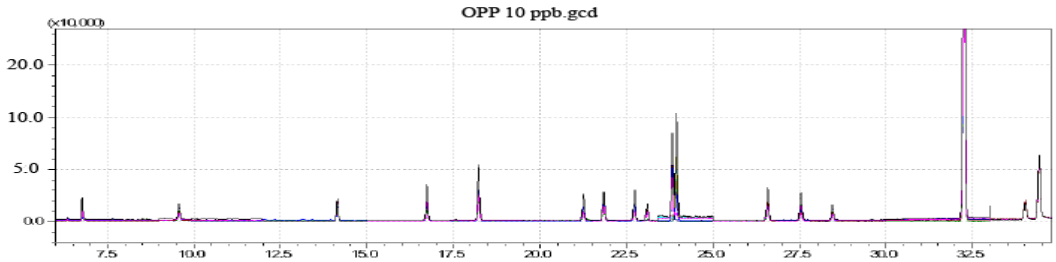


Figure-38: SIM chromatogram of 10 µg/L calibration standard

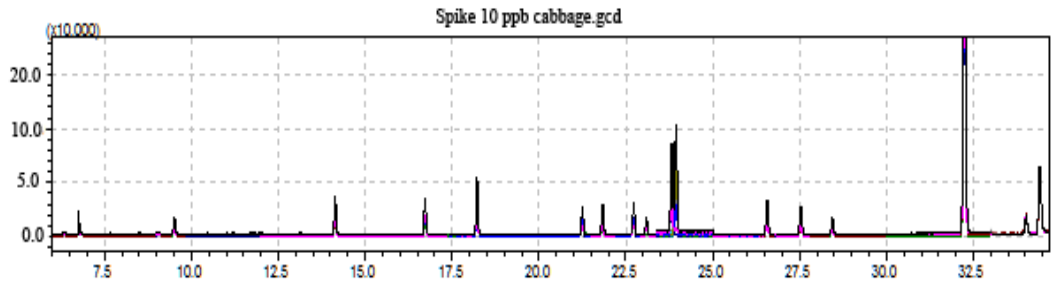


Figure-39: SIM chromatogram of spiked cabbage control matrix (10 µg/L) with 16 CRM standards of OPPs

Table 22: Recovery of 16 organophosphorus pesticides

10 µg/L was spiked in cabbage		
Serial Number	**Name of Pesticide**	**Average Recovery %**
01	Methamidophos	97±1.11
02	Acephate	94±1.81
03	Ethoprophos	94±2.41
04	Dimethoate	95±3.51
05	Diazinon	91±4.10
06	Methyl parathion	96±3.48
07	Metalaxyl	94±2.99
08	Fenitrothion	92±3.84
09	Malathion	92±5.99
10	Fenthion	92±4.83
11	Chlorpyrifos	90±1.92
12	Quinalphos	96±4.75
13	Methidathion	92±6.99
14	Fenamiphos	89±4.80
15	Ethion	92±6.66
16	Propiconazole	95±2.80

3.1.2.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked cabbage sample with CRM standard of 16 organophosphorus pesticides at concentration 20 µg/kg. RSD% of 16 organophosphorus pesticides was given in Table 23.

Table 23: Relative standard deviation of 16 organophosphorus pesticides

Serial Number	Name of Pesticide	RSD%
01	Methamidophos	1.70
02	Acephate	2.41
03	Ethoprophos	3.74
04	Dimethoate	1.85
05	Diazinon	3.00
06	Methyl Parathion	1.62
07	Metalaxyl	1.84
08	Fenitrothion	1.70
09	Malathion	3.17
10	Fenthion	2.90
11	Chlorpyrifos	1.82
12	Quinalphos	2.57
13	Methidathion	1.97
14	Fenamiphos	2.10
15	Ethion	1.64
16	Propiconazole	2.56

3.1.2.2 Analysis of Sample

Fruit (n=280) and vegetable (n=455) samples were analyzed for identification and quantitation of sixteen (16) different organophosphorus pesticide residues. Table 24 and 25 shows the analysis results of fruits and vegetable samples. Figure 40-45 shows the chromatogram of pesticide detected samples of vegetable.

Table 24: Amount of organophosphorous pesticides in different fruit sample

Sl No.	Sample Name	Number of sample Analyzed	Results (mg/kg)
01	Banana	35	Not Detected
02	Red apple	35	Not Detected
03	Green apple	35	Not Detected
04	Dates	35	Not Detected
05	Orange	35	Not Detected
06	Grape	35	Not Detected
07	Pineapple	35	Not Detected
08	Mango	35	Not Detected

Table 25: Amount of organophosphorous pesticides in different vegetable samples

Sample Name	Number of sample	Number of pesticide detected sample	Name of Pesticide	Sample ID	Analysis Results [µg/kg]	BFSA MRL [µg/kg]
Cabbage	35	02	Chlorpyrifos	Cab 03	15.21±0.17	1000
				Cab 19	8.10±0.24	
Green Chili	35	04	Dimethoate	GC 04	14.91±0.51	500
				GC 10	19.60±0.17	
				GC 12	8.91±0.11	
				GC 17	34.64±1.07	
Tomato	35	0			Not Detected	
Carrot	35	0			Not Detected	
Cauliflower	35	0			Not Detected	
Potato	35	0			Not Detected	
Green Bean	35	0			Not Detected	
Long Bean	35	0			Not Detected	
Coriander Leaf	35	0			Not Detected	
Eggplant	35	0			Not Detected	
Red Amaranth	35	0			Not Detected	
Lettuce	35	0			Not Detected	
Capsicum	35	0			Not Detected	

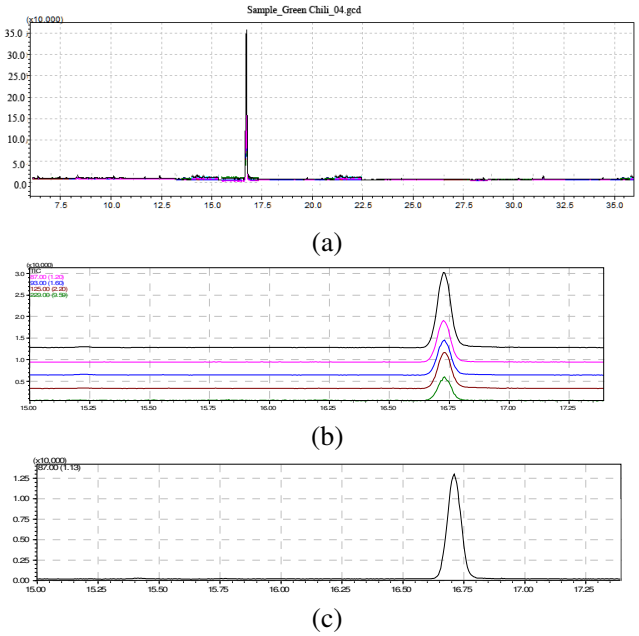


Figure-40: (a) SIM, (b) total ion and (c) target ion chromatogram of dimethoate for green chili sample GC 04

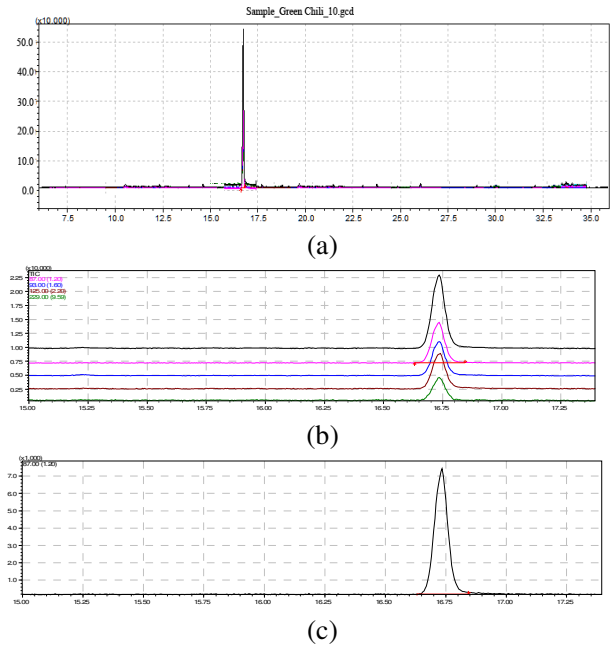


Figure-41: (a) SIM, (b) total ion and (c) target ion chromatogram of dimethoate for green chili sample GC 10

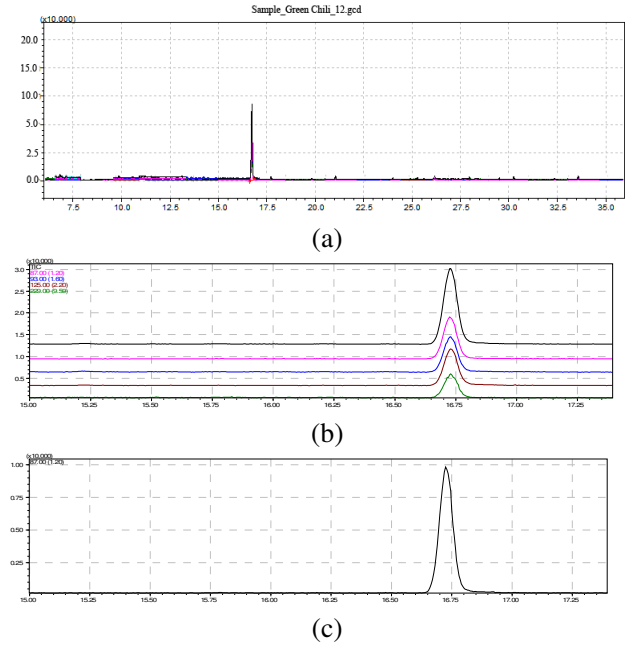


Figure-42: (a) SIM, (b) total ion and (c) target ion chromatogram of dimethoate for green chili sample GC 12

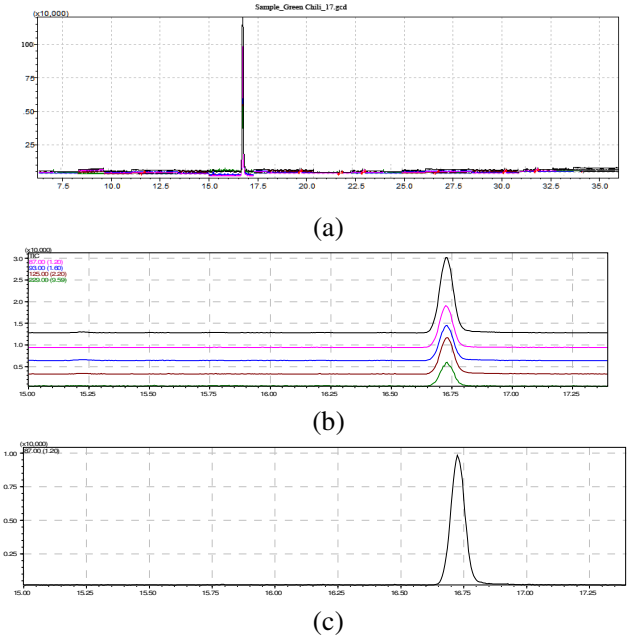


Figure-43: (a) SIM, (b) total ion and (c) target ion chromatogram of dimethoate for green chili sample GC 17

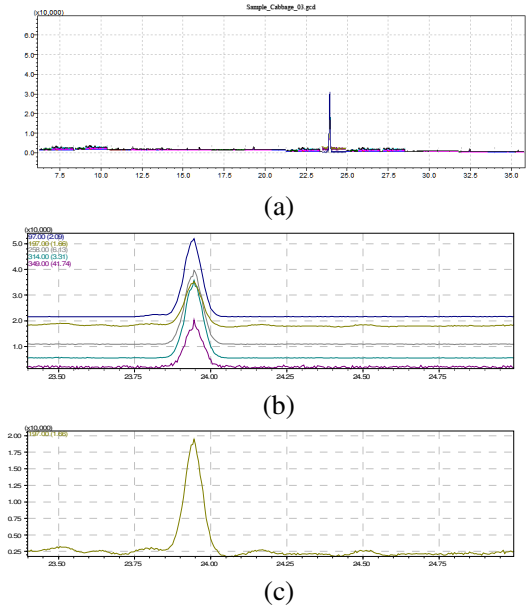


Figure-44: (a) SIM, (b) total ion and (c) target ion chromatogram of chlorpyrifos for cabbage sample Cab 03

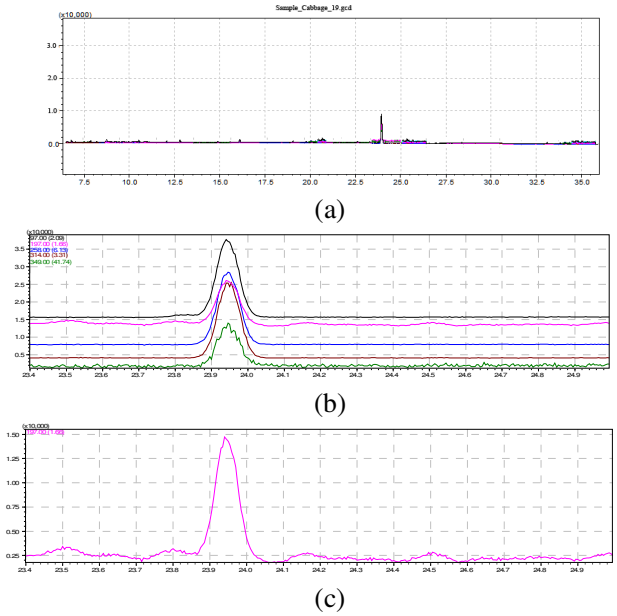


Figure-45: (a) SIM, (b) total ion and (c) target ion chromatogram of chlorpyrifos for cabbage sample Cab 19

3.1.2.3 Discussion

Sixteen organophosphorus pesticides were analyzed in fruits (n=280) and vegetables (n=455) sample by GC-MS. LOD and LOQ were in the range of 0.40-0.80 µg/kg and 0.80-2.66 µg/kg, respectively. Working range was 5-50 µg/L and linear correlation coefficient (R^2) value was in the range of 0.996-0.999. Recovery (%) for cabbage was in the range of 89-97%. Relative standard deviation (RSD %) for repeatability was in the range of 1.70-3.74. Dimethoate was found in four green chili samples and chlorpyrifos was found in two cabbage samples which were within the maximum residue limit (MRL) set by BFSA **[69].** Dimethoate and Chlorpyrifos are approved pesticide in Bangladesh by the Department of Agricultural Extension (DAE) **[70].**

Dimethoate is a post harvest insecticide. It was considered as a high priority compound by different countries of the world. They concern about unacceptable dietary exposure risks. It is resulting from post harvest dipping of fruits and vegetables for obstruction the pest and growth of micro organism. Dimethoate was categorized as a group C carcinogen for human. It was concluded based on the observations of haemolymphoreticular tumours in male mice at the highest dose of 30 mg/kg bw/d. In some toxicological study of dimethoate, experiment with male rats shows positive results in some mutagenicity assays **[71].** So, it is important to determine dimethoate in fruits and vegetable.

Previously it was reported in Bangladesh that dimethoate was found in hyacinth bean at concentration range of 303-961 µg/kg and in eggplant at concentration range of 23-217 µg/kg **[72].** In this study, dimethoate was found in green chili at concentration range of 8.91-34.64 µg/kg.

With Comet assay in human lymphocytes, an *in vitro* study shows that chlorpyrifos can damage the DNA extensively **[73].** Chlorpyrifos is considered as a genotoxicant **[74].** Some *in vitro* studies shows that chlorpyrifos can induce the developmental of neurotoxicity **[75].**

It was reported earlier in Bangladesh that chlorpyrifos was found in eggplant at concentration 200 µg/kg, in tomato at concentration range of 40-700 µg/kg, in cauliflower at concentration range of 62-80 µg/kg, in cabbage at concentration range of 20-50 µg/kg, in potato at concentration 26 µg/kg, in cucumber at concentration range of 18-270 µg/kg, in carrot at concentration range of 30-400 µg/kg, and in onion at concentration 130 µg/kg **[76].** In this study chlorpyrifos was found in cabbage at concentration range 8.91-34.64 µg/kg.

As dimethoate is a post harvest insecticide and there is a perception that farmers often dip their crops in dimethoate solution. In this study dimethoate was found in green chili which was within the MRL set by BFSA. Chlorpyrifos were found in cabbage and it was also within the MRL set by BFSA. As these two organophosphorus pesticides are approved and heavily used in Bangladesh, it might be conclude that the farmers maintain the withdrawal period before harvesting their crops. Although the dimethoate and chlorpyrifos pesticides were found within the MRL, a continuous monitoring should be maintain to observe the trend of these pesticide contamination in fruits and vegetable in Bangladesh.

3.1.3 Determination of 85 organophosphorus pesticides in fruits and vegetables by LC-MS/MS

For determination of 85 organophosphorus pesticides in fruit (n=280) and vegetable (n=455) samples by LC-MS/MS**,** the QuEChERS method was validated using tomato sample T 01 as a representative control matrix. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of 85 organophosphorus pesticides (OPPs) in different fruits and vegetables analyses. Liquid chromatograph was used for separation of compounds and tandem mass spectrometer was used for detection and quantification of compounds. Targeted compounds in the analytes were identified in comparison with the retention time and multiple reaction monitoring (MRM) transition of precursor ion into fragment ion of CRM standards with the retention time and MRM transition of components to found present in samples.

3.1.3.1 Method Validation Performance Characteristics

3.1.3.1.1 Selectivity

Blank of control tomato matrix T01, mixed CRM standard of acephate, acetamiprid, buprofezin, carbaryl, clothianidin, cymoxanil, dicrotophos, dimethomorph, dinotefuran, formetanate HCl, hexythiazox, imazalil, imidacloprid, linuron, metalaxyl, methamidophos, methomyl, monocrotophos, omethoate, piperonyl butoxide, prochloraz, propamocarb, propargite, pyraclostrobin, pyridaben, pyrimethanil, spinosad, spiromesifen, thiabendazole, thiamethoxam and trifloxystrobin in mix-1. Mixed CRM standard of aldicarb sulfoxide, aldicarb, benalaxyl, bendiocarb, bifenazate, carbetamide, carbofuran, carboxin, carfentrazone, diflubenzuron, dioxacarb, diuron, fenamidone, fenazaquin, fenhexamid, furalaxyl, furathiocarb, iprovalicarb, isoprocarb, mefenacet, metconazole, methiocarb, oxamyl, propham, propoxur, spiroxamine and zoxamide in mix-2. Mixed CRM standard of 3-hydroxy carbofuran, aminocarb, bitertanol, bupirimate, clofentezine, difenoconazole, epoxiconazole, fenbuconazole, fenuron, flusilazole, flutriafol, fuberidazole, isoproturon, metobromuron, mevinphos, nitenpyram, paclobutrazol, phoxim, pymetrozine, tebuconazole, tebuthiuron, temephos, thiacloprid, triadimefon, triazophos, tricyclazole and triflumizole in mix-3 and spiked sample were analyzed by this method. This highly selective and highly sensitive confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. The below MRM chromatograms in Figure 46-49 are the supporting evidence which give sufficient reliability for selectivity. The chromatograms show that compounds are well resolved and there is no significant interference of matrix with targeted OPPs. Representative MRM

transition of acephate, aldicarb and 3-hydroxy carbofuran were given in Figure 50, 51 and 52. MRM transition of all other pesticides were given in the annexure in Figure 161-182. Table 26 shows retention time (RT) of 85 organophosphorus pesticides.

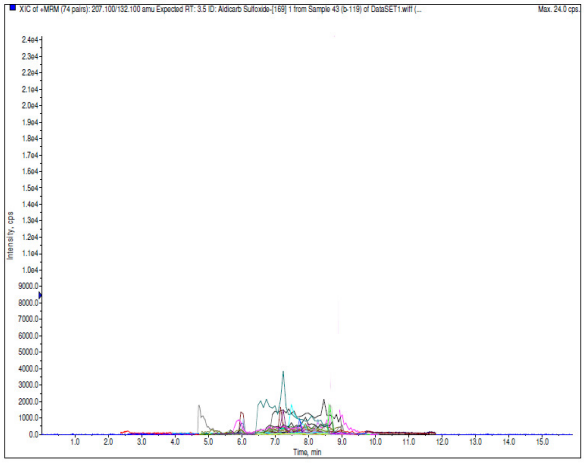


Figure-46: MRM chromatogram of blank control tomato matrix

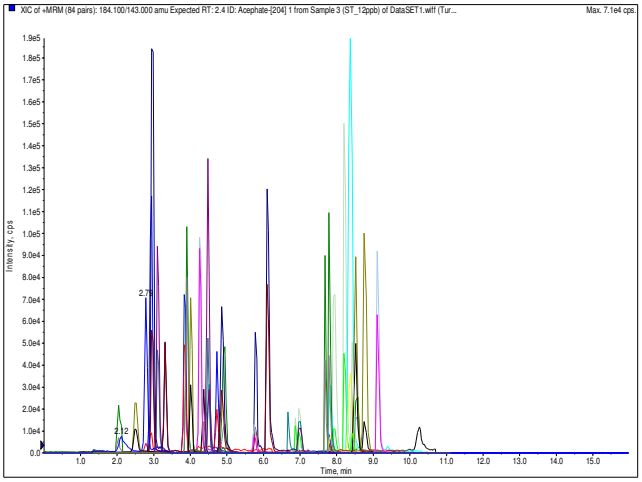


Figure-47: MRM chromatogram of spiked tomato control matrix with 31 pesticide CRM standard at concentration of 12 µg/L (Mix-1)

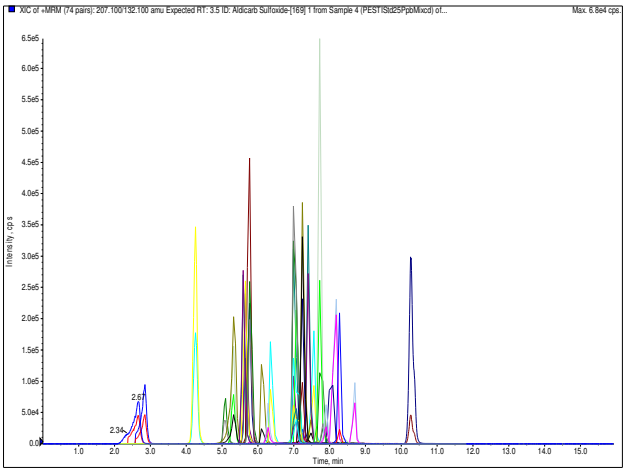


Figure-48: MRM chromatogram of spiked tomato control matrix with 27 pesticide CRM standard at concentration of 25 µg/L (Mix-2)

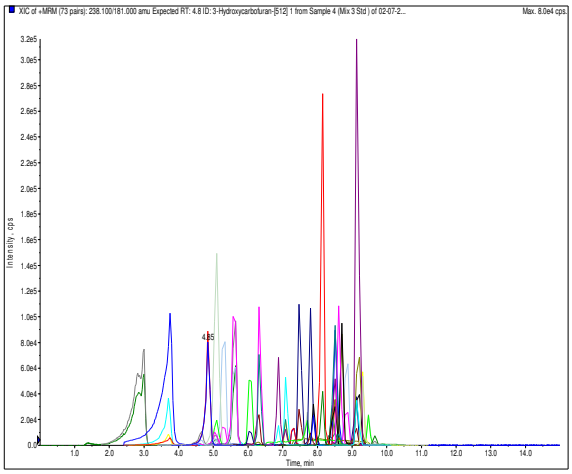
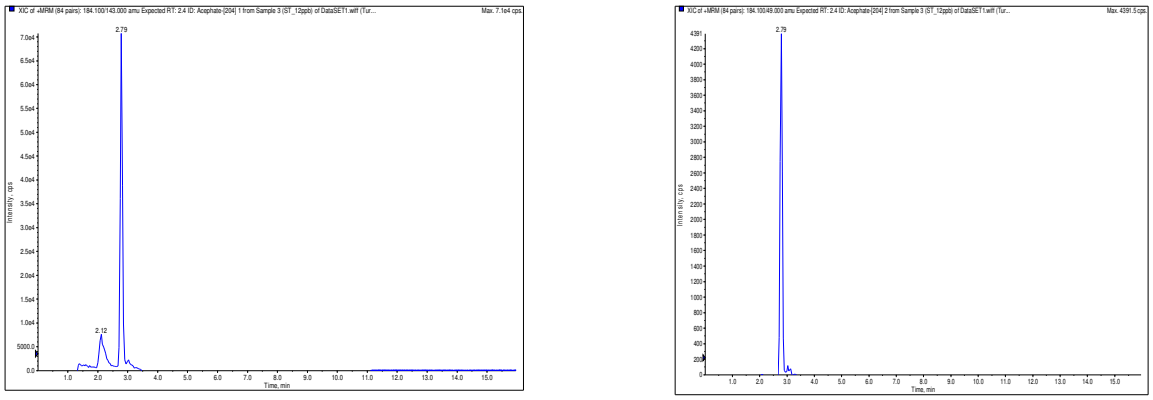


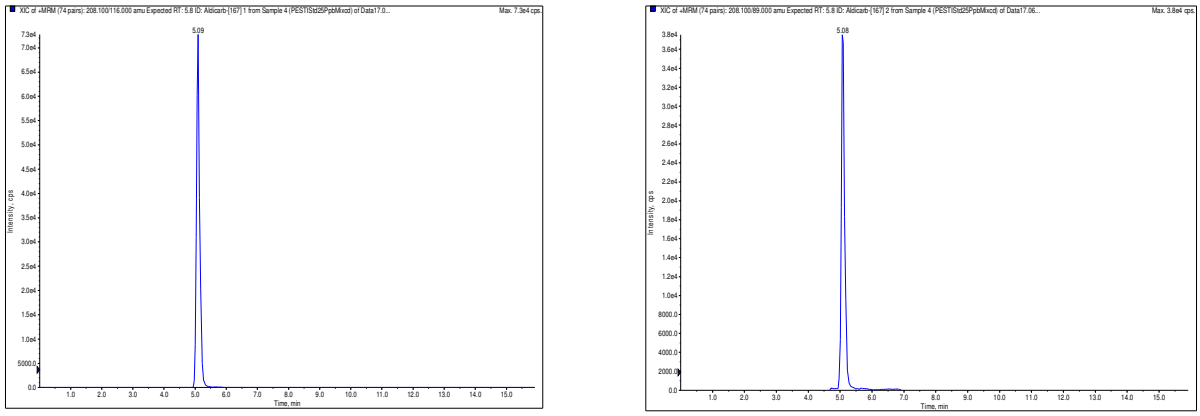
Figure-49: MRM chromatogram of spiked tomato control matrix with 27 pesticide CRM standard at concentration of 15 µg/L (Mix-3)

MRM Transition of Pesticides



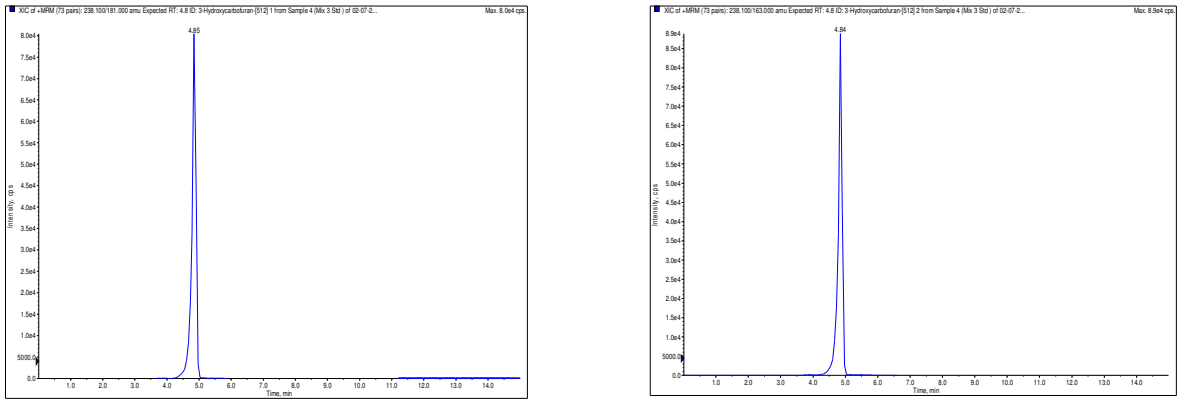
(a) (b)

Figure-50: MRM transition of acephate (a) 184.100>143.000 amu and (b) 184.100>49.000 amu



(a) (b)

Figure-51: MRM transition of aldicarb (a) 208.100>116.000 amu and (b) 208.100>89.000 amu



(a) (b)

Figure-52: MRM transition of 3-Hydroxycarbofuran (a) 238.100>181.000 amu and (b) 238.100>163.000 amu

Table 26: Retention time (RT) of 85 organophosphorus pesticides.

Mix-1(31 pesticides)		Mix-2(27 pesticides)		Mix-3(27 pesticides)	
Compound Name	**RT(min)**	**Compound Name**	**RT(Min)**	**Compound Name**	**RT(Min)**
Methamidophos	2.50	Aldicarb sulfoxide	2.67	Aminocarb	2.99
Acephate	2.79	Oxamyl	2.85	Nitenpyram	3.71
Formetanate HCl	2.93	Dioxacarb	4.26	Pymetrozine	3.75
Propamocarb	2.96	Aldicarb	5.09	3-Hydroxycarbofuran	4.85
Omethoate	3.11	Propoxur	5.30	Fuberidazole	5.09
Dinotefuran	3.31	Bendiocarb	5.30	Fenuron	5.30
Methomyl	3.85	Carbetamide	5.32	Mevinphos	5.60
Monocrotophos	3.91	Carbofuran	5.76	Thiacloprid	6.06
Thiamethoxam	4.02	Carboxin	6.12	Tricyclazole	6.33
Dicrotophos	4.26	Propham	6.29	Tebuthiuron	6.88
Clothianidin	4.37	Isoprocarb	6.36	Flutriafol	7.09
Imidacloprid	4.52	Spiroxamine Isomer	7.01	Metobromuron	7.30
Cymoxanil	4.73	Furalaxyl	7.02	Isoproturon	7.48
Thiabendazole	4.87	Diuron	7.05	Paclobutrazol	7.80
Acetamiprid	4.93	Fenamidone	7.09	Triadimefon	7.88
Imazalil	5.76	Methiocarb	7.24	Triazophos	8.15
Carbaryl	5.79	Iprovalicarb Isomer	7.41	Fenbuconazole	8.43
Metalaxyl	6.11	Fenhexamid	7.48	Bupirimate	8.50
Linuron	6.87	Bifenazate	7.56	Flusilazole	8.51
Dimethomorph	6.98	Mefenacet	7.73	Epoxiconazole	8.52
Pyrimethanil	6.99	Carfentrazone	7.83	Tebuconazole	8.62
Pyraclostrobin	7.69	Diflubenzuron	7.91	Phoxim	8.70
Trifloxystrobin	7.79	Zoxamide	8.05	Bitertanol	8.86
Spinosad (Spinosyn A)	7.83	Benalaxyl	8.17	Clofentezine	9.12
Prochloraz	7.93	Metconazole	8.28	Triflumizole	9.14
Buprofezin	8.21	Furathiocarb	8.70	Difenoconazole	9.18
Piperonyl butoxide	8.37	Fenazaquin	10.26	Temephos	9.31
Spiromesifen	8.43				
Propargite	8.52				
Hexythiazox	8.56				
Pyridaben	9.12				

3.1.3.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked tomato sample with CRM standard of 85 organophosphorus pesticides at concentration 3 µg/kg for mix-1, 6.25 µg/kg for mix-2 and 3.75 µg/kg for mix-2. LOD and LOQ of 85 organophosphorus pesticides were given in Table 27.

Table 27: LOD and LOQ of Mix-1(31 pesticides), Mix-2(27 pesticides) and Mix-3 (27 pesticide)

Mix-1(31 pesticides)			**Mix-2(27 pesticides)**			**Mix-3(27 pesticides)**		
Compound	**LOD (µg/kg)**	**LOQ (µg/kg)**	**Compound**	**LOD (µg/kg)**	**LOQ (µg/kg)**	**Compound**	**LOD (µg/kg)**	**LOQ (µg/kg)**
Acephate	0.07	0.22	Aldicarb sulfoxide	0.16	0.53	3-Hydroxy carbofuran	0.10	0.32
Acetamiprid	0.03	0.10	Aldicarb	0.16	0.52	Aminocarb	0.09	0.30
Buprofezin	0.07	0.26	Benalaxyl	0.10	0.33	Bitertanol	0.26	0.87
Carbaryl	0.09	0.32	Bendiocarb	0.11	0.36	Bupirimate	0.23	0.79
Clothianidin	0.06	0.21	Bifenazate	0.12	0.40	Clofentezine	0.25	0.83
Cymoxanil	0.09	0.31	Carbetamide	0.27	0.89	Difenoconazole	0.21	0.72
Dicrotophos	0.16	0.53	Carbofuran	0.38	1.25	Epoxiconazole	0.20	0.68
Dimethomorph	0.06	0.20	Carboxin	0.22	0.74	Fenbuconazole	0.33	1.11
Dinotefuran	0.08	0.29	Carfentrazone	0.28	0.93	Fenuron	0.21	0.72
Formetanate HCl	0.07	0.24	Diflubenzuron	0.34	1.12	Flusilazole	0.18	0.60
Hexythiazox	0.11	0.37	Dioxacarb	0.11	0.37	Flutriafol	0.50	1.67
Imazalil	0.15	0.51	Diuron	0.07	0.21	Fuberidazole	0.16	0.54
Imidacloprid	0.02	0.08	Fenamidone	0.13	0.43	Isoproturon	0.08	0.26
Linuron	0.07	0.24	Fenazaquin	0.11	0.37	Metobromuron	0.81	2.70
Metalaxyl	0.05	0.19	Fenhexamid	0.46	1.54	Mevinphos	0.15	0.48
Methamidophos	0.09	0.30	Furalaxyl	0.10	0.33	Nitenpyram	0.41	1.38
Methomyl	0.09	0.33	Furathiocarb	0.49	1.62	Paclobutrazol	0.27	0.92
Monocrotophos	0.09	0.32	Iprovalicarb	0.19	0.63	Phoxim	0.33	1.10
Omethoate	0.14	0.48	Isoprocarb	0.30	1.01	Pymetrozine	0.27	0.92
Piperonyl butoxide	0.07	0.23	Mefenacet	0.40	1.31	Tebuconazole	0.29	0.97
Prochloraz	0.06	0.22	Metconazole	0.47	1.58	Tebuthiuron	0.34	1.15
Propamocarb	0.15	0.52	Methiocarb	0.11	0.35	Temephos	0.41	1.37
Propargite	0.11	0.36	Oxamyl	0.40	1.32	Thiacloprid	0.34	1.13
Pyraclostrobin	0.11	0.37	Propham	0.43	1.44	Triadimefon	0.42	1.43
Pyridaben	0.11	0.37	Propoxur	0.10	0.32	Triazophos	0.06	0.19
Pyrimethanil	0.07	0.26	Spiroxamine	0.16	0.51	Tricyclazole	0.20	0.68
Spinosad	0.07	0.25	Zoxamide	0.31	1.04	Triflumizole	0.11	0.38
Spiromesifen	0.09	0.30						
Thiabendazole	0.13	0.44						
Thiamethoxam	0.13	0.44						
Trifloxystrobin	0.09	0.31						

3.1.3.1.3 Working Range and Linearity

The working range for 31 organophosphorus pesticides in mix-1 was 3-12 µg/L, for 27 organophosphorus pesticides in mix-2 was 6.25-25 µg/L, for 27 organophosphorus pesticides in mix-3 was 3.75-15 µg/L. Representative calibration curves of some OPPs were given in Figure 53. Linear correlation coefficient (R^2) value of 85 organophosphorus pesticides was given in Table 28.

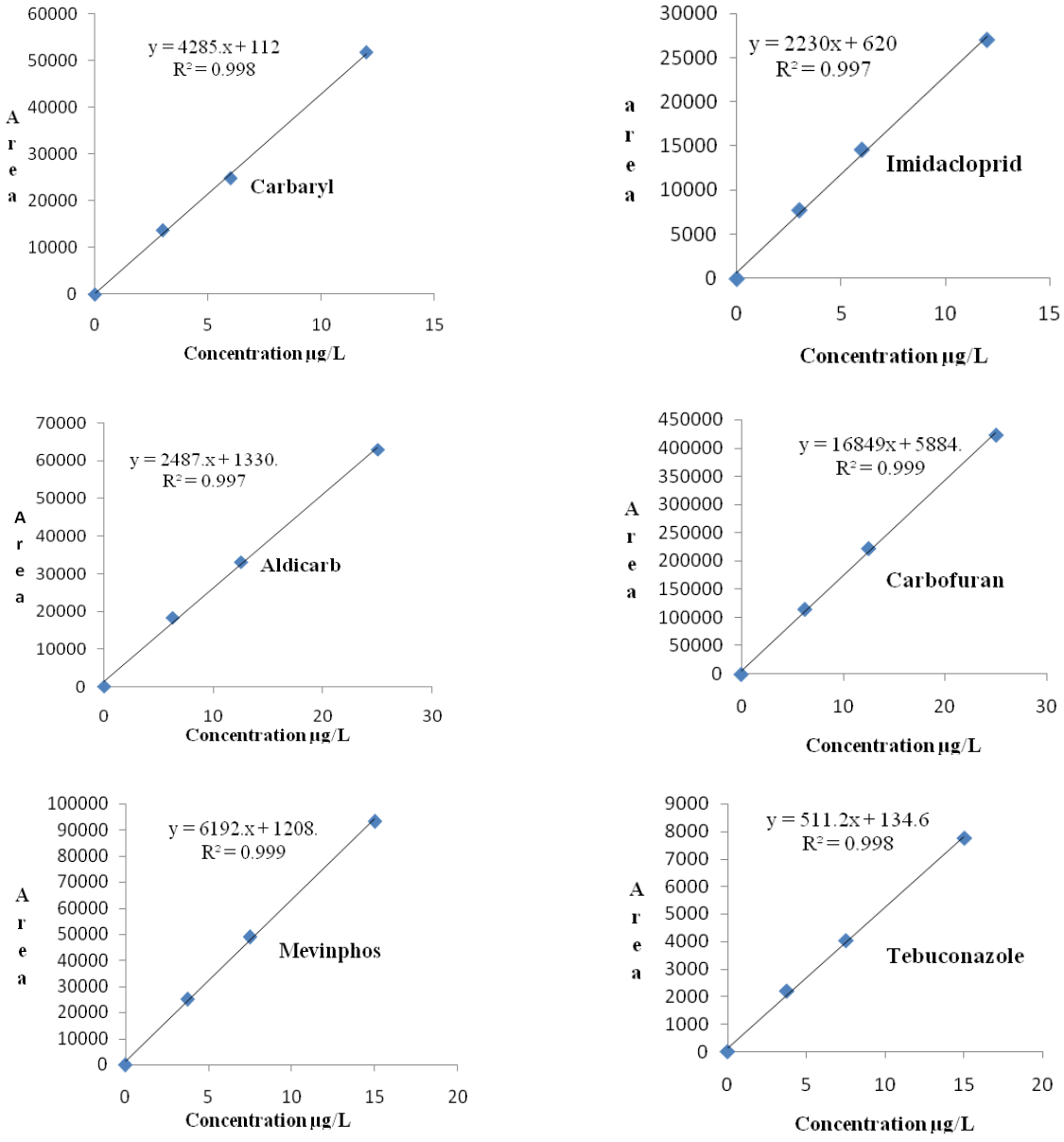


Figure-53: Calibration curve of carbaryl, imidachloprid, aldicarb, carbofuran, mevinphos and tebuconazol

Table 28: Linear correlation coefficient (R^2) of Mix-1(31 pesticides), Mix-2(27 pesticides) and Mix-3 (27 pesticide)

Mix-1(31 Pesticide)		**Mix-2(27 Pesticide)**		**Mix-3(27 Pesticide)**	
Compound	**Linear correlation coefficient (R^2)**	**Compound**	**Linear correlation coefficient (R^2)**	**Compound**	**Linear correlation coefficient (R^2)**
Acephate	0.999	Aldicarb sulfoxide	0.999	3-Hydroxy carbofuran	0.998
Acetamiprid	0.997	Aldicarb	0.997	Aminocarb	0.998
Buprofezin	0.999	Benalaxyl	0.999	Bitertanol	0.999
Carbaryl	0.998	Bendiocarb	0.999	Bupirimate	0.997
Clothianidin	0.998	Bifenazate	0.999	Clofentezine	0.998
Cymoxanil	0.999	Carbetamide	0.997	Difenoconazole	0.997
Dicrotophos	0.999	Carbofuran	0.999	Epoxiconazole	0.999
Dimethomorph	0.997	Carboxin	0.998	Fenbuconazole	0.998
Dinotefuran	0.998	Carfentrazone	0.998	Fenuron	0.999
Formetanate HCl	0.999	Diflubenzuron	0.998	Flusilazole	0.999
Hexythiazox	0.998	Dioxacarb	0.999	Flutriafol	0.997
Imazalil	0.998	Diuron	0.998	Fuberidazole	0.998
Imidacloprid	0.997	Fenamidone	0.999	Isoproturon	0.999
Linuron	0.997	Fenazaquin	0.999	Metobromuron	0.998
Metalaxyl	0.999	Fenhexamid	0.997	Mevinphos	0.999
Methamidophos	0.998	Furalaxyl	0.999	Nitenpyram	0.998
Methomyl	0.998	Furathiocarb	0.998	Paclobutrazol	0.998
Monocrotophos	0.999	Iprovalicarb	0.999	Phoxim	0.999
Omethoate	0.998	Isoprocarb	0.999	Pymetrozine	0.999
Piperonyl butoxide	0.999	Mefenacet	0.999	Tebuconazole	0.998
Prochloraz	0.999	Metconazole	0.998	Tebuthiuron	0.999
Propamocarb	0.998	Methiocarb	0.999	Temephos	0.999
Propargite	0.999	Oxamyl	0.998	Thiacloprid	0.998
Pyraclostrobin	0.999	Propham	0.999	Triadimefon	0.997
Pyridaben	0.998	Propoxur	0.999	Triazophos	0.999
Pyrimethanil	0.997	Spiroxamine	0.999	Tricyclazole	0.998
Spinosad	0.999	Zoxamide	0.998	Triflumizole	0.998
Spiromesifen	0.999				
Thiabendazole	0.999				
Thiamethoxam	0.999				
Trifloxystrobin	0.999				

3.1.3.1.4 Accuracy (Recovery)

3 µg/ L CRM standard of mix-1 was spiked in tomato sample, 6.25 µg/ L CRM standard of mix-2 was spiked in tomato sample and 3.75 µg/ L CRM standard of mix-3 was spiked in tomato sample. MRM chromatogram of CRM standards and spiked control matrix tomato was given in Figure 54. Table 29 shows the recovery study.

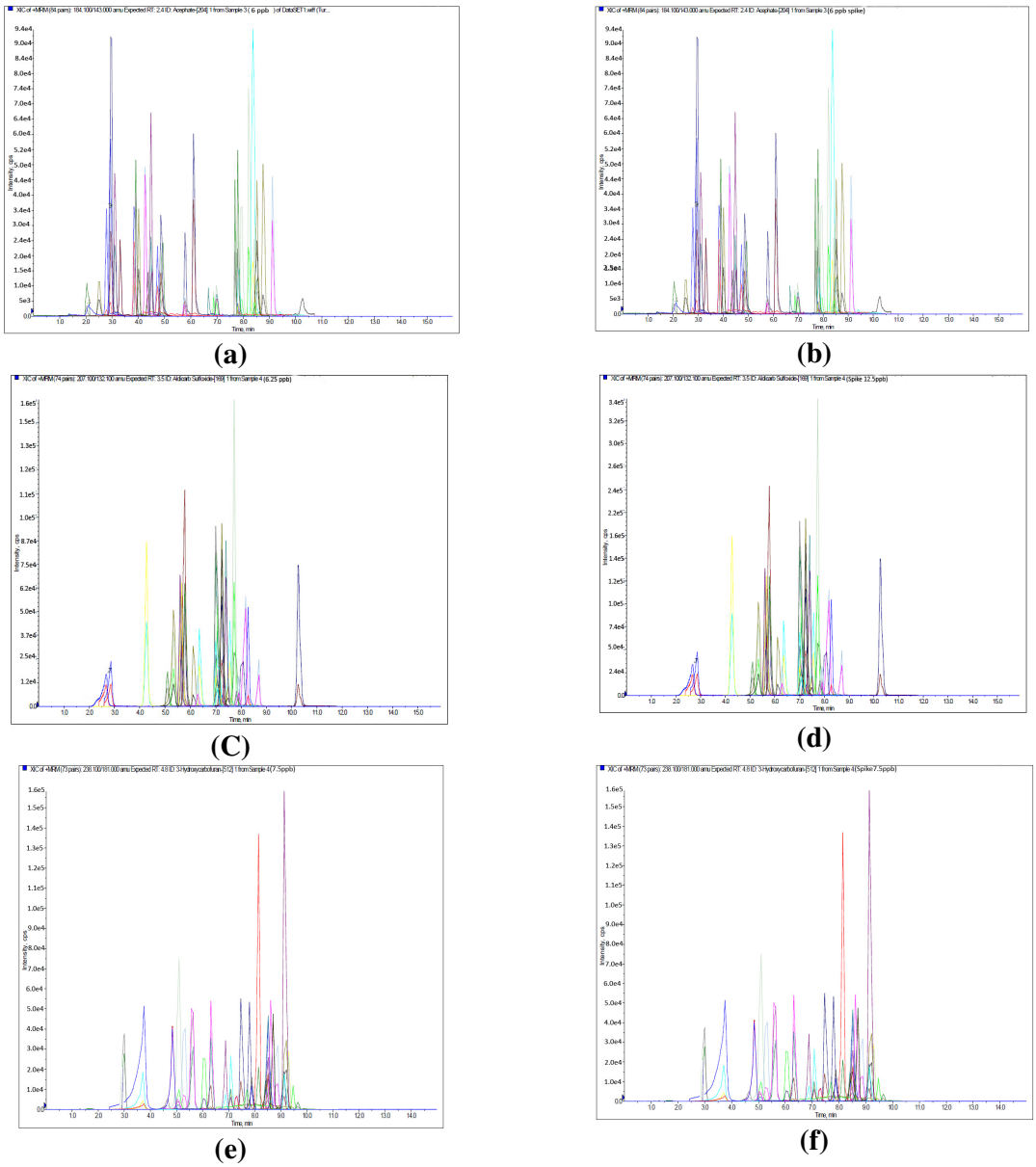


Figure 54: **(a)** MRM chromatogram of 31 pesticide CRM standard at concentration of 6 µg/L, mix-1 **(b)** MRM chromatogram of spiked tomato control matrix with 31 pesticide CRM standard (6 µg/L) **(c)** MRM chromatogram of 27 pesticide CRM standard at concentration of 12.5 µg/L, mix-2 **(d)** MRM chromatogram of spiked tomato control matrix with 27 pesticide CRM standard (12.5 µg/L) **(e)** MRM chromatogram of 27 pesticide CRM standard at concentration of 7.5 µg/L, mix-3 **(f)** MRM chromatogram of spiked tomato control matrix with 27 pesticide CRM standard (7.5 µg/L)

Table 29: Recovery of Mix-1(31 pesticides), Mix-2(27 pesticides) and Mix-3 (27 pesticide)

Mix-1(31 Pesticide)		Mix-2(27 Pesticide)		Mix-3(27 Pesticide)	
Compound	**Recovery% With Tomato**	**Compound**	**Recovery% With Tomato**	**Compound**	**Recovery% With Tomato**
Acephate	93	Aldicarb sulfoxide	94	3-Hydroxycarbofuran	92
Acetamiprid	89	Aldicarb	92	Aminocarb	90
Buprofezin	86	Benalaxyl	89	Bitertanol	87
Carbaryl	85	Bendiocarb	90	Bupirimate	85
Clothianidin	92	Bifenazate	95	Clofentezine	92
Cymoxanil	88	Carbetamide	90	Difenoconazole	88
Dicrotophos	95	Carbofuran	92	Epoxiconazole	84
Dimethomorph	84	Carboxin	90	Fenbuconazole	92
Dinotefuran	93	Carfentrazone	94	Fenuron	82
Formetanate HCl	90	Diflubenzuron	85	Flusilazole	83
Hexythiazox	90	Dioxacarb	92	Flutriafol	90
Imazalil	94	Diuron	88	Fuberidazole	85
Imidacloprid	92	Fenamidone	85	Isoproturon	85
Linuron	91	Fenazaquin	87	Metobromuron	91
Metalaxyl	87.	Fenhexamid	88	Mevinphos	85
Methamidophos	90	Furalaxyl	90	Nitenpyram	85
Methomyl	90	Furathiocarb	89	Paclobutrazol	83
Monocrotophos	94	Iprovalicarb	91	Phoxim	81
Omethoate	91	Isoprocarb	92	Pymetrozine	88
Piperonyl butoxide	88	Mefenacet	85	Tebuconazole	82
Prochloraz	90	Metconazole	92	Tebuthiuron	83
Propamocarb	90	Methiocarb	89	Temephos	91
Propargite	95	Oxamyl	85	Thiacloprid	87
Pyraclostrobin	95	Propham	94	Triadimefon	85
Pyridaben	92	Propoxur	86	Triazophos	85
Pyrimethanil	88	Spiroxamine	84	Tricyclazole	87
Spinosad	95	Zoxamide	90	Triflumizole	85
Spiromesifen	97				
Thiabendazole	92				
Thiamethoxam	90				
Trifloxystrobin	87				

3.1.3.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked tomato sample with CRM standard of mix-1 at concentration 3 µg/kg, with CRM standard of mix-2 at concentration 6.25 µg/kg, with CRM standard of mix-3 at concentration 3.75 µg/kg. Table 30 shows the RSD% of 85 organophosphorus pesticides.

Table 30: RSD% of Mix-1(31 pesticides), Mix-2(27 pesticides) and Mix-3 (27 pesticide)

Mix-1(31 Pesticide)		**Mix-2(27 Pesticide)**		**Mix-3(27 Pesticide)**	
Compound	**RSD%**	**Compound**	**RSD%**	**Compound**	**RSD%**
Acephate	0.13	Aldicarb sulfoxide	0.33	3-Hydroxy carbofuran	0.20
Acetamiprid	0.06	Aldicarb	0.32	Aminocarb	0.18
Buprofezin	0.16	Benalaxyl	0.20	Bitertanol	0.54
Carbaryl	0.20	Bendiocarb	0.22	Bupirimate	0.49
Clothianidin	0.13	Bifenazate	0.25	Clofentezine	0.52
Cymoxanil	0.19	Carbetamide	0.56	Difenoconazole	0.45
Dicrotophos	0.33	Carbofuran	0.78	Epoxiconazole	0.42
Dimethomorph	0.12	Carboxin	0.46	Fenbuconazole	0.69
Dinotefuran	0.18	Carfentrazone	0.58	Fenuron	0.45
Formetanate HCl	0.15	Diflubenzuron	0.70	Flusilazole	0.38
Hexythiazox	0.23	Dioxacarb	0.23	Flutriafol	1.04
Imazalil	0.32	Diuron	0.13	Fuberidazole	0.34
Imidacloprid	0.05	Fenamidone	0.27	Isoproturon	0.16
Linuron	0.15	Fenazaquin	0.23	Metobromuron	1.69
Metalaxyl	0.12	Fenhexamid	0.96	Mevinphos	0.30
Methamidophos	0.19	Furalaxyl	0.21	Nitenpyram	0.86
Methomyl	0.20	Furathiocarb	1.02	Paclobutrazol	0.57
Monocrotophos	0.20	Iprovalicarb	0.40	Phoxim	0.69
Omethoate	0.30	Isoprocarb	0.63	Pymetrozine	0.57
Piperonyl butoxide	0.14	Mefenacet	0.82	Tebuconazole	0.61
Prochloraz	0.14	Metconazole	0.99	Tebuthiuron	0.72
Propamocarb	0.32	Methiocarb	0.22	Temephos	0.86
Propargite	0.23	Oxamyl	0.82	Thiacloprid	0.71
Pyraclostrobin	0.23	Propham	0.90	Triadimefon	0.89
Pyridaben	0.23	Propoxur	0.20	Triazophos	0.12
Pyrimethanil	0.16	Spiroxamine	0.32	Tricyclazole	0.42
Spinosad	0.16	Zoxamide	0.65	Triflumizole	0.23
Spiromesifen	0.19				
Thiabendazole	0.28				
Thiamethoxam	0.28				
Trifloxystrobin	0.20				

3.1.3.2 Analysis of Sample

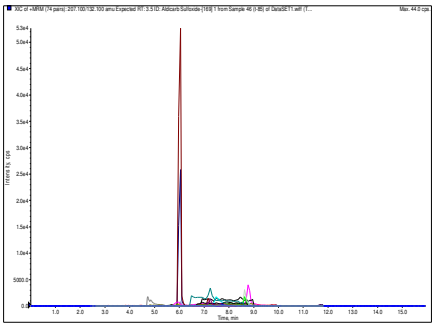
Eighty five (85) organophosphorus pesticides were analyzed in fruits (n=280) and vegetables (n=455) sample. Table 31 and 32 shows the analysis results of fruits and vegetable samples. Figure 55-58 shows the chromatograms of pesticide detected vegetable samples.

Table 31: Amount of 85 organophosphorous pesticides in different fruit samples

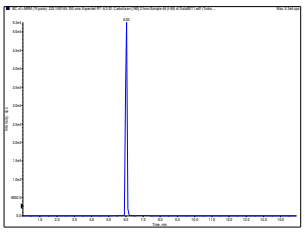
Sample Name	Number of sample Analyzed	Analysis Results [mg/kg]
Banana	35	Not Detected
Red apple	35	Not Detected
Green apple	35	Not Detected
Dates	35	Not Detected
Orange	35	Not Detected
Grape	35	Not Detected
Pineapple	35	Not Detected
Mango	35	Not Detected

Table 32: Amount of 85 organophosphorous pesticides in different vegetable samples

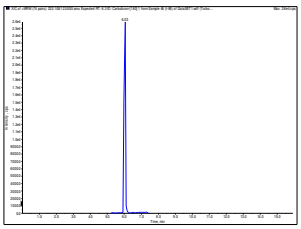
Sample Name	Number of sample Analyzed	Number of pesticide detected sample	Name of Pesticide	Sample ID	Analysis Results [µg/kg]	EU MRL [µg/kg]
Cabbage	35	0			Not Detected	
Green Chili	35	0			Not Detected	
Tomato	35	2	Carbofuran	T14	2.74±0.06	10.00
				T17	1.07±0.01	
Carrot	35	0			Not Detected	
Cauliflower	35	0			Not Detected	
Potato	35	0			Not Detected	
Green Bean	35	0			Not Detected	
Long Bean	35	0			Not Detected	
Coriander Leaf	35	0			Not Detected	
Eggplant	35	2	Carbofuran	B19	0.53±0.02	10.00
				B28	1.22±0.06	
Red Amaranth	35	0			Not Detected	
Lettuce	35	0			Not Detected	
Capsicum	35	0			Not Detected	



(a)

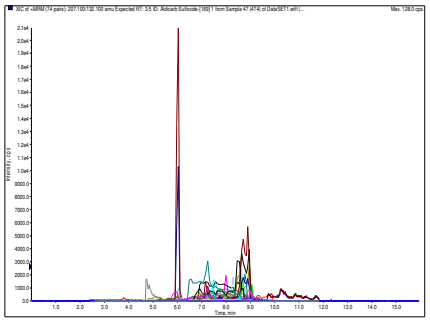


(b)

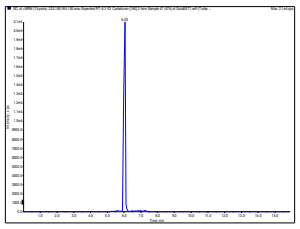


(c)

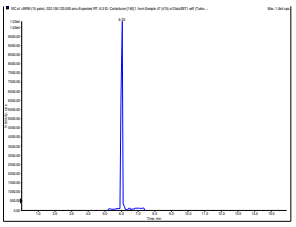
Figure-55: (a) MRM chromatogram (b) MRM transition 222.1>165.1 (c) MRM transition 222.1>123 of carbofuran for tomato sample T 14



(a)

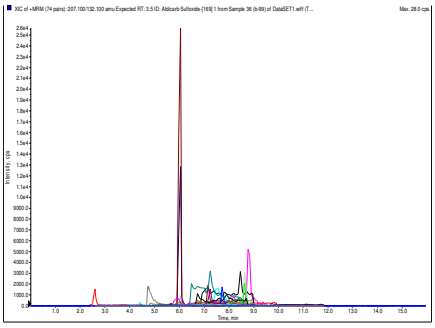


(b)

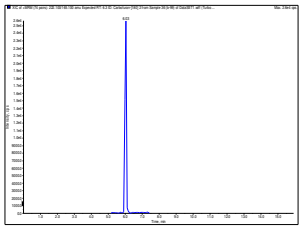


(c)

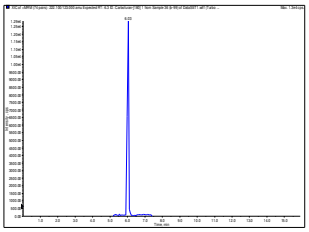
Figure-56: (a) MRM chromatogram (b) MRM transition 222.1>165.1 (c) MRM transition 222.1>123 of carbofuran for tomato sample T 17



(a)

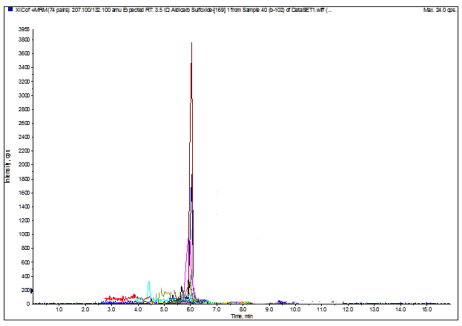


(b)

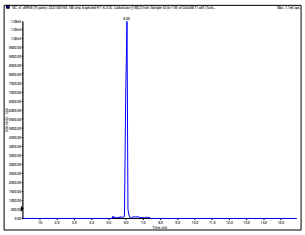


(c)

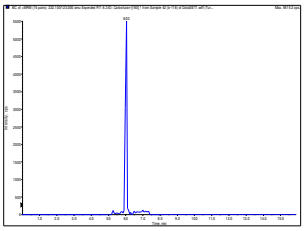
Figure-57: (a) MRM chromatogram (b) MRM transition 222.1>165.1 (c) MRM transition 222.1>123 of carbofuran for eggplant sample B 19



(a)



(b)



(c)

Figure-58: (a) MRM chromatogram (b) MRM transition 222.1>165.1 (c) MRM transition 222.1>123 of carbofuran for eggplant sample B 28

3.1.3.3 Discussion

Eighty five organophosphorus pesticides in fruits (n=280) and vegetables (n=455) sample were analyzed by LC-MS/MS. LOD were in the range of 0.02-0.16 µg/kg, 0.07-0.49 µg/kg and 0.06-0.81 µg/kg for mix-1, mix-2 and mix-3, respectively. LOQ were in the range of 0.08-0.53 µg/kg, 0.21-1.62 µg/kg and 0.19-2.70 µg/kg for mix-1, mix-2 and mix-3, respectively. Calibration range were 3-12 µg/L, 6.25-25 µg/L and 3.75-15 µg/L for mix-1,mix-2 and mix-3, respectively. Linear correlation coefficient (R^2) value was in the range of 0.997-0.999 for mix-1, mix-2 and mix-3. Recovery (%) for tomato was in the range of 81-97%. Relative standard deviation (RSD%) for repeatability was in the range of 0.05-1.69. Carbofuran was found in two tomato samples and in two eggplant samples which were within the MRL 0.01 mg/kg set by European Commission **[77].**

Carbofuran is one of the most toxic broad-spectrum pesticide. It is systemic *N*-methyl carbamate pesticide. Carbofuran is extensively used as insecticide, nematicide and acaricide for agricultural production. For anticholinesterase activity carbofuran is extremely toxic to mammals, birds, fish and wildlife. It inhibits acetyl-cholinesterase and butyrylcholinesterse activity. Carbofuran is responsible for endocrine disrupting activity, reproductive disorders, cytotoxic and genotoxic abnormalities in human **[78].** The presence of carbofuran in the urine, feces, bile, any other body tissue or fluid is considered as a most specific biomarker of recent or continuing exposure. The effect of carbamate exposure can be measure by monitoring of pre-exposure and post exposure levels of AChE in erythrocytes **[79].** Hussain et al. (1990) also explain significant inhibition of blood AChE in grain farmers who were exposed to carbofuran **[80].** Carbofuran is widely used pesticide in vegetable farming. Carbofuran has a broad spectrum of activity and it is relatively cheap **[81].** Carbofuran now banned in the European Union, United States and Canada for the several incident of bird poisoning **[82].** However carbofuran is approved pesticide in Bangladesh by DAE **[83].** It is heavily used in different agricultural production.

It was reported previously in Bangladesh that carbofuran was found in eggplant at the range of 5-50 µg/kg, in tomato at the range of 4-50 µg/kg and in cabbage at the range of 13-1000 µg/kg **[84].** In this study carbofuran was found in tomato in the range of 1.01-2.74 µg/kg and in eggplant in the range of 0.53-1.22 µg/kg which were within the MRL 10 µg/kg set by European Commission. Carbofuran is highly toxic to human. Although in tomato and in eggplant it was found within the MRL, a careful monitoring should be establish to see the level of carbofuran in fruits and vegetables in Bangladesh.

3.1.4 Determination of arsenic, lead, cadmium in vegetables and fruit by atomic absorption spectrophotometer(AAS) equipped with graphite furnace atomizer(GFA)

The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of As, Pb and Cd in different fruits and vegetables analyses. This method was validated using potato as a representative control matrix. Samples were analyzed by injection into a GFA of AAS. Fruit (n=280) and vegetable (n=455) samples were dried by freeze drier and digested by microwave digester using HNO_3 (65%) and H_2O_2 (30%). Metals released by the digestion and then diluted with De-ionized water. 0.2% $Mg(NO_3)_2$ and 0.1% palladium were used as matrix modifiers.

3.1.4.1 Method Validation Performance Characteristics

3.1.4.1.1 Selectivity

Blank of potato control matrix P 01, CRM standard of As, Pb and Cd and spiked potato sample were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. Figure 59-65 shows absorption spectrums of blank, CRM standard of As, Pb and Cd and spiked potato control matrix which gives sufficient reliability for selectivity.

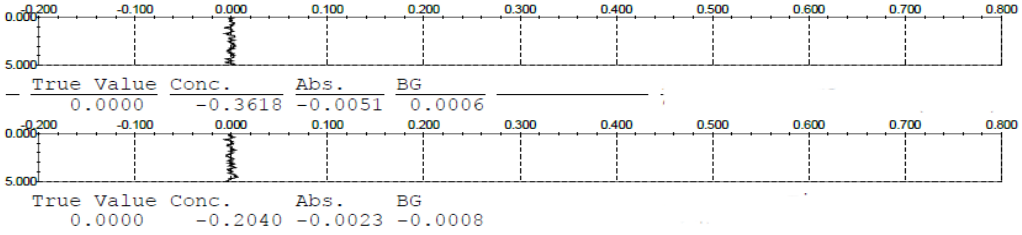


Figure-59: Absorption spectrum of blank potato control matrix

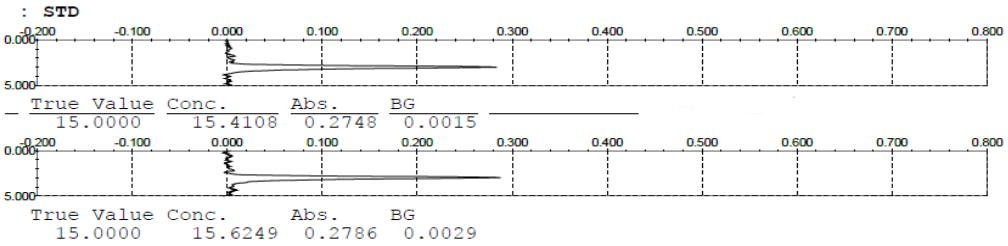


Figure-60: Absorption spectrum of CRM standard of As at concentration 15 µg/L

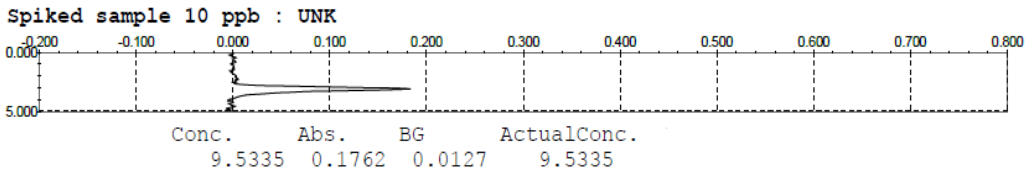


Figure-61: Absorption spectrum of spiked potato control matrix with CRM standard of As at concentration 10 µg/L

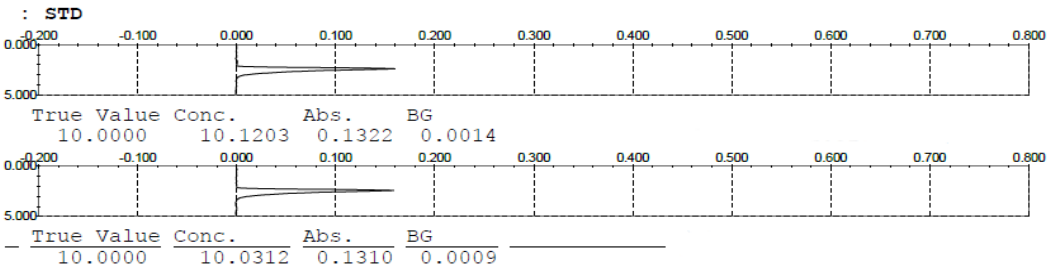


Figure-62: Absorption spectrum of CRM standard of Pb at concentration 10 µg/L

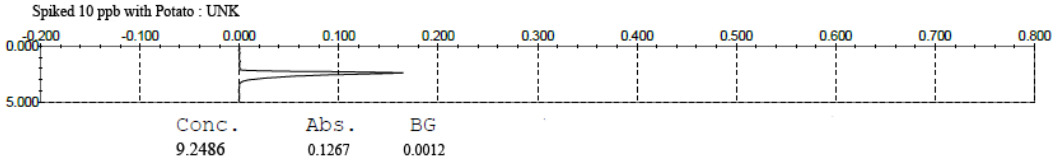


Figure-63: Absorption spectrum of spiked potato control matrix with CRM standard of Pb at concentration 10 µg/L

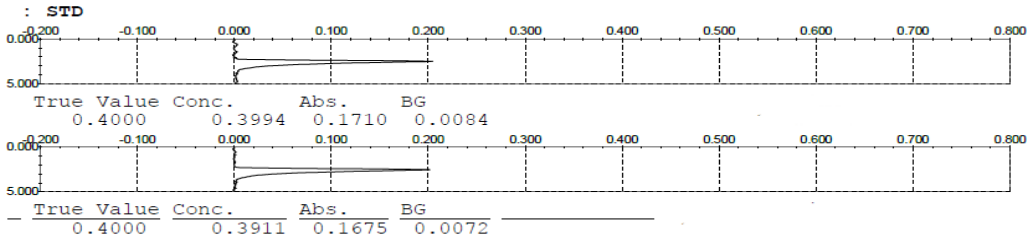


Figure-64: Absorption spectrum of CRM standard of Cd at concentration 0.4 µg/L

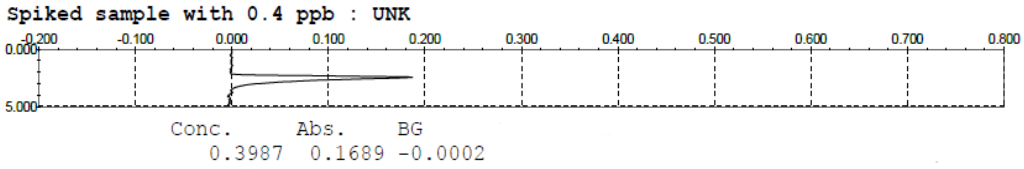


Figure-65: Absorption spectrum of spiked potato control matrix with CRM standard of Cd at concentration 0.4 µg/L

3.1.4.1.2 LOD and LOQ for As, Pb and Cd

LOD and LOQ were calculated from ten replicates of spiked potato sample with CRM standard of arsenic and lead at concentration 100 µg/kg and cadmium at concentration 4 µg/kg. LOD and LOQ of As, Pb and Cd were given in Table 33.

Table 33: LOD and LOQ of As, Pb and Cd

Name of Heavy Metal	LOD (µg/kg)	LOQ (µg/kg)
Arsenic	2.49	8.30
Lead	2.39	7.96
Cadmium	0.09	0.29

3.1.4.1.3 Working Range and Linearity

Working range of As, Pb and Cd were 5-20 µg/L, 5-20 µg/L and 0.2-0.8 µg/L, respectively. Figure 66 shows the calibration curve of As, Pb and Cd.

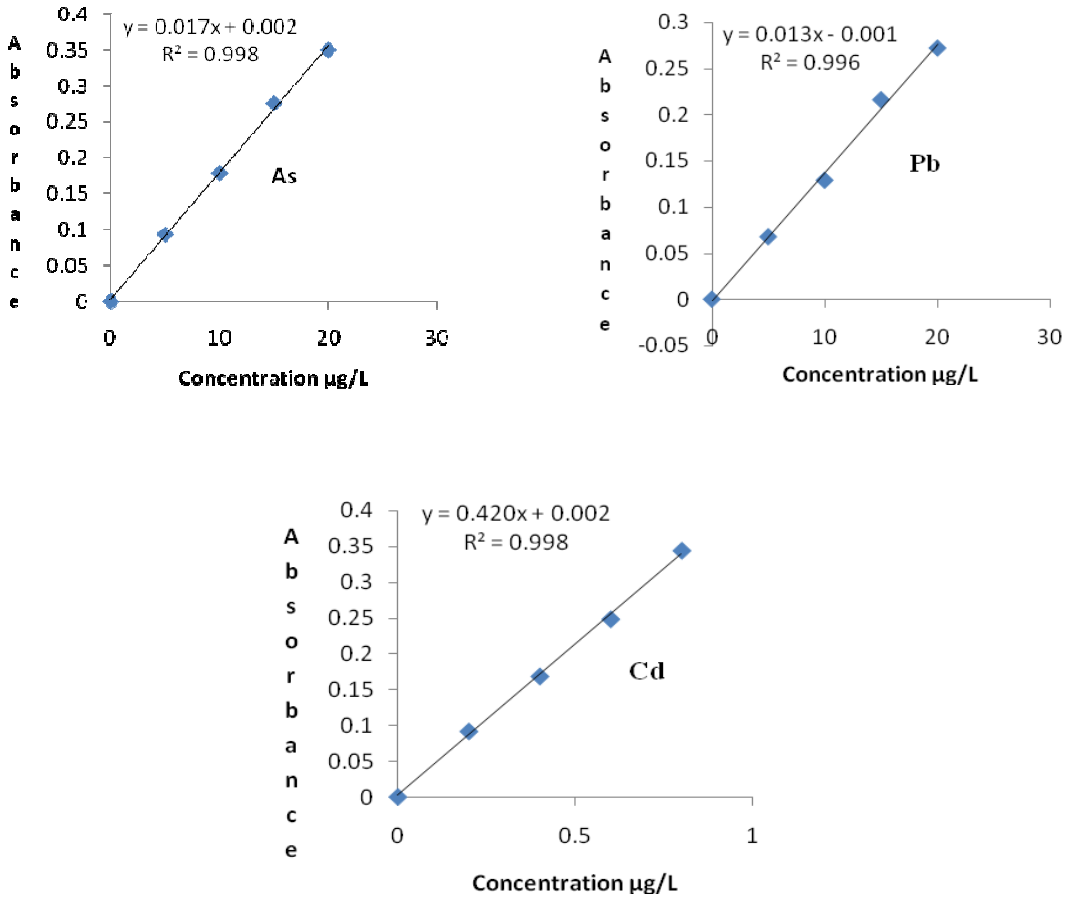


Figure-66: Calibration Curve of As, Pb and Cd

3.1.4.1.4 Accuracy (Recovery %)

Potato control sample was spiked with CRM standard of As, Pb (10 µg/L) and Cd (0.4 µg/L) for recovery experiment. Absorption spectrum of CRM standard of As, Pb and Cd ; and spiked potato control matrix were given in Figure 67-72.

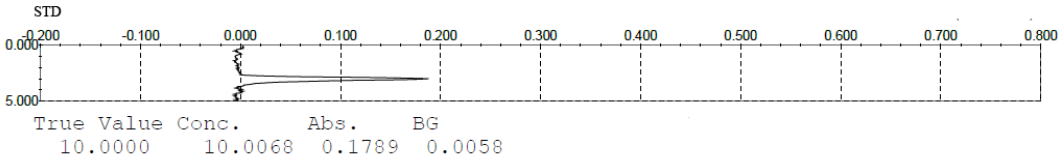


Figure-67: Absorption spectrum of CRM standard of As at concentration 10 µg/L

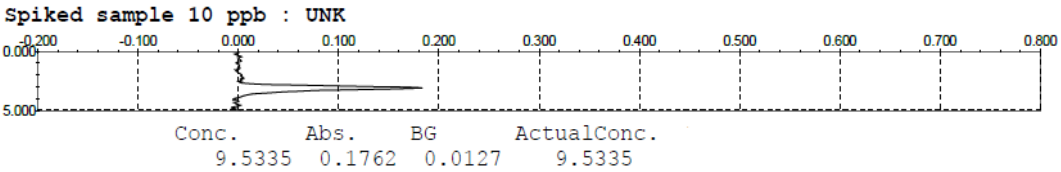


Figure-68: Absorption spectrum of spiked potato control matrix with CRM standard of As at concentration 10 µg/L

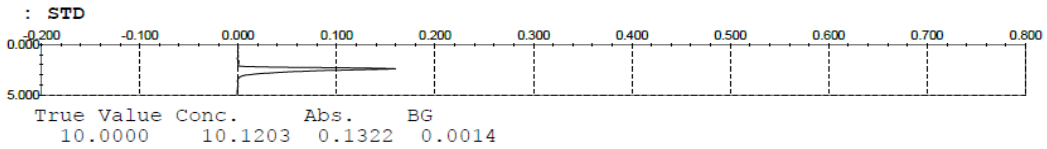


Figure-69: Absorption spectrum of CRM standard of Pb at concentration 10 µg/L

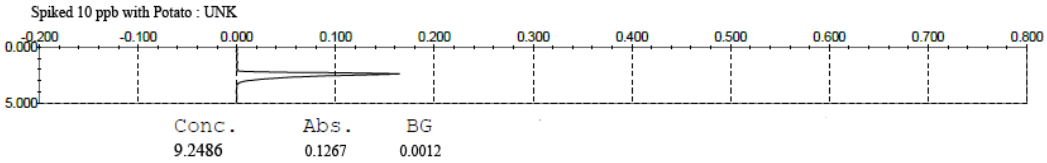


Figure-70: Absorption spectrum of spiked potato control matrix with CRM standard of Pb at concentration 10 µg/L

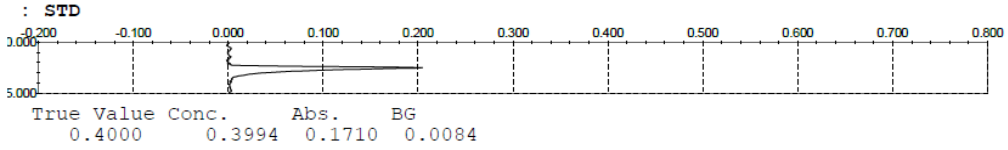
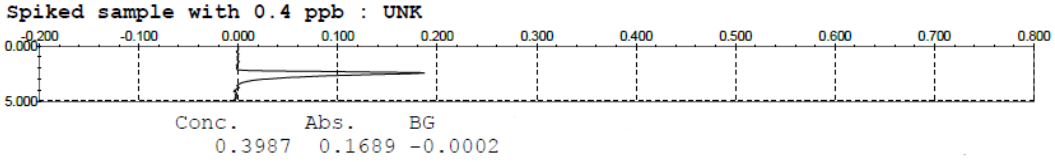


Figure-71: Absorption spectrum of CRM standard of Cd at concentration 0.4 µg/L



72: Absorption spectrum of spiked potato control matrix with CRM standard of Cd at concentration 0.4 µg/L

For recovery study As CRM standard was spiked in control potato sample with six replication at concentration 10 µg/L. The average recovery was found for As is 98±0.66 %. Recovery study of Pb was done by spiking the CRM standard of Pb in control potato sample with six replication at concentration 10 µg/L. The average recovery was found for Pb is 95±2.55%. Recovery study of Cd was performed by spiking the CRM standard of Cd with six replication at concentration 0.4 µg/L and the average recovery was found 96±1.43%.

3.1.4.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked potato sample with CRM standard of As and Pb at concentration 100 µg/kg and Cd at concentration 4 µg/kg. RSD% of As, Pb and Cd was given in Table 34.

Table 34: RSD% of As, Pb and Cd

Name of Heavy Metal	RSD%
Arsenic	1.86
Lead	1.80
Cadmium	1.51

3.1.4.2 Analysis of Sample

Arsenic, lead and cadmium were analyzed in fruits (n=280) and vegetable (n=455) samples. Table 35, 36 and 37 shows the analysis results of fruits and vegetable samples. All absorption spectrum of As and Cd detected sample were attached in the annexure in Figure 183-187.

Table 35: Amount of As, Pb and Cd for fruit sample

Sl No	Sample	Number of Sample	Result (µg/kg)		
			Arsenic	Lead	Cadmium
1	Banana	35	ND	ND	ND
2	Red apple	35	ND	ND	ND
3	Green apple	35	ND	ND	ND
4	Dates	35	ND	ND	ND
5	Orange	35	ND	ND	ND
6	Grape	35	ND	ND	ND
7	Pineapple	35	ND	ND	ND
8	Mango	35	ND	ND	ND

Table 36: Amount of As and Cd in detected vegetable sample

Sample ID	Result of As (µg/kg)	Sample ID	Result of Cd (µg/kg)
P 14	10.47±0.75	P11	0.69±0.05
P 16	27.54±1.29	P12	0.81±0.03
P 17	28.74±0.69	P24	2.19±0.01
P 18	33.91±0.99	P31	1.23±0.04
P 19	36.81±0.12	P32	2.66±0.08
P 20	42.51±1.12	P33	3.19±0.09
P 21	22.07±0.96		
P 22	24.89±1.23		
P 23	24.54±1.05		
P 24	27.35±0.47		
P 25	49.73±1.41		
P 32	16.26±0.95		
P 33	18.83±0.18		
T 13	5.15±0.25		
B 1	8.12±0.50		
B 2	13.11±0.66		
B 6	2.00±0.28		
B 7	4.63±0.33		
B 8	5.59±0.39		
B 17	13.22±0.09		
B 18	7.96±0.12		
B 24	7.63±0.16		
B 26	6.32±0.61		
B 29	17.40±0.68		
B 30	8.19±0.29		
C 13	5.12±0.31		

Table 37: Number of vegetable samples in which As, Pb and Cd was not detected

Analysis result of As			Analysis result of Pb		Analysis result of Cd	
Sample	Number of Sample	Result of As (µg/kg)	Number of Sample	Result of Pb (µg/kg)	Number of Sample	Result of Cd (µg/kg)
Cabbage	35	ND	35	ND	35	ND
Green Chili	35	ND	35	ND	35	ND
Tomato	34	ND	35	ND	35	ND
Carrot	34	ND	35	ND	35	ND
Cauliflower	35	ND	35	ND	35	ND
Potato	22	ND	35	ND	29	ND
Green Bean	35	ND	35	ND	35	ND
Long Bean	35	ND	35	ND	35	ND
Coriander Leaf	35	ND	35	ND	35	ND
Eggplant	24	ND	35	ND	35	ND
Red Amaranth	35	ND	35	ND	35	ND
Lettuce	35	ND	35	ND	35	ND
Capsicum	35	ND	35	ND	35	ND

3.1.4.3 Discussion

As, Pb and Cd in fruit (n=280) and vegetable (n=455) samples were analyzed by AAS-GFA. LOD for As, Pb and Cd was 2.49, 2.39, 0.09 and LOQ was 8.3, 7.96, 0.29 µg/kg, respectively. Calibration range for As and Pb was 5-20 µg/L and calibration range for Cd was 0.2-0.8 µg/L. Linear correlation coefficient (R^2) value for As, Pb and Cd was 0.998, 0.996 and 0.998, respectively. Recovery (%) was 98%, 95% and 96% for As, Pb and Cd in potato, respectively. Relative standard deviation (RSD%) for repeatability of As, Pb and Cd was 1.86, 1.80 and 1.51, respectively. Arsenic was found in 13 potato, 1 tomato, 11 eggplant and 1 carrot samples. Lead was not detected in any sample of fruits and vegetables. Cadmium was detected in 6 potato samples which were within the maximum limit of 0.1 mg/kg set by BFSA **[85].**

Chronic arsenic toxicity is connected with various medical symptom called arsenicosis. Some particular skin lesion is caused by the chronic arsenic toxicity. Arsenicosis is also associated with chronic lung disease such as chronic bronchitis, chronic obstructive pulmonary disease and bronchiectasis, liver disease such as non-cirrhotic portal fibrosis, polyneuropathy and cerebrovascular disease, peripheral vascular disease, hypertension and ischemic heart disease, diabetes mellitus, weakness and anemia, congestion of eyes, pterygium and cataract. Cancer of skin, lung, and urinary bladder are significant cancers linked with chronic arsenic toxicity **[86].**

Lead poisoning has been present from the beginning of the history of mankind. Ingestion of contaminated food is one of the important pathway of lead exposure. Lead poisoning also caused by occupational exposure. In children, anemia and neurological disorder can be occurred from lead toxicity **[87].**

The carcinogenic effects of cadmium toxicity have been reported in human where cadmium has entered in food chain **[88].** Cadmium toxicity is occurred through food chain via contaminated food crops and the contaminated drinking water **[89].** In South and Southeast Asian countries including China, India, Thailand, Bangladesh and Sri Lanka it has been observed that a prevalence of cadmium contaminated rice **[90].** Therefore, the growth in cadmium toxicity has attracted the interest of worldwide research.

Earlier it was reported in Bangladesh that arsenic was found in potato at the range of 4-6 µg/kg and in eggplant at the range of 3-9 µg/kg, cadmium was found in eggplant at average

concentration of 27 µg/kg **[91].** In this study arsenic was found in potato at the range of 10.47-49.73 µg/kg, in eggplant at the range of 2.00-17.40 µg/kg, in tomato it was 5.15 µg/kg and in carrot it was 5.12 µg/kg. Cadmium was found in potato at the range of 0.69-3.19 µg/kg.

It can be seen from the results that concentration of arsenic is increasing over the time in potato and eggplant. There is no maximum limit available worldwide for arsenic in potato, eggplant, tomato and carrot. This is why it cannot be evaluated that it is within or exceed the maximum limit. Results of this study can give a good thought to the global food safety authorities for setting the maximum limit of arsenic in different vegetables. Cadmium also found in potato samples which is within the maximum limit set by BFSA. A regular monitoring system should be adopted to avoid heavy metal contamination in fruits and vegetables in Bangladesh.

3.2 Determination of Lead and Chromium in Turmeric Powder by Atomic Absorption Spectrophotometer(AAS) Equipped with Graphite Furnace Atomizer(GFA)

Turmeric powder sample (n=17) were collected from 5 city corporation market of Dhaka. Turmeric powder samples were dried by laboratory oven and digested by microwave digester using HNO_3 (65%) and H_2O_2 (30%). Metals released by the digestion and then diluted with De-ionized water. $Mg(NO_3)_2$ (0.2%) and palladium (0.1%) were used as matrix modifiers. Samples were analyzed by injection into a GFA of AAS. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of Pb and Cr in turmeric powder analysis. This method was validated using turmeric powder as a representative control matrix.

3.2.1 Method Validation Performance Characteristics

3.2.1.1 Selectivity

Blank of control sample of turmeric powder TP 09, CRM standard of Pb and Cr and spiked turmeric powder control sample were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. Figure 73-77 shows absorption spectrum of blank, CRM standard of Pb and Cr and spiked control matrix of turmeric powder which gives sufficient reliability for selectivity.

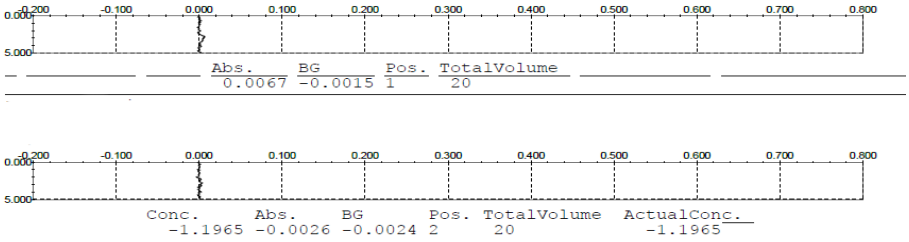


Figure 73: Absorption spectrum of control sample blank of turmeric powder

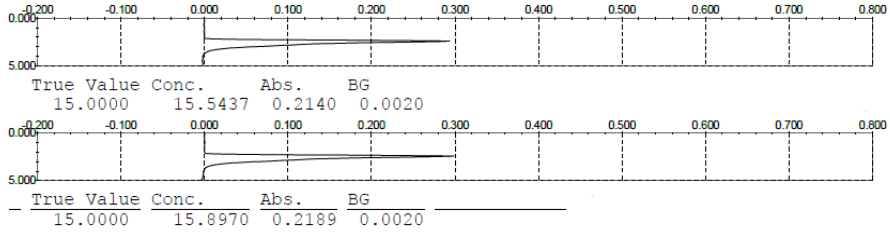


Figure-74: Absorption spectrum of CRM standard of 15 µg/L Pb

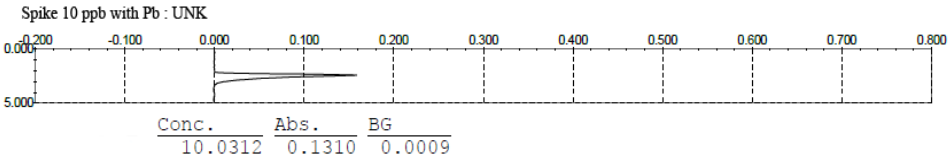


Figure-75: Absorption spectrum of spiked turmeric powder with CRM standard of Pb at concentration 10 µg/L

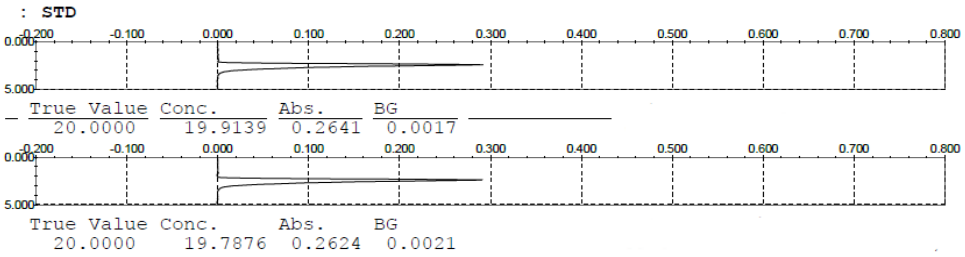


Figure-76: Absorption spectrum of CRM standard of 20 µg/L Cr

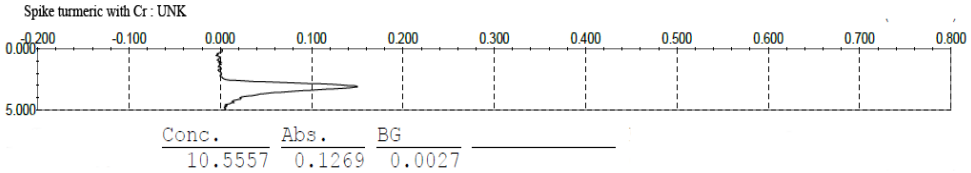


Figure-77: Absorption spectrum of spiked turmeric powder with CRM standard of Cr at concentration 10 µg/L

3.2.1.2 LOD and LOQ for Pb and Cr

LOD and LOQ were calculated from ten replicates of spiked potato sample with CRM standard of lead and chromium at concentration 100 µg/kg (table 38).

Table 38: LOD and LOQ of Pb and Cr

Name of Heavy Metal	LOD (µg/kg)	LOQ (µg/kg)
Lead	1.71	5.69
Chromium	2.17	7.22

3.2.1.3 Working Range and Linearity

Working range of Pb and Cr was 5-20 µg/L. Figure 78 shows the calibration curve of Pb and Cr.

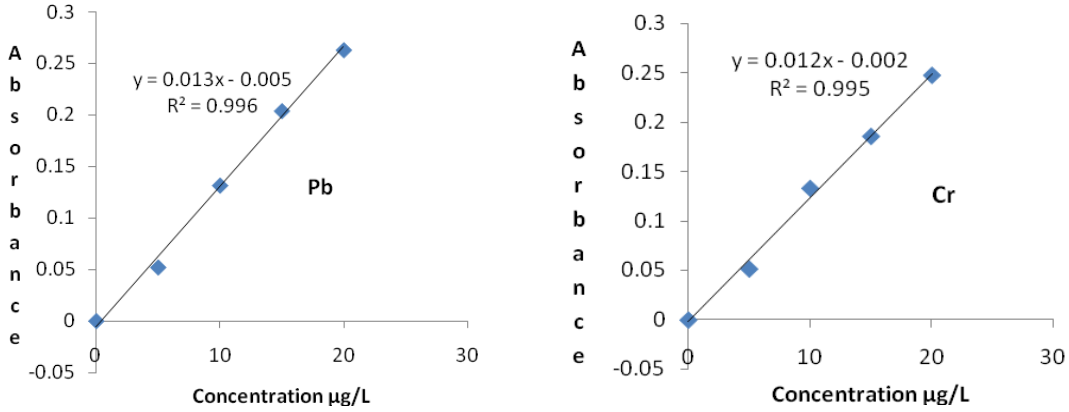


Figure 78: Calibration curve of Pb and Cr

3.2.1.4 Accuracy (Recovery %)

Turmeric powder control sample was spiked with CRM standard Pb and Cr (10 µg/L) for recovery experiment. Absorption spectrum of CRM standard of Pb and Cr ; and spiked turmeric powder control matrix were given in Figure 79-82.

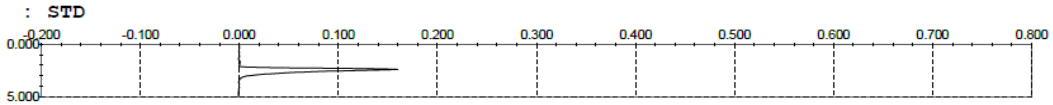


Figure-79: Absorption spectrum of CRM standard of Pb at concentration of 10 µg/L

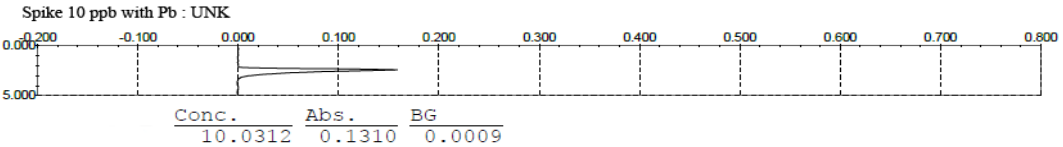


Figure-80: Absorption spectrum of spiked turmeric powder with CRM standard of Pb at concentration 10 µg/L

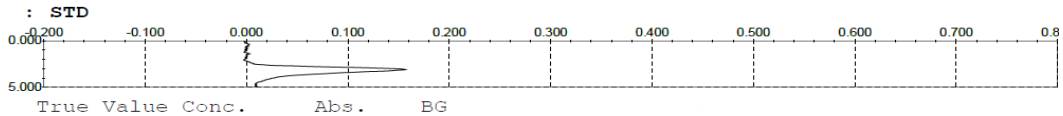


Figure-81: Absorption spectrum of CRM standard of Cr at concentration of 10 µg/L

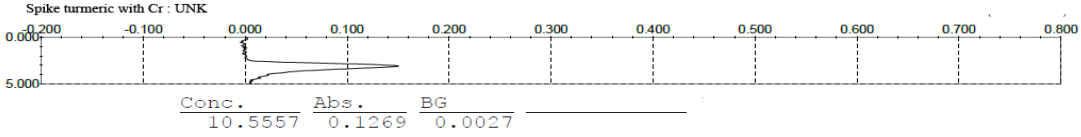


Figure-82: Absorption spectrum of spiked turmeric powder with CRM standard of Cr at concentration 10 µg/L

Recovery study of Pb was done by spiking the CRM standard of Pb in control turmeric powder sample with six replication at concentration 10 µg/L. The average recovery was found for Pb is 98±2.78%. Recovery study of Cr was performed by spiking the CRM standard of Cr with six replication at concentration 10 µg/L and the average recovery was found 94±2.09%

3.2.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked turmeric powder sample with CRM standard of Pb and Cr at concentration 100 µg/kg. RSD% of Pb and Cr was given in table 39.

Table 39: RSD% of Pb and Cr

Name of Heavy Metal	RSD%
Lead	1.30
Chromium	1.70

3.2.2 Analysis of Sample

Pb and Cr were analyzed in 17 turmeric powder samples. Table 40 shows the analysis results of 17 turmeric powder samples. Absorption spectrum of Pb and Cr detected sample of turmeric powder sample were attached in the annexure in Figure 188-189.

Table 40: Amount of Pb and Cr for turmeric powder

Sample ID	Result of Lead (mg/kg)	Result of Chromium (mg/kg)
TP 01	44.65±1.05	12.36±0.24
TP 02	48.15±0.74	11.63±0.48
TP 03	46.54±1.20	8.90±0.25
TP 04	45.89±1.49	17.09±0.53
TP 05	47.80±0.39	20.62±0.23
TP 06	41.89±0.75	17.00±0.23
TP 07	46.46±0.55	19.40±0.46
TP 08	45.56±0.43	18.18±0.89
TP 09	ND	ND
TP 10	ND	ND
TP 11	ND	ND
TP 12	ND	ND
TP 13	ND	ND
TP 14	ND	ND
TP 15	ND	ND
TP 16	ND	ND
TP 17	ND	ND

3.2.3 Discussion

Lead and chromium were determined in turmeric powder by AAS-GFA. LOD for Pb and Cr was 1.71 and 2.17, respectively. LOQ was 5.69 and 7.22 µg/kg for Pb and Cr, respectively. Calibration range of Pb and Cr was 5-20 µg/L. Linear correlation coefficient (R^2) value for Pb and Cr was 0.996 and 0.995, respectively. Recovery (%) for Pb and Cr in turmeric powder was 98% and 94%, respectively. Relative standard deviation (RSD%) for repeatability of Pb and Cr was 1.30 and 1.70, respectively. High amount of Pb and Cr was found in 8 samples of turmeric powder out of 17 samples. Eight turmeric powder samples exceeded the maximum limit of Pb (2.5 mg/kg) set by Bangladesh Standards and Testing Institution (BSTI) **[92].**

The brain is the most sensitive organ for lead exposure**.** In a developing brain of children, synapse formation is highly affected in the cerebral cortex by lead. Lead also interferes with the development of neurochemicals, including neurotransmitters and management of ion channels **[93].** Lead poisoning also causes loss of neuron myelin sheath, reduction of neurons, it interferes the neurotransmission and decreases neuronal growth. Chronic lead nephropathy occurred due to long time exposure **[94].**

The toxicities of chromium compounds were established with epidemiological studies and with animal studies. Oxidation state is a critical factor in evaluating the activities of chromium compounds. Hexavalent chromium compounds are more toxic than the trivalent chromium compounds. This observation is recognized to the stronger oxidizing power. Respiratory tract and cell-mediated allergic reactions, tissue damage, irritative lesions of the skin are caused by exposure to hexavalent chromium **[95].**

In 2017, United States Food and Drug Administration (US-FDA) found high amount of lead in a specific brand of turmeric powder of Bangladesh ranging from 28 to 53 mg/kg **[96].** In this study also high amount of Pb were found in 8 samples of turmeric powder ranging from 42 to 48 mg/kg. Although this sample sized does not represent the whole Bangladesh but this study can give an idea about the intensity of lead and chromium contamination in turmeric powder. It is a major concern that from where this high amount of Pb and Cr enter into turmeric powder. It is might be from the soil where turmeric plant was cultivated or from processing industry. For the root cause of this high amount of Pb and Cr in turmeric powder further investigation is required with soil and processing industry.

3.3 Determination of Aflatoxin B_1, B_2, G_1 and G_2 in wheat and Maize by HPLC-FLD

For determination of aflatoxins, wheat samples (n=25) were collected from five government silos of Narayangonj, Chattogram, Santahar, Khulna and Ashugonj. Maize samples (n=25) were collected form Bogra and Dinajpur government food storage. Aflatoxins B_1, B_2, G_1 and G_2were extracted from the wheat and maize sample with aqueous methanol. The sample extract was filtered, diluted with water and applied to an immunoaffinity column (IAC) containing antibodies specific for aflatoxins B_1, B_2, G_1 and G_2. The aflatoxins were separated, purified and concentrated on the column then removed from the antibodies with methanol. The aflatoxins were quantified by reverse-phase HPLC with fluorescence detection and post-column derivatization (PCD). The PCD was achieved with electrochemically generated bromine. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of aflatoxins B_1, B_2, G_1 and G_2 in wheat and maize. Targeted compounds in the analytes were identified in comparison with the retention time of CRM standards of aflatoxins B_1, B_2, G_1 and G_2 with the retention time of components to found present in samples. For method validation wheat was used as a representative control matrix.

3.3.1 Method Validation Performance Characteristics

3.3.1.1 Selectivity

Blank of wheat control sample Wt 01, CRM standard of aflatoxin G_2, G_1, B_2 and B_1 and spiked wheat control sample matrix were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. Figure 83, 84 and 85 shows chromatograms of blank of wheat control matrix, CRM standard of aflatoxin G_2, G_1, B_2 and B_1 and spiked sample of wheat which gives sufficient reliability for selectivity. The chromatograms (83, 84 and 85) are given below showed that compounds were well resolved and there is no significant interference of matrix with targeted aflatoxin G_2, G_1, B_2 and B_1.

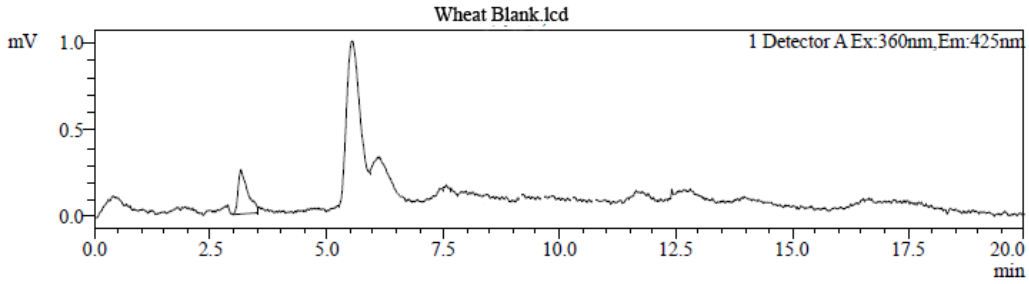


Figure-83: Blank of wheat control sample

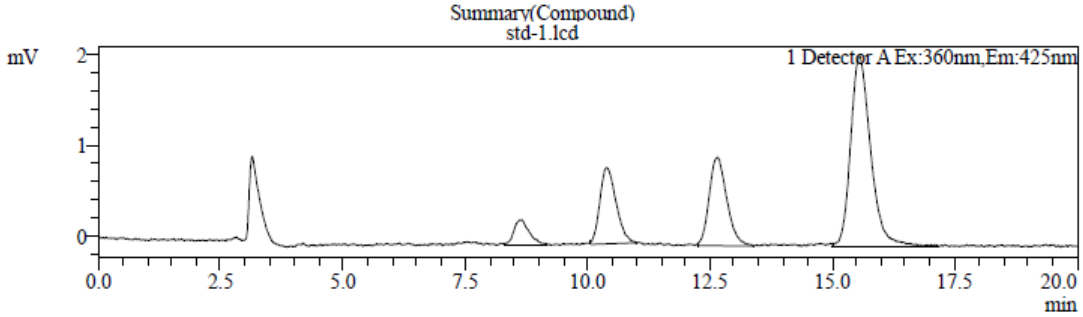


Figure-84: Chromatogram of CRM standard aflatoxin G2 (RT 8.65), G1 (RT 10.384) , B2 (RT 12.65) and B1 (RT 15.55) at concentration 0.252, 1.010, 0.252, 1.015 µg/L, respectively

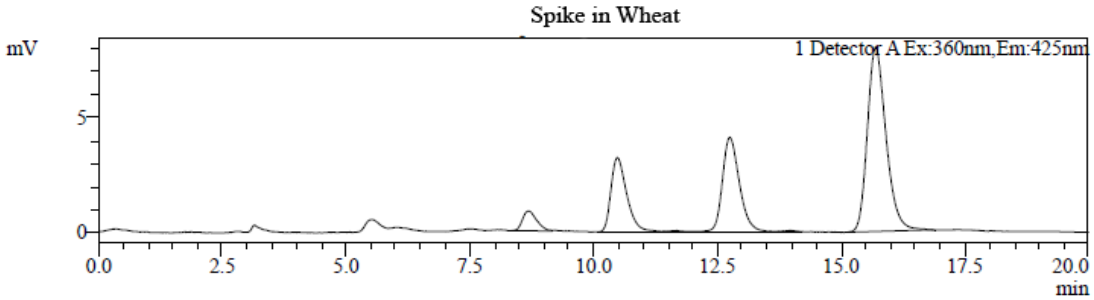


Figure-85: Chromatogram of Aflatoxin G_2 (RT 8.68), G_1 (RT 10.48), B_2 (RT 12.75) and B_1 (RT 15.69) spiked with wheat at concentration 0.756, 5.050, 0.755, 3.045 µg/L, respectively

3.3.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked wheat sample with CRM standard of aflatoxin G_2, G_1, B_2 and B_1at concentration 0.50, 2.00, 0.50 and 2.00 µg/kg, respectively (Table 41).

Table 41: LOD and LOQ of aflatoxin G_2, G_1, B_2 and B_1

Name of Aflatoxin	LOD (µg/kg)	LOQ (µg/kg)
G2	0.006	0.020
G1	0.021	0.069
B2	0.020	0.066
B1	0.046	0.153

3.3.1.3 Working Range and Linearity

Working range for aflatoxin G_2, G_1, B_2 and B_1 was 0.252-2.520 µg/L, 1.010-10.100 µg/L, 0.252-2.520 µg/L and 1.015-10.150 µg/L, respectively. Figure 86 and 87 shows the chromatograms of calibration standards and Figure 88 shows the calibration curve of aflatoxin G_2, G_1, B_2 and B_1.

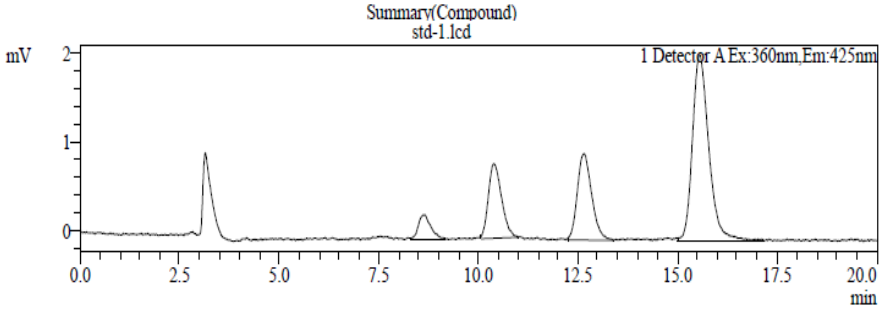


Figure-86: Chromatograms of aflatoxin G_2, G_1 , B_2 and B_1 at concentration 0.252, 1.010, 0.252, 1.015 µg/L , respectively

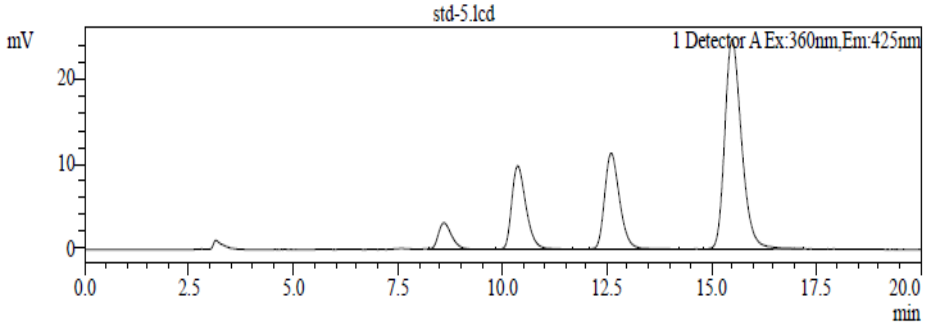


Figure-87: Chromatograms of aflatoxin G_2, G_1 , B_2 and B_1 at concentration 2.520, 10.100, 2.515, 10.150 µg/L , respectively

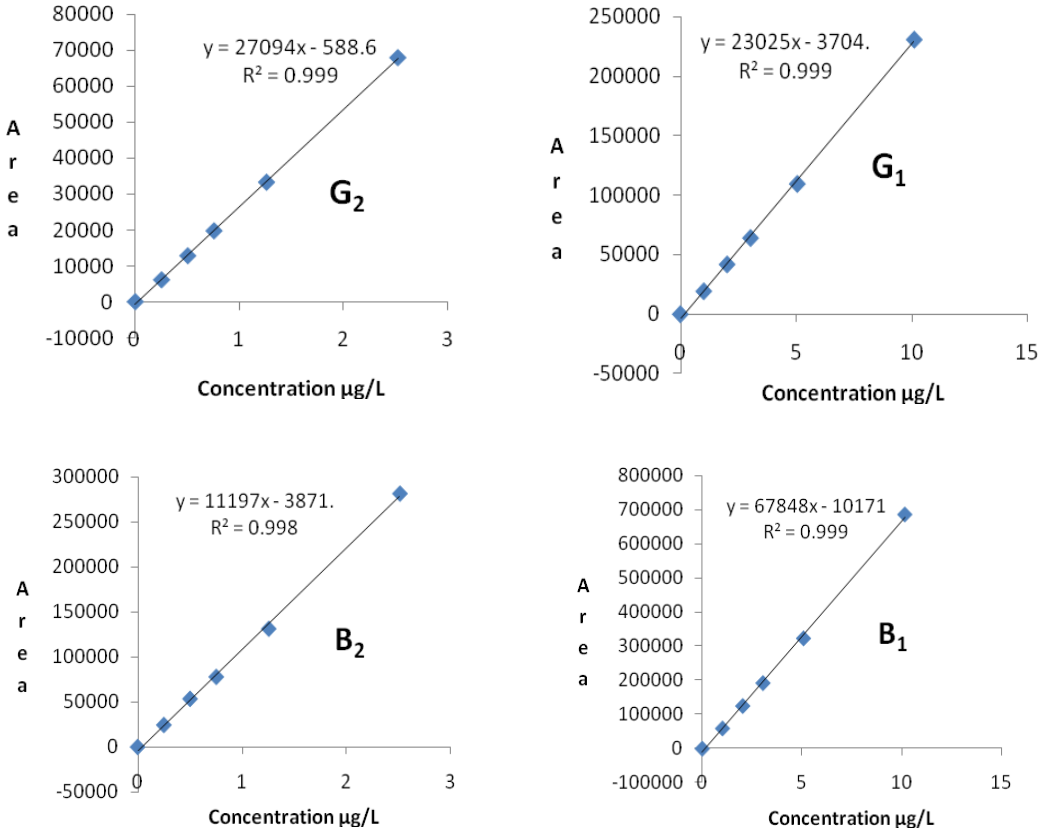


Figure-88: Calibration curve of aflatoxin G_2, G_1 , B_2 and B_1

3.3.1.4 Accuracy (Recovery)

CRM standard of aflatoxin G_2, G_1, B_2 and B_1were spiked in wheat sample at concentration 0.756, 5.050, 0.755 and 3.045 µg/L, respectively. Chromatogram of CRM standard of aflatoxin G_2, G_1, B_2 and B_1 and spike wheat control matrix were given in Figure 89 and 90. Recovery of aflatoxin G_2, G_1, B_2 and B_1 were given in Table 42.

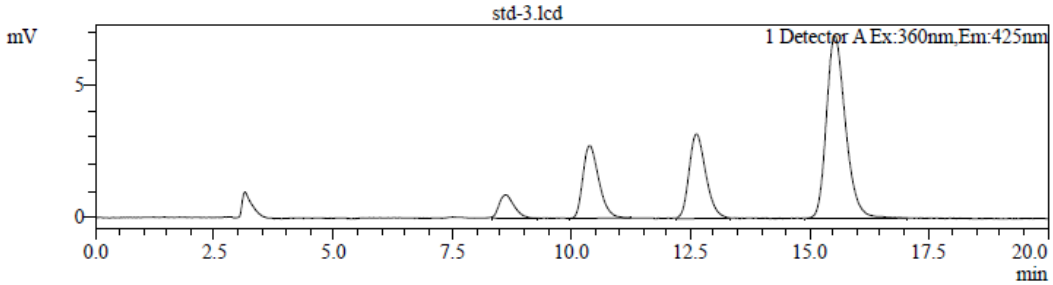


Figure-89: Chromatogram of CRM standard of aflatoxin G_2 (RT 8.62), G_1 (RT 10.38), B_2 (RT 12.62) and B_1 (RT 15.53) at concentration 0.756, 5.050, 0.755, 3.045 µg/L , respectively

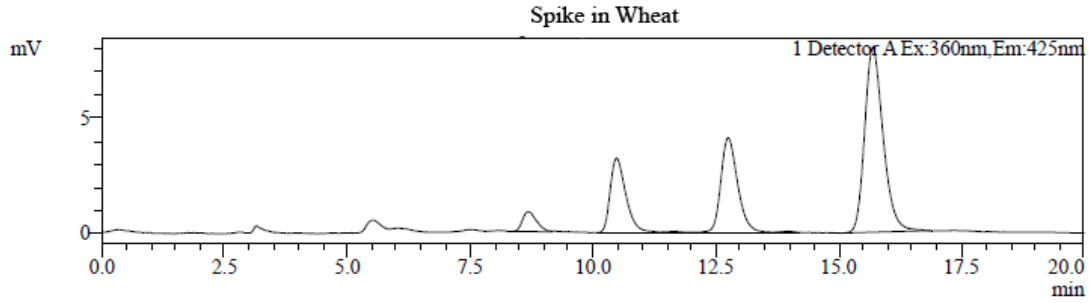


Figure-90: Chromatogram of Aflatoxin G_2 (RT 8.68), G_1 (RT 10.48) , B_2 (RT 12.75) and B_1 (RT 15.69) spiked with wheat at concentration 0.756, 5.050, 0.755, 3.045 µg/L , respectively

Table 42: Recovery of aflatoxin G_2, G_1, B_2 and B_1.

Name of Aflatoxin	Recovery% with wheat
G_2	87±1.96
G_1	94±2.30
B_2	96±1.49
B_1	92±3.27

3.3.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked with wheat sample with CRM standard of aflatoxin G_2, G_1 , B_2 and B_1at concentration 2.5, 10.0, 2.5 and 10.0 µg/kg , respectively. RSD% of aflatoxin G_2, G_1, B_2 and B_1 was given in Table 43.

Table 43: RSD% of aflatoxin G_2, G_1, B_2 and B_1

Name of Aflatoxin	RSD%
G_2	1.41
G_1	2.45
B_2	1.60
B_1	2.92

3.3.2 Analysis of Sample

Aflatoxin G_2, G_1, B_2 and B_1were analyzed in 25 wheat and 25 maize samples. Figure 91 and 92 shows the representative chromatogram of samples. Similar Chromatogram was found for all other samples of wheat and maize. Analysis results of 25 wheat and 25 maize samples was given in Table 44.

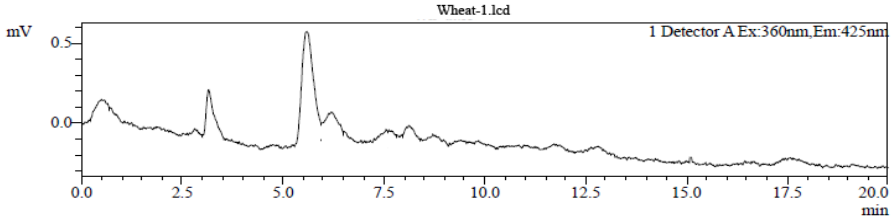


Figure-91: Chromatograms of sample wheat-1 for aflatoxin G_2, G_1 , B_2 and B_1

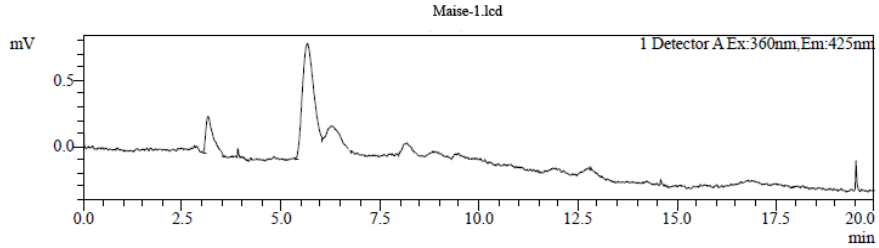


Figure-92: Chromatograms of sample maize-1 for aflatoxin G_2, G_1 , B_2 and B_1

Table 44: Amount of aflatoxin G_2, G_1, B_2 and B_1 for wheat and maize sample

Sl. No	Sample ID of Wheat	Result(µg/kg)	Sample ID of Maize	Result(µg/kg)
01	Wt 01	Not Detected	Mz 01	Not Detected
02	Wt 02	Not Detected	Mz 02	Not Detected
03	Wt 03	Not Detected	Mz 03	Not Detected
04	Wt 04	Not Detected	Mz 04	Not Detected
05	Wt 05	Not Detected	Mz 05	Not Detected
06	Wt 06	Not Detected	Mz 06	Not Detected
07	Wt 07	Not Detected	Mz 07	Not Detected
08	Wt 08	Not Detected	Mz 08	Not Detected
09	Wt 09	Not Detected	Mz 09	Not Detected
10	Wt 10	Not Detected	Mz 10	Not Detected
11	Wt 11	Not Detected	Mz 11	Not Detected
12	Wt 12	Not Detected	Mz 12	Not Detected
13	Wt 13	Not Detected	Mz 13	Not Detected
14	Wt 14	Not Detected	Mz 14	Not Detected
15	Wt 15	Not Detected	Mz 15	Not Detected
16	Wt 16	Not Detected	Mz 16	Not Detected
17	Wt 17	Not Detected	Mz 17	Not Detected
18	Wt 18	Not Detected	Mz 18	Not Detected
19	Wt 19	Not Detected	Mz 19	Not Detected
20	Wt 20	Not Detected	Mz 20	Not Detected
21	Wt 21	Not Detected	Mz 21	Not Detected
22	Wt 22	Not Detected	Mz 22	Not Detected
23	Wt 23	Not Detected	Mz 23	Not Detected
24	Wt 24	Not Detected	Mz 24	Not Detected
25	Wt 25	Not Detected	Mz 25	Not Detected

3.3.3 Discussion

Aflatoxin G_2, G_1, B_2 and B_1 were determined in wheat (n=25) and maize (n=25) samples by HPLC equipped with fluorescence detector. LOD of G_2, G_1, B_2 and B_1 were 0.006, 0.021, 0.020, 0.046 µg/kg and LOQ of G_2, G_1, B_2 and B_1 were 0.020, 0.069, 0.066, 0.153 µg/kg, respectively. Calibration range of G_2, G_1, B_2 and B_1 were 0.252-2.52, 1.010-10.100, 0.252-2.515 and 1.015-10.150 µg/L, respectively. Linear correlation coefficient (R^2) value for G_2, G_1, B_2 and B_1 were 0.999, 0.999, 0.998 and 0.999, respectively. Recovery (%) of G_2, G_1, B_2 and B_1 for wheat were 87, 94, 96, 92%, respectively. Relative standard deviation (RSD%) for repeatability of G_2, G_1, B_2 and B_1 were 1.41, 2.45, 1.60 and 2.92, respectively. Aflatoxin G_2, G_1, B_2 and B_1 were analyzed in 25 wheat and 25 maize samples. No targeted aflatoxin G_2, G_1, B_2 and B_1 was detected in any wheat and maize sample.

The main source of aflatoxins is *Aspergillus* species in the environment. These species are universal in distribution. *Aspergillus* species has high ecological, biological and metabolic diversity for exploration of secondary metabolites among these species. Chronic aflatoxicosis includes terratogenic effect linked with congenital malformation. Aflatoxins are mutagenic and carcinogenic. Mutagenic effect creates mutation in genetic code and DNA alteration which lead to chromosomal breaks, rearrangements, loss or gain of chromosome or changes within a gene **[97].**

It was reported previously in Bangladesh that aflatoxin B_1 was found in wheat in the range of 0.9-1.5 µg/kg **[98].** In this study no targeted aflatoxin G_2, G_1, B_2 and B_1 was detected in any wheat and maize samples.

Although no targeted aflatoxin G_2, G_1, B_2 and B_1 were found in any sample of wheat and maize, a continuous monitoring is required to avoid aflatoxin contamination in wheat and maize.

3.4 Quantitative measurement of benzoic acid and sorbic acid in fruit drink and tomato ketchup by HPLC

For quantitative measurement of benzoic acid and sorbic acid, fruit drink samples (n=25) and tomato ketchup samples (n=27) were collected and from local market of Dhaka city. Extraction of benzoic acid and sorbic acid from a test portion was done using a mixture of ammonium acetate buffer solution and methanol, under pH 4.5. The concentration of benzoic acid and sorbic acid were determined by means of HPLC using a reverse phase column and ultraviolet (UV) detector. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of benzoic and sorbic acid in fruit drink and tomato ketchup. Targeted compounds in the analytes were identified in comparison with the retention time of CRM standards of benzoic and sorbic acid with the retention time of components to found present in samples. Method validation was performed using apple fruit drink as a representative control matrix.

3.4.1 Method Validation Performance Characteristics

3.4.1.1 Selectivity

Blank of control apple fruit drink sample FD 07, CRM standard of benzoic acid and sorbic acid and spiked control apple fruit drink sample were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. Figure 93, 94 and 95 shows chromatograms of blank control sample, CRM standard of benzoic acid and sorbic acid and spiked apple fruit drink control sample which gives sufficient reliability for selectivity. The chromatograms (Figure 93, 94 and 95) are given below showed that compounds were well resolved and there is no significant interference of matrix with targeted benzoic acid and sorbic acid.

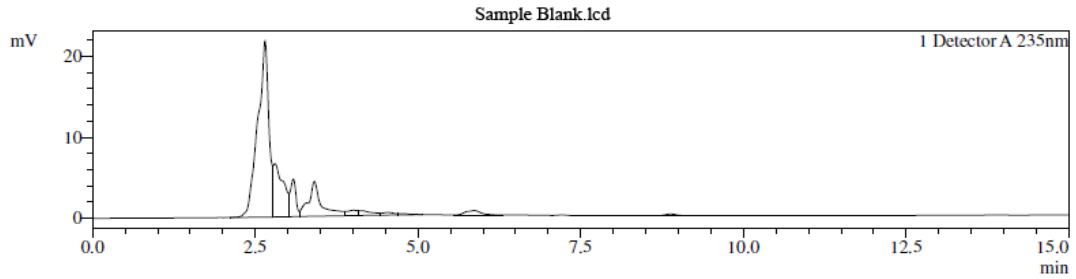


Figure-93: Chromatogram of blank control apple fruit drink sample

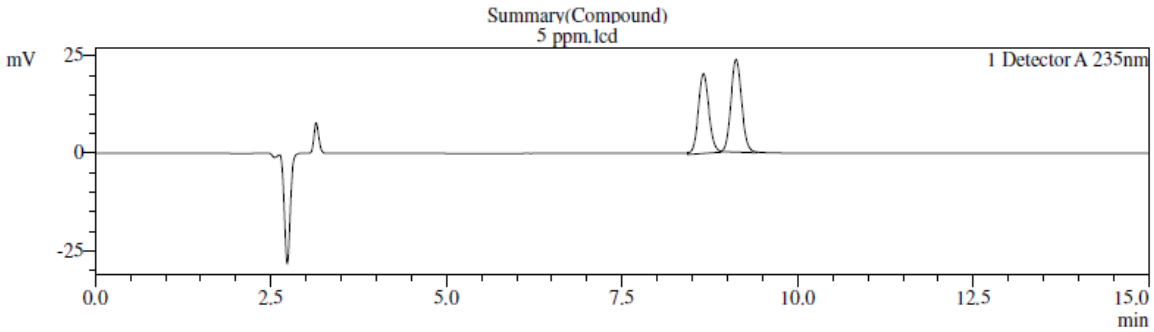


Figure-94: Chromatogram of benzoic acid (RT 8.64) and sorbic acid (RT 9.12) at concentration 5 mg/L

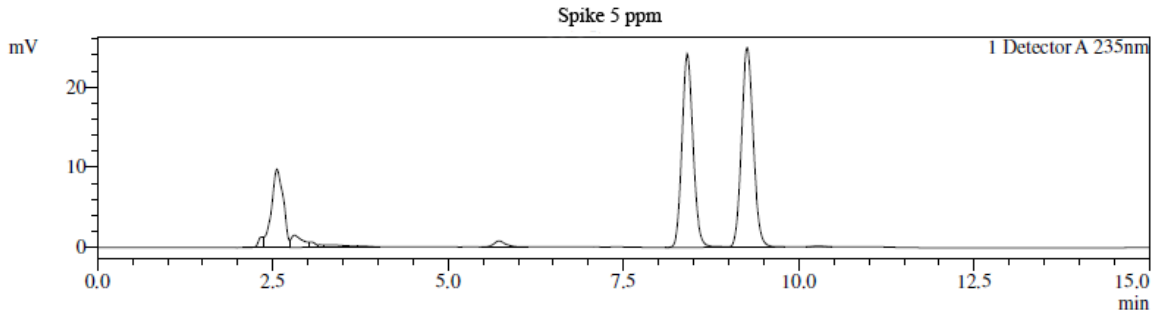


Figure-95: Chromatogram of benzoic acid (RT 8.63) and sorbic acid (RT 9.13) spiked in apple fruit drink at concentration 5 mg/L

3.4.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked apple fruit drink sample with CRM standard of benzoic acid and sorbic acid at concentration 5.0 mg/kg (Table 45).

Table 45: LOD and LOQ of benzoic acid and sorbic acid.

Name of Preservative	LOD (mg/kg)	LOQ (mg/kg)
Benzoic Acid	0.15	0.49
Sorbic Acid	0.09	0.30

3.4.1.3 Working Range and Linearity

Working range for benzoic acid and sorbic acid was 5-100 mg/L. Figure 96 and 97 shows the chromatograms of calibration standards and Figure 98 shows the calibration curve of benzoic acid and sorbic acid.

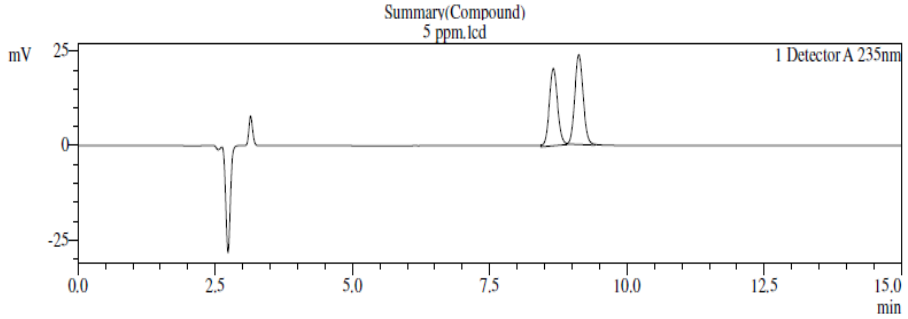


Figure-96: Chromatogram of benzoic acid and sorbic acid at concentration 5 mg/L

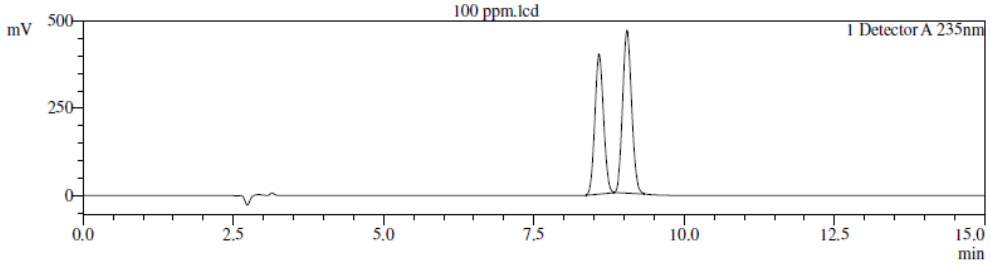


Figure-97: Chromatogram of benzoic acid and sorbic acid at concentration 100 mg/L

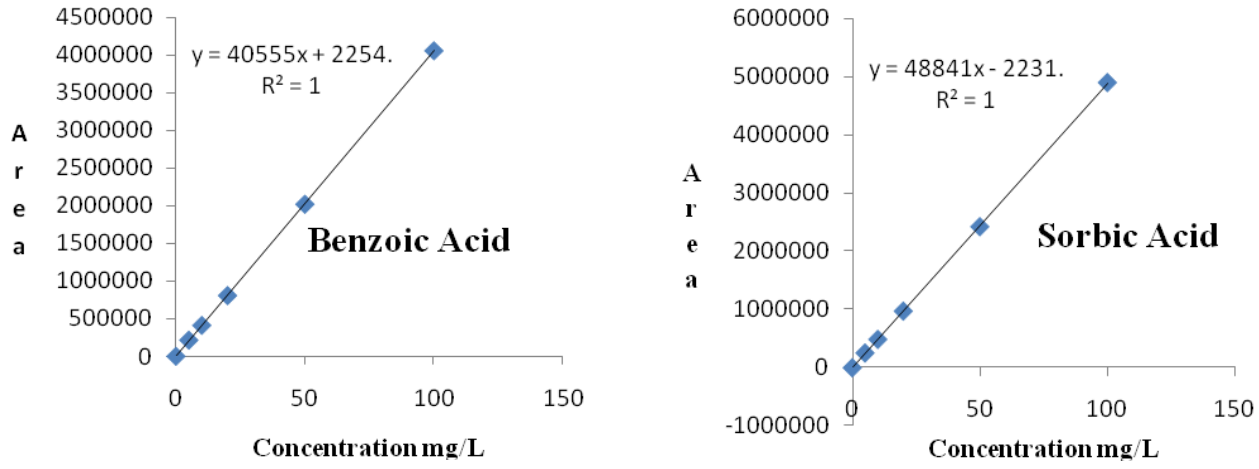


Figure-98: Calibration curve of benzoic acid and sorbic acid

3.4.1.4 Accuracy (Recovery %)

CRM standard of for benzoic acid and sorbic acid were spiked in apple fruit drink sample at concentration 50.0 mg/L. Chromatogram of CRM standard of benzoic acid and sorbic acid and spike apple fruit drink were given in Figure 99 and 100. Table 46 shows the recovery of benzoic acid and sorbic acid.

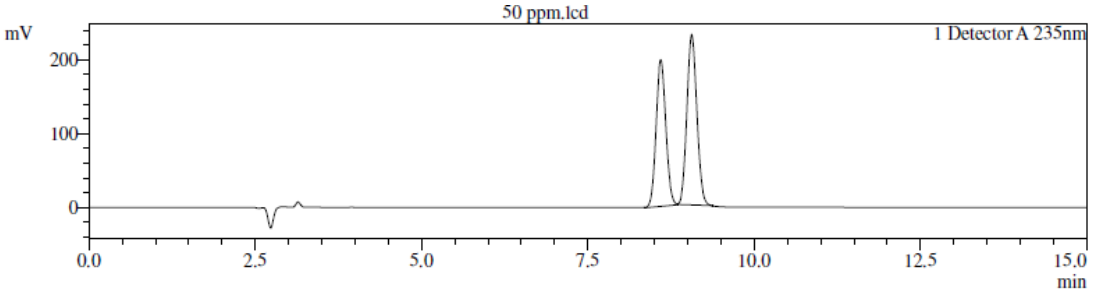


Figure-99: Chromatogram of CRM standard of benzoic acid and sorbic acid at concentration 50 mg/L

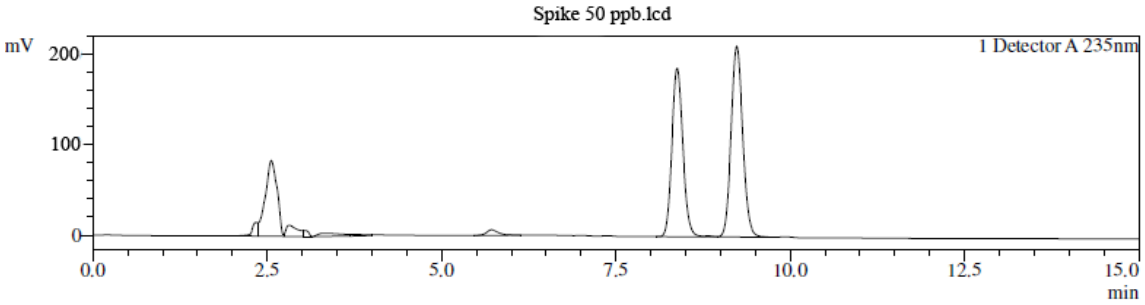


Figure-100: Chromatogram of spiked apple fruit drink control matrix with CRM standard of benzoic acid and sorbic acid at concentration 50 mg/L

Table 46: Recovery of benzoic acid and sorbic acid.

Name of Analyte	Recovery %
Benzoic Acid	99±0.65
Sorbic Acid	99±0.57

3.4.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked with apple fruit drink sample with CRM standard of benzoic acid and sorbic acid at concentration 50.0 mg/kg. Table 47 shows the RSD% of benzoic acid and sorbic acid.

Table 47: RSD% of benzoic acid and sorbic acid

Name of Analyte	RSD%
Benzoic Acid	1.57
Sorbic Acid	2.73

3.4.2 Analysis of Sample

Benzoic acid and sorbic acid were analyzed in 25 fruit drink and 27 tomato ketchup samples. Figure 101-104 shows representative chromatograms of some detected samples. Chromatogram of all other benzoic acid detected sample of fruit drink and tomato ketchup are attached in the annexure in Figure 190-200. The analysis results of 25 fruit drink and 27 tomato ketchup samples were given in Table 48.

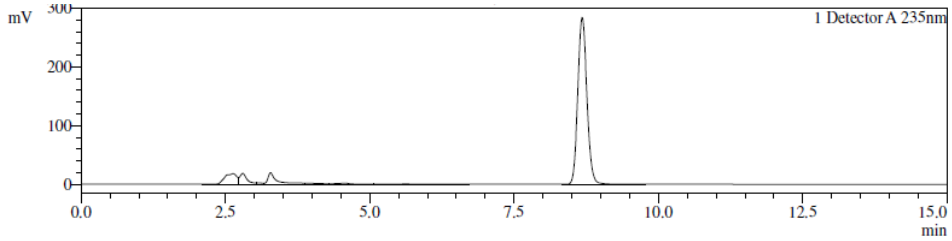


Figure 101: Chromatogram of fruit drink sample FD 01

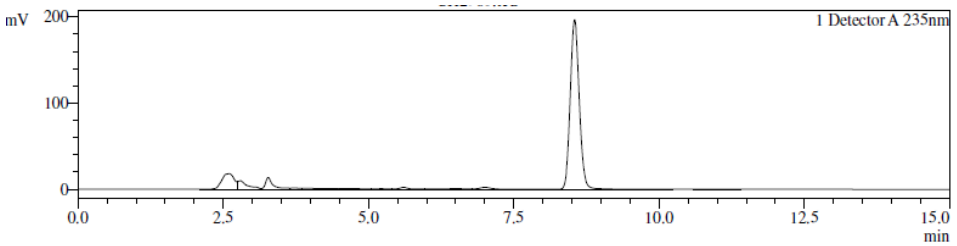


Figure 102: Chromatogram of fruit drink sample FD 02

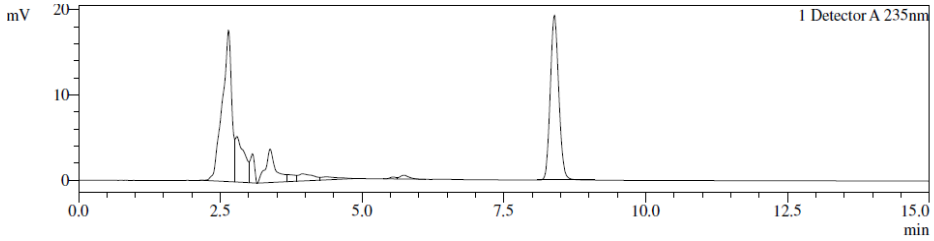


Figure 103: Chromatogram of tomato ketchup sample TK 06

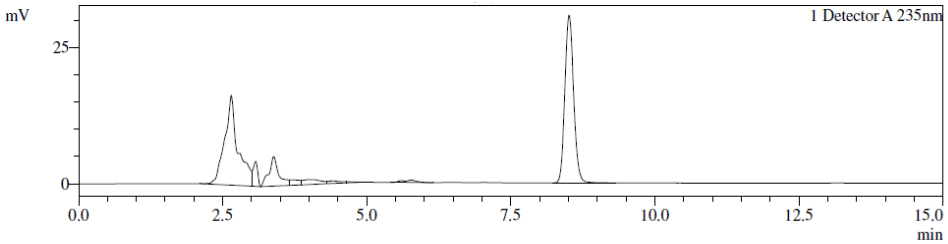


Figure 104: Chromatogram of tomato ketchup sample TK 07

Table 48: Amount of benzoic acid in fruit drinks and tomato ketchup sample

Sample analysis result of fruit drinks		**Sample analysis result of tomato ketchup**	
Sample ID	Concentration (mg/kg)	Sample ID	Concentration (mg/kg)
FD 01	761±1.21	TK 01	67±1.44
FD 02	526±2.02	TK 02	630±1.87
FD 03	559±1.99	TK 03	0
FD 04	91±1.13	TK 04	154±2.64
FD 05	315±0.67	TK 05	0
FD 06	134±1.83	TK 06	50±2.12
FD 07	0	TK 07	77±1.93
FD 08	32±0.93	TK 08	0
FD 09	31±0.13	TK 09	0
FD 10	0	TK 10	0
FD 11	0	TK 11	90±2.04
FD 12	0	TK 12	0
FD 13	117±1.62	TK 13	84±1.64
FD 14	105±1.58	TK 14	1248±2.44
FD 15	0	TK 15	88±1.89
FD 16	0	TK 16	669±2.18
FD 17	0	TK 17	127±2.41
FD 18	195±2.63	TK 18	228±1.48
FD 19	160±1.67	TK 19	137±2.42
FD 20	0	TK 20	126±2.06
FD 21	208±1.56	TK 21	153±1.93
FD 22	126±1.02	TK 22	150±2.26
FD 23	87±1.90	TK 23	91±1.62
FD 24	221±2.29	TK 24	224±2.57
FD 25	1352±2.40	TK 25	50±2.16
		TK 26	62±1.87
		TK 27	122±2.87

3.4.3 Discussion

Benzoic acid and sorbic acid were determined in fruit drink and tomato ketchup by HPLC equipped with UV detector. LOD of benzoic acid and sorbic acid was 0.15, 0.09 mg/kg and LOQ was 0.49 and 0.30 mg/kg, respectively. Calibration range of benzoic acid and sorbic acid was 5-100 mg/L. Linear correlation coefficient (R^2) value for benzoic acid and sorbic acid was 1. Recovery (%) of benzoic acid and sorbic acid with apple fruit drink was 99%. Relative standard deviation (RSD%) for repeatability of benzoic acid and sorbic acid was 1.57 and 2.73, respectively. Benzoic acid and sorbic acid were analyzed in 25 fruit drink and 27 tomato ketchup samples. Benzoic acid was detected in 17 fruit drink samples and in 21 tomato ketchup samples. Eleven fruit drink sample exceeded maximum limit of benzoic acid concentration 120 mg/kg set

by BSTI **[99].** One tomato ketchup sample exceeded maximum limit of benzoic acid concentration 750 mg/kg set by BSTI **[100]**.

Benzoic acid is used as antimicrobial preservative in food and beverages because it shows strongest antibacterial activity at the pH range of 2.5-4.0. Benzoic acid has inhibitory effects on the proliferation of bacteria which is a major cause of food degradation. Addition of benzoic acid can extend the shelf life of fruit drinks and tomato ketchup. It also prevents the loss of nutritional value of processed fruit products. The excessive ingestion of benzoic acid may cause diarrhea, abdominal pain and other clinical symptoms **[101].** For this reason the maximum limit of benzoic acid in every variety of food are restricted by legislation. In fruit juices or fruit drinks carcinogenic compound benzene might be produced for the presence of benzoic acid and ascorbic acid. It can be stimulated by the exposure of light and heat.

Previously it was reported in Bangladesh that benzoic acid in fruit drinks samples was in the range of 96-467 mg/kg **[102].** In this study benzoic acid was found at the range of 87-1352 mg/kg in fruit drinks samples and in tomato ketchup samples it was found at the range of 50-1248 mg/kg. It can be seen from the present study that the concentration of benzoic acid in fruit drink is increasing from the previous study. For this reason benzoic and sorbic acid should continuously be monitored carefully in processed fruit product in Bangladesh.

3.5 Quantitative determination of Sudan Red-I, II, III, IV in Chili Powder by HPLC

For determination of sudan red I, II, III and IV, chili powder samples (n=20) were collected from local market of Dhaka city. Sudan dyes were extracted by ethanol from chili powder. HPLC was used for the separation of sudan red I, II, III, IV as they elute at different rate under isocratic condition. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of sudan red I, II, III, IV in chili powder. Targeted compounds in the analytes were identified in comparison with the retention time of CRM standards of sudan red I, II, III and IV with the retention time of components to found present in samples. Method validation was performed using chili powder as a represetative control matrix.

3.5.1 Method Validation Performance Characteristics

3.5.1.1 Selectivity

Blank of control chili powder sample CP 02, CRM standard of Sudan I, II, III and IV and spiked chili powder control sample were analyzed by this method. This confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analyte in isolation from other interference. Figure 105, 106 and 107 shows the chromatograms of blank control chili powder sample, CRM standard of sudan I, II, III and IV and spiked sample of chili powder control matrix which gives sufficient reliability for selectivity. The chromatograms (Figure 105, 106 and 107) are given below showed that compounds were well resolved and there is no significant interference of matrix with targeted sudan red I, II, III and IV.

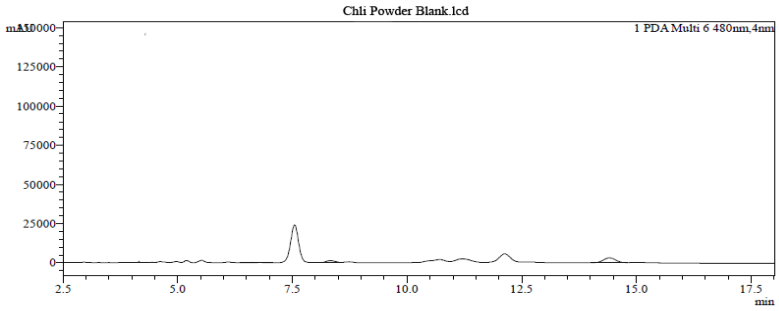


Figure-105: Chromatogram of blank of control chili powder sample

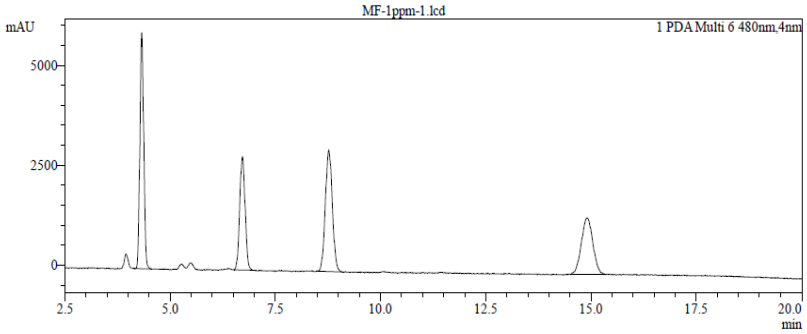


Figure-106: Chromatogram of sudan I (RT 4.35), II (RT 6.77), III (RT 8.85) and IV (RT 15.04) CRM standard at concentration 1 mg/L

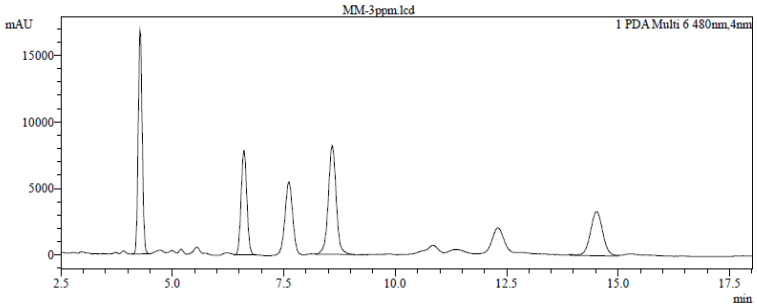


Figure-107: Chromatogram of sudan I (RT 4.28), II (RT 6.62), III (RT 8.61) and IV (RT 14.57) CRM standard spiked with chili powder at concentration 3 mg/L

3.5.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked chili powder sample with CRM standard of Sudan I, II, III and IV at concentration 20.0 mg/kg (Table 49)

Table 49: LOD and LOQ of sudan I, II, III and IV

Name of Sudan Dye	LOD (mg/kg)	LOQ (mg/kg)
Sudan I	0.22	0.72
Sudan II	0.50	1.66
Sudan III	0.38	1.25
Sudan IV	1.49	4.96

3.5.1.3 Working Range and Linearity

Working range for sudan I, II, III and IV is 0.05-5 mg/L. Figure 108 and 109 shows the chromatograms of calibration standards and Figure 110 shows the calibration curve of Sudan I, II, III and IV.

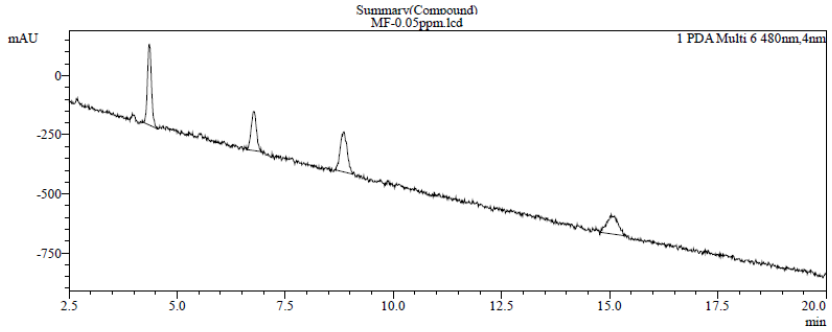


Figure-108: Chromatogram of sudan I, II, III and IV CRM standard at concentration 0.05 mg/L

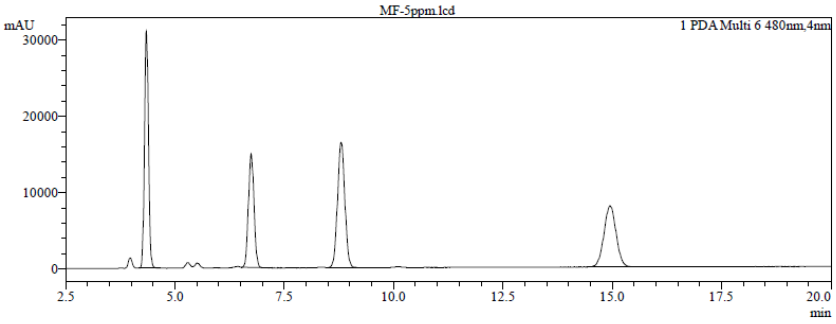


Figure-109: Chromatogram of sudan I, II, III and IV CRM standard at concentration 5 mg/L

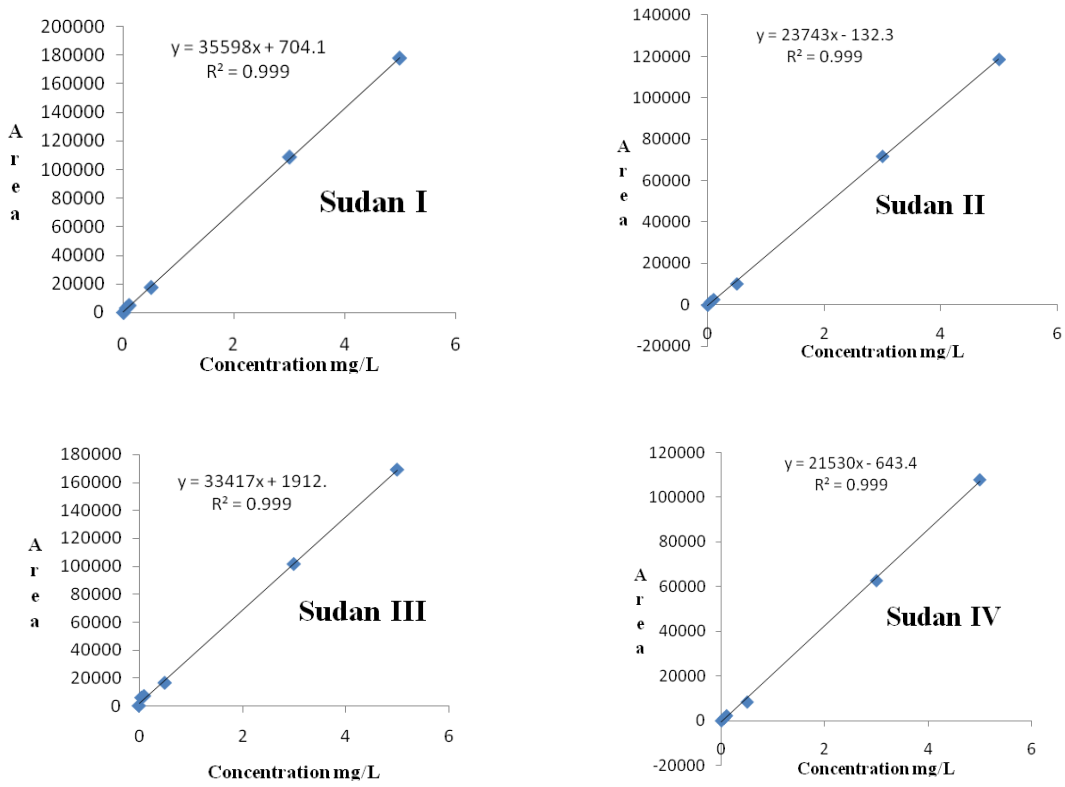


Figure 110: Calibration curve of sudan I, II, III and IV

3.5.1.4 Accuracy (Recovery %)

CRM standard of sudan I, II, III and IV were spiked in chili powder sample at concentration 3.0 mg/L. Chromatogram of CRM standard of sudan I, II, III & IV and spiked chili powder control matrix were given in Figure 111 and 112. Recovery of sudan I, II, III and IV was given in Table 50.

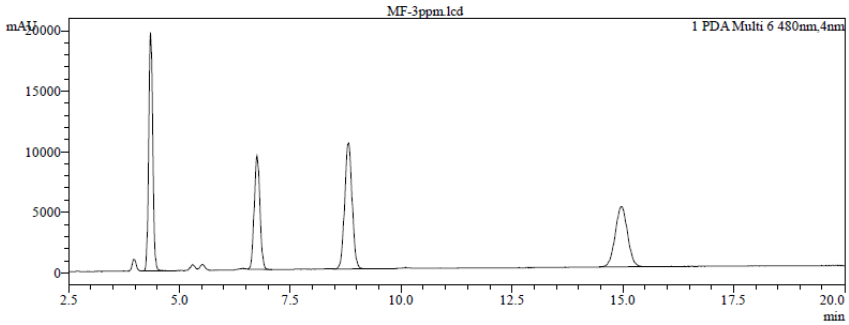


Figure-111: Chromatogram of sudan I (RT 4.35), II (RT 6.75), III (RT 8.81) and IV (RT 14.97) CRM standard at concentration 3 mg/L

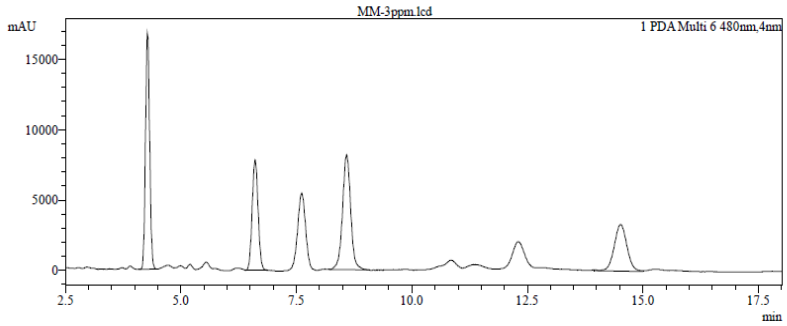


Figure-112: Chromatogram of Sudan I (RT 4.28), II (RT 6.62), III (RT 8.61) and IV (RT 14.57) spiked chili powder control matrix at concentration 3 mg/L

Table 50: Recovery of Sudan I, II, III and IV.

Name of Sudan Dye	Recovery %
Sudan I	98±1.58
Sudan II	97±1.38
Sudan III	99±1.14
Sudan IV	93±2.37

3.5.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of spiked chili powder sample with CRM standard of sudan I, II, III and IV at concentration 20.0 mg/kg. RSD% of Sudan I, II, III and IV was given in Table 51.

Table 51: RSD% of Sudan I, II, III and IV

Name of Sudan Dye	RSD%
Sudan I	3.38
Sudan II	1.91
Sudan III	1.47
Sudan IV	2.79

3.5.2 Analysis of Sample

Sudan I, II, III and IV were analyzed in 20 chili powder samples. In Figure 113 the chromatogram shows that sudan III was detected in one chili powder sample. Analysis results of 20 chili powder samples was given in Table 52.

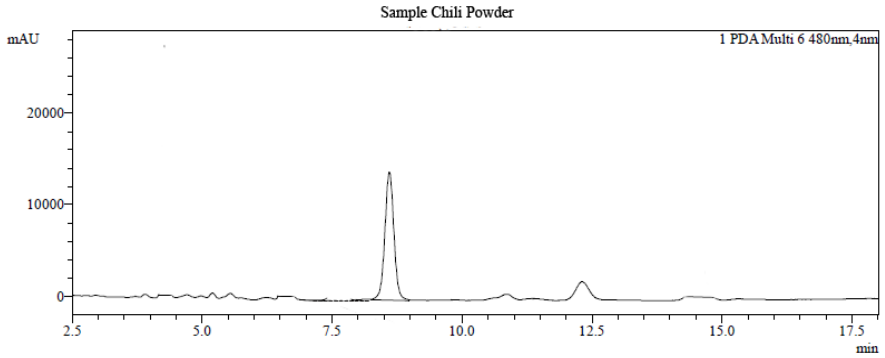


Figure-113: Chromatogram of Chili powder sample CP-14

Table 52: Amount of sudan I, II, III and IV in 20 chili powder samples

Sample ID	Result(mg/kg)	Sample ID	Result(mg/kg)
CP-01	Not Detected	CP-11	Not Detected
CP-02	Not Detected	CP-12	Not Detected
CP-03	Not Detected	CP-13	Not Detected
CP-04	Not Detected	CP-14	53±1.91
CP-05	Not Detected	CP-15	Not Detected
CP-06	Not Detected	CP-16	Not Detected
CP-07	Not Detected	CP-17	Not Detected
CP-08	Not Detected	CP-18	Not Detected
CP-09	Not Detected	CP-19	Not Detected
CP-10	Not Detected	CP-20	Not Detected

3.5.3 Discussion

Sudan I, II, III and IV were determined in chili powder by HPLC equipped with UV detector. LOD of sudan red I, II, III and IV were 0.22, 0.50, 0.38 and 1.49 mg/kg, respectively. LOQ of sudan red I, II, III and IV were 0.72, 1.66, 1.25 and 4.96 mg/kg, respectively. Calibration range of sudan red I, II, III and IV were 0.05-5.0 mg/kg. Linear correlation coefficient (R^2) value for sudan red I, II, III and IV was 0.999. Recovery (%) of sudan red-I, II, III and IV with chili powder were 98, 97, 99 and 93%, respectively. Relative standard deviation (RSD%) for repeatability of sudan red I, II, III and IV were 3.38, 1.91, 1.47 and 2.79, respectively. Sudan III was detected in 1 sample out of 20 samples.

Sudan I is genotoxic with metabolic activation which was shown both *in vitro* and *in vivo* study. Bio-assays revealed that sudan I is carcinogenic in the rat. The *in vitro* data shows there are sufficient evidence that sudan II is genotoxic. About genotoxicity of sudan III, the result is

inconclusive. From the mutagenicity data it was revealed that sudan IV is potentially genotoxic [**103].**

Illegal presence of sudan I in food of EU was first reported in May 2003. It was found in chili powder and in foods which contains chili powder. Since then there were many notifications from several EU Member States via the Rapid Alert System (RSAFF). Primarily sudan I and sudan IV were found in chili powder, curry powder, processed products containing chili or curry powder, sumac, curcuma and palm oil. There were occasional notifications of sudan II and Sudan III in the same range of products. The origin of contaminated processed products has generally been within the EU. But it was thought that the contaminated raw products enter from outside the EU **[104].**

Sudan red I, II, III and IV are genotoxic and carcinogenic. Sudan III was detected in 1 sample out of 20 samples. Although this sample size does not represent the whole Bangladesh but it can give an idea about the sudan red contamination in chili powder. Sudan red should be analyzed in chili powder regularly to avoid contamination.

3.6 Determination of Antibiotic Residues in Pasteurized milk by LC-MS/MS

For determination of antibiotic residues, pasteurized milk samples (n=42) were collected from local market of Dhaka city. Antibiotic were extracted from pasteurized milk by modified QuEChERS method. Antibiotics were separated by liquid chromatograph and then detected and quantified by tandem mass spectrometer utilizing multiple reaction monitoring (MRM) which is highly selective and highly sensitive technique for residue analysis. The method was validated using pasteurized milk as representative control matrix. The purpose of this validation was to prove that the method was sufficiently accurate, sensitive, repeatable and reproducible for the determination of antibiotic residues in pasteurized milk. Targeted compounds in the analytes were identified in comparison with the retention time and MRM transition of precursor ion to fragment ion of CRM standards of antibiotics with the retention time and MRM transition of components to found present in samples. For method validation, pasteurized milk was taken as representative matrix.

3.6.1 Method Validation Performance Characteristics

3.6.1.1 Selectivity

Blank of pasteurized milk control sample PM 01, CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline and spiked pasteurized milk control sample were analyzed by this method. This highly selective and highly sensitive confirmatory technique has the ability to confirm analyte identity and it has the ability to measure the analytes in isolation from other interference. Figure 114, 115 and 116 shows MRM chromatograms of blank control pasteurized milk sample, CRM standard of antibiotics and spiked sample of pasteurized milk control matrix. Figure 117-122 shows MRM chromatogram and mass spectrum of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline which gives sufficient reliability for selectivity. The MRM chromatograms and mass spectrum (Figure 114-122) showed that compounds were well resolved and there is no significant interference of matrix with targeted antibiotics.

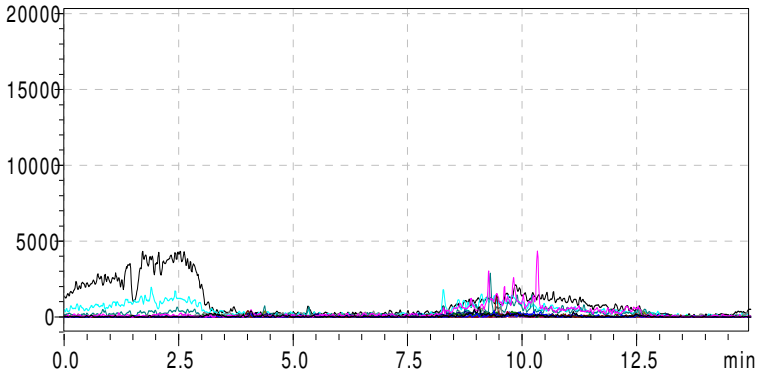


Figure-114: MRM chromatogram of blank of pasteurized milk control sample

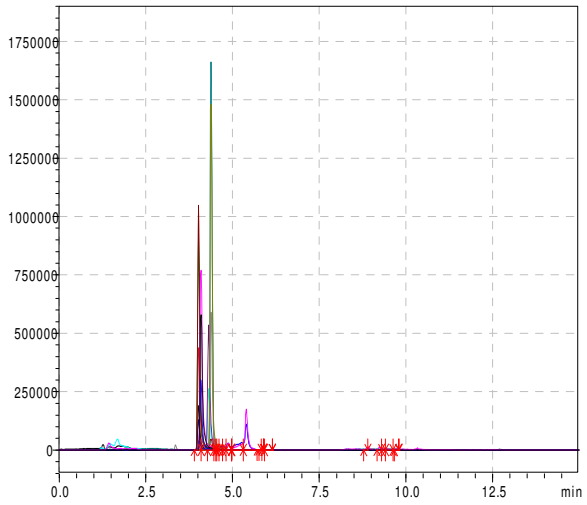


Figure-115: MRM chromatogram of CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration of 100 µg/L

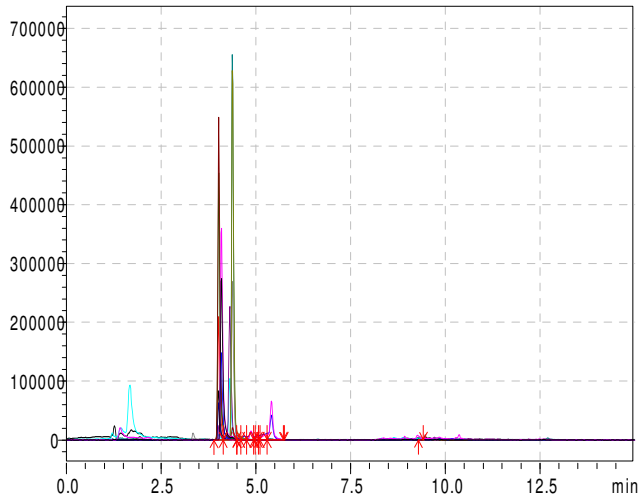
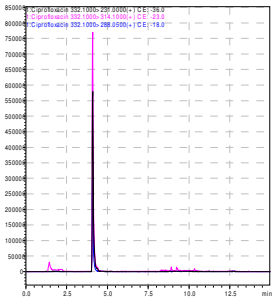
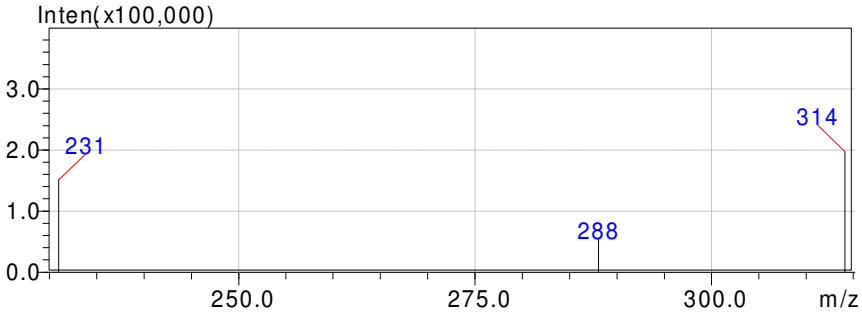


Figure-116: MRM chromatogram of pasteurized milk spiked with CRM standards of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration of 50 µg/L

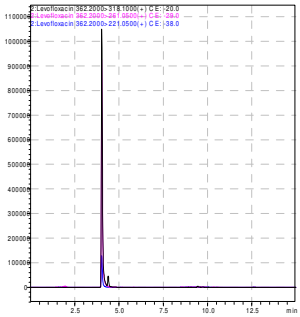


(a)

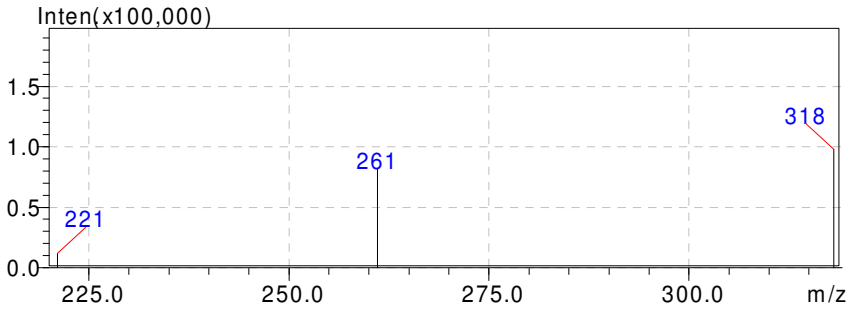


(b)

Figure-117: (a) MRM chromatogram and (b) mass spectrum of ciprofloxacin (RT 4.101) 332.1>314, 288, 231

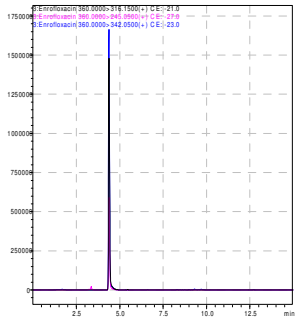


(a)

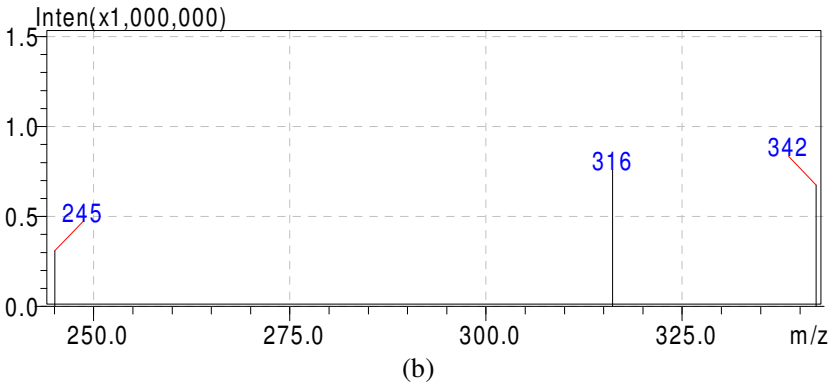


(b)

Figure-118: (a) MRM chromatogram and (b) mass spectrum of levofloxacin (RT 4.025) 362.2>318.1, 261.05, 221.05

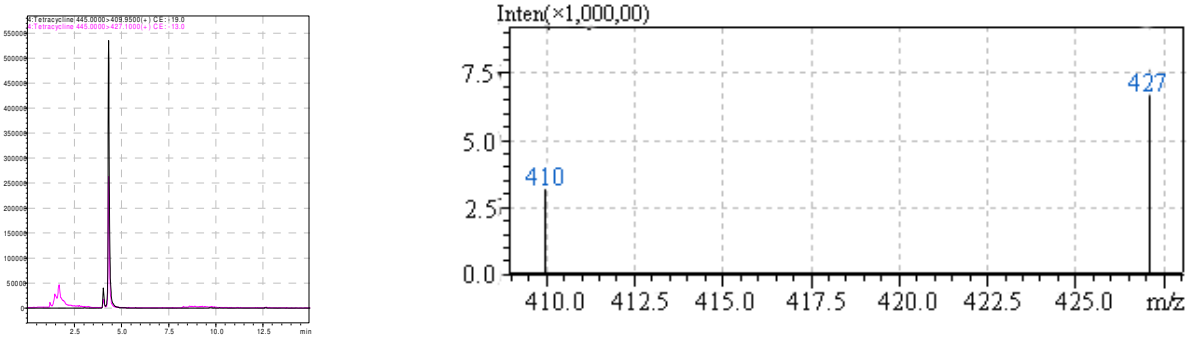


(a)



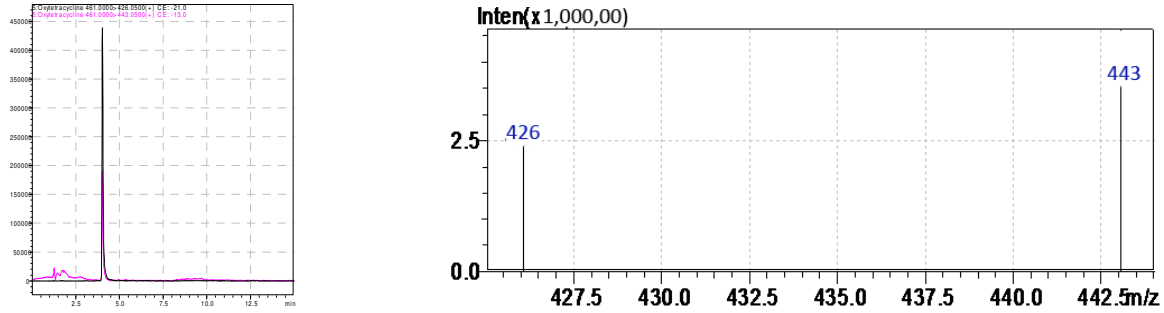
(b)

Figure-119: (a) MRM chromatogram and (b) mass spectrum of enrofloxacin (RT 4.386) 360.0>342.05, 316.15, 245.05



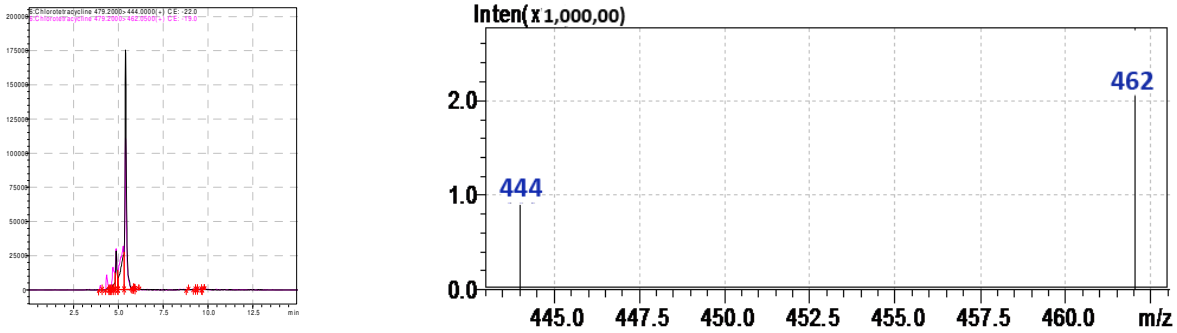
(a) (b)

Figure-120: (a) MRM chromatogram and (b) mass spectrum of tetracycline (RT 4.320) 445.0>427.1, 409.95



(a) (b)

Figure-121: (a) MRM chromatogram and (b) mass spectrum of oxytetracycline (RT 4.027) 461.0>443.05, 426.05



(a) (b)

Figure-122: (a) MRM chromatogram and (b) mass spectrum of chlortetracycline (RT 5.393) 479.2>462.05, 444.00

3.6.1.2 LOD and LOQ

LOD and LOQ were calculated from ten replicates of spiked pasteurized milk sample with CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration 50.0 µg/kg (Table 53).

Table 53: LOD and LOQ of six antibiotics.

Name of Antibiotic	LOD (µg/kg)	LOQ (µg/kg)
Ciprofloxacin	4.20	14.01
Levofloxacin	1.53	5.09
Enrofloxacin	2.66	8.87
Tetracycline	3.89	12.96
Oxytetracycline	4.87	16.25
Chlortetracycline	3.43	11.43

3.6.1.3 Working Range and Linearity

Working range for ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline was 5.0-100 µg/L. Figure 123 and 124 shows the chromatograms of calibration standards and Figure 125 shows the calibration curves of six antibiotics.

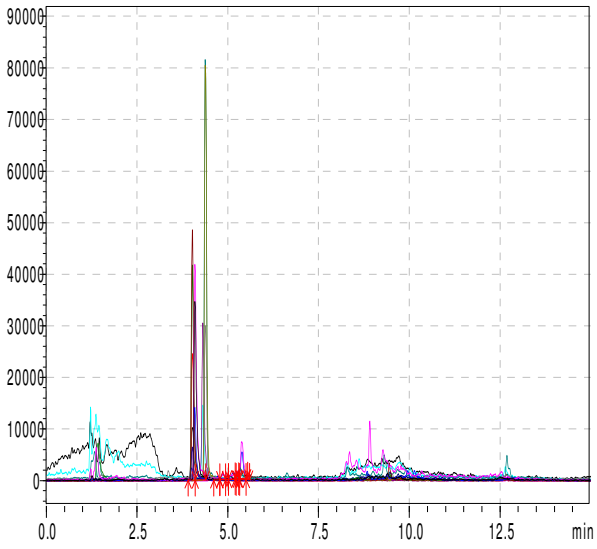


Figure-123: MRM of six antibiotic matrix matched CRM with pasteurized milk at concentration 5 µg/L

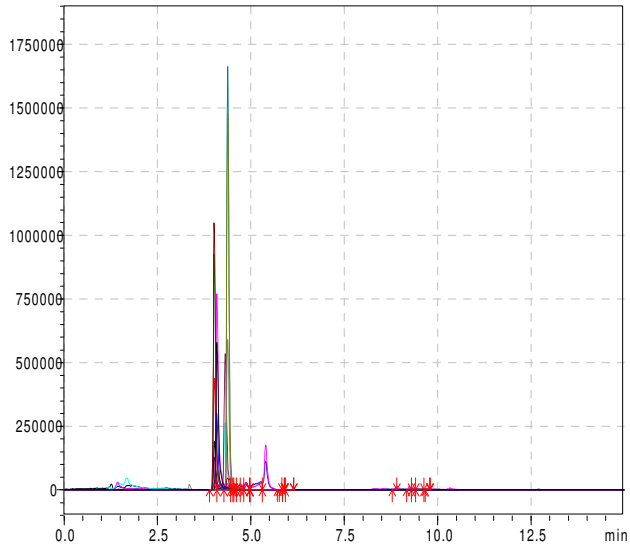


Figure-124: MRM of six antibiotic matrix matched CRM with pasteurized milk at concentration 100 µg/L

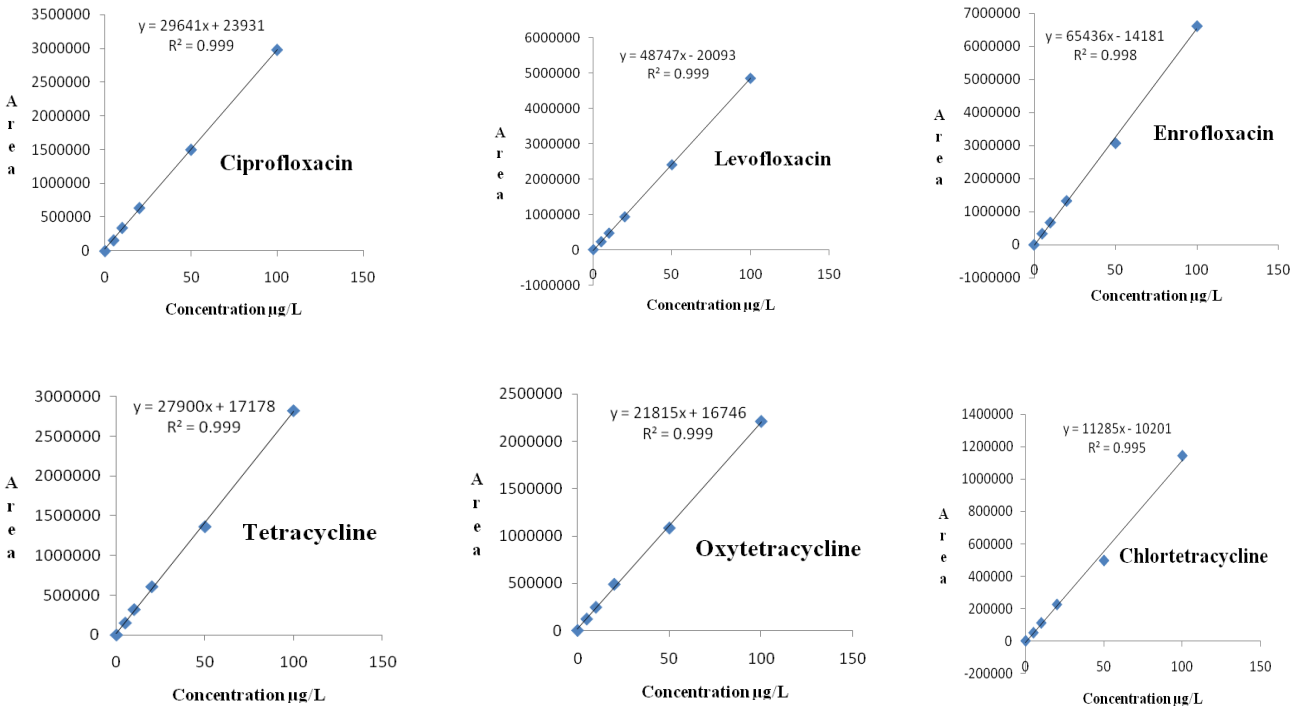


Figure-125: Calibration curve of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline

3.6.1.4 Accuracy (Recovery)

CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were spiked in pasteurized milk sample at concentration 50.0 µg/L. MRM chromatogram of CRM standard of antibiotics at concentration 50.0 µg/L and MRM chromatogram of spike pasteurized milk (50.0 µg/L) were given in Figure 126. Recovery of six antibiotics was given in Table 54.

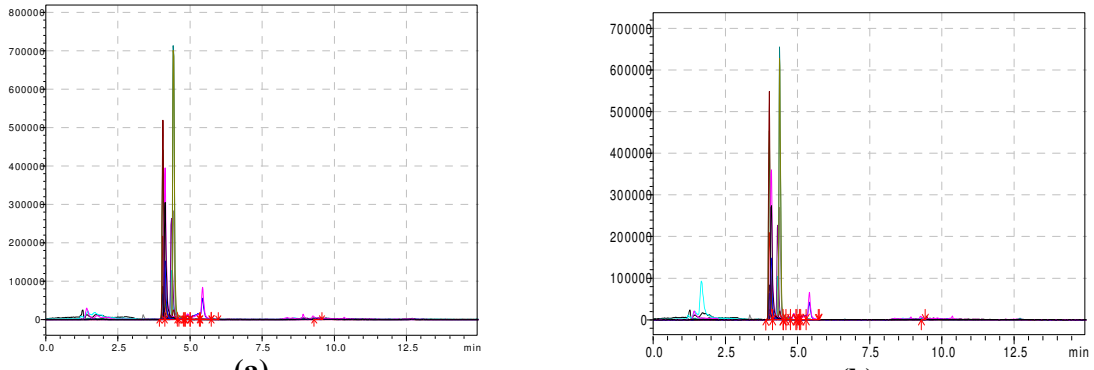


Figure-126: **(a)** MRM chromatogram of CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration of 50 µg/L and **(b)** MRM chromatogram of spiked pasteurized milk control matrix at concentration of 50 µg/L

Table 54: Recovery of six antibiotics

Name of Antibiotic	Recovery %
Ciprofloxacin	89±4.27
Levofloxacin	94±2.40
Enrofloxacin	93±2.15
Tetracycline	92±2.62
Oxytetracycline	84±1.16
Chlortetracycline	92±1.73

3.6.1.5 Precision (Repeatability)

Precision were calculated from ten replicates of pasteurized milk sample spiked with CRM standard of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline at concentration 50.0 µg/kg. RSD% of six antibiotics was given in Table 55.

Table 55: RSD% of six antibiotics

Name of Antibiotic	RSD%
Ciprofloxacin	7.93
Levofloxacin	2.59
Enrofloxacin	4.73
Tetracycline	7.04
Oxytetracycline	9.40
Chlortetracycline	5.52

3.6.2 Analysis of Sample

Ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were analyzed in 42 pasteurized milk samples. No targeted antibiotics were detected in any pasteurized milk sample. Figure 127 shows representative chromatogram of one pasteurized milk sample for six antibiotics. Similar chromatograms were found for all other samples of pasteurized milk. Analysis results of six antibiotics in 42 pasteurized milk samples was given in Table 56.

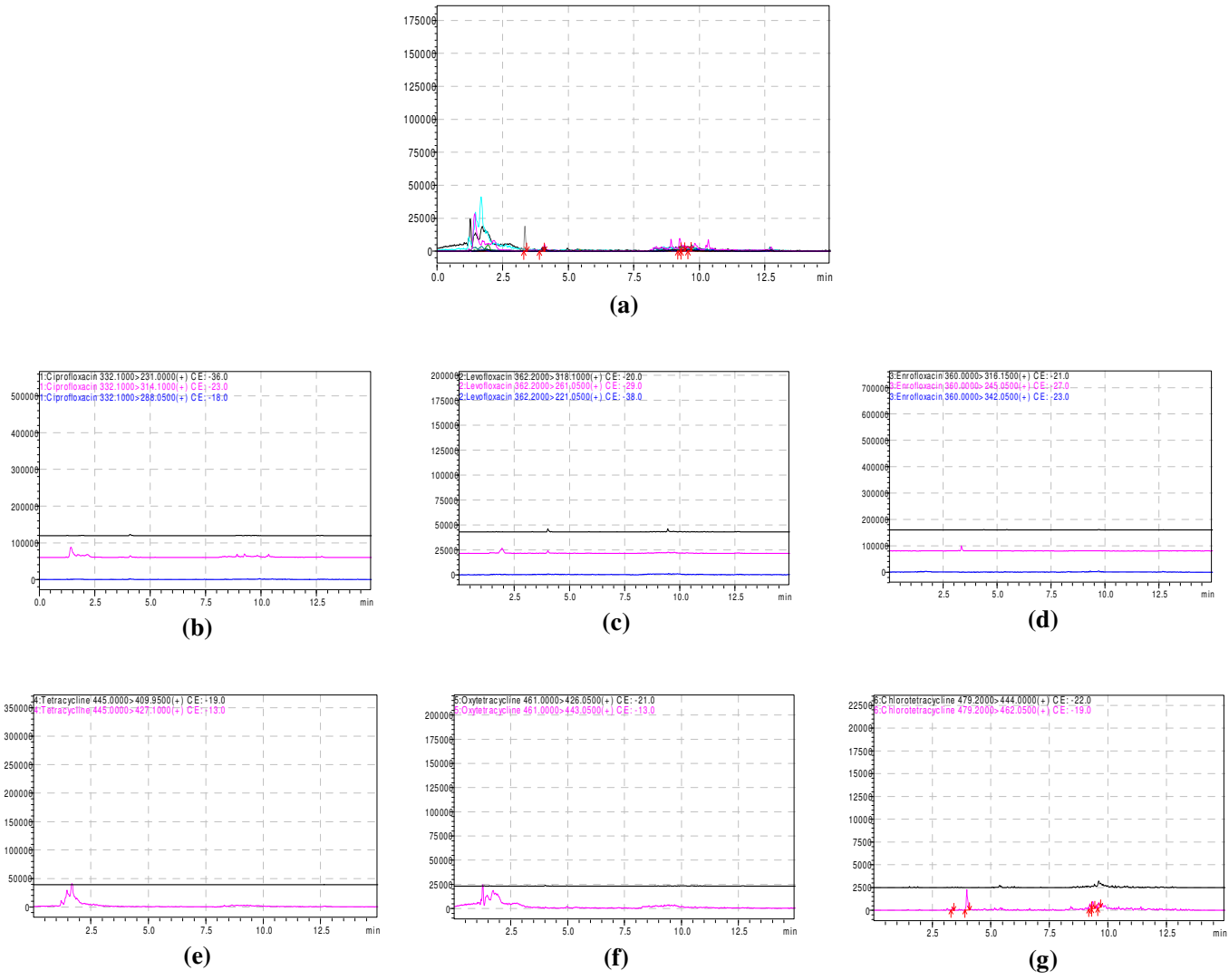


Figure-127: (**a**) MRM total ion chromatogram, (**b**) MRM transition of ciprofloxacin 332.1>314, 288, 231 (**c**) MRM transition of levofloxacin 362.2>318.1, 261.05, 221.05 (**d**) MRM transition of enrofloxacin 360.0>342.05, 316.15, 245.05 (**e**) MRM transition of tetracycline 445.0>427.1, 409.95 (**f**) MRM transition of oxytetracycline 461.0>443.05, 426.05 and (**g**) MRM transition of chlortetracycline 479.2>462.05, 444.00 of sample PM-02

Table 56: Amount of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline in pasteurized milk sample

Sample ID	Concentration (µg/kg)	Sample ID	Concentration (µg/kg)	Sample ID	Concentration (µg/kg)
PM-01	Not Detected	PM-15	Not Detected	PM-29	Not Detected
PM-02	Not Detected	PM-16	Not Detected	PM-30	Not Detected
PM-03	Not Detected	PM-17	Not Detected	PM-31	Not Detected
PM-04	Not Detected	PM-18	Not Detected	PM-32	Not Detected
PM-05	Not Detected	PM-19	Not Detected	PM-33	Not Detected
PM-06	Not Detected	PM-20	Not Detected	PM-34	Not Detected
PM-07	Not Detected	PM-21	Not Detected	PM-35	Not Detected
PM-08	Not Detected	PM-22	Not Detected	PM-36	Not Detected
PM-09	Not Detected	PM-23	Not Detected	PM-37	Not Detected
PM-10	Not Detected	PM-24	Not Detected	PM-38	Not Detected
PM-11	Not Detected	PM-25	Not Detected	PM-39	Not Detected
PM12	Not Detected	PM-26	Not Detected	PM-40	Not Detected
PM-13	Not Detected	PM-27	Not Detected	PM-41	Not Detected
PM-14	Not Detected	PM-28	Not Detected	PM-42	Not Detected

3.6.3 Discussion

Ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were determined in pasteurized milk by LC-MS/MS. LOD of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were 4.20, 1.53, 2.66, 3.89, 4.87 and 3.43 µg/kg, respectively. LOQ of ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were 14.01, 5.09, 8.87, 12.96, 16.25 and 11.43 µg/kg, respectively. Calibration range of six antibiotic was 5-100 µg/L. Linear correlation coefficient (R^2) value for ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were 0.999, 0.999, 0.998, 0.999, 0.999 and 0.995, respectively. Recovery (%) of ciprofloxacin, levofloxacin, enrofloxacin tetracycline, oxytetracycline and chlortetracycline with pasteurized milk were 89, 94, 93, 92, 84 and 101%, respectively. Relative standard deviation (RSD%) for repeatability of ciprofloxacin, levofloxacin, enrofloxacin tetracycline, oxytetracycline and chlortetracycline were 7.93, 2.59, 4.73, 7.04, 9.40 and 5.52, respectively. No targeted antibiotic was detected in any pasteurized milk sample.

For the treatment of dairy cattle with antibiotics may cause milk contamination. This antibiotic residues can pose a risks to human health. In 2019 a series of news was reported in daily newspaper in Bangladesh that antibiotic residues were found in pasteurized milk. Then high court directed the authorities concerned to stop production, distribution, sale, purchase and consumption of pasteurized milk of 14 companies for five weeks for presence of antibiotics **[105].** This was a huge business loss for the country. After this incident, determination of antibiotic residues in pasteurized milk was included in this study. LC-MS/MS is the perfect technique for determination of antibiotic residues in pasteurized milk. Forty two (42) pasteurized milk samples of that 14 company were analyzed. No targeted antibiotic residues were detected in any pasteurized milk sample.

Although this sample size does not represent the whole Bangladesh but it can give an idea about the occurrence of antibiotic residue contamination in pasteurized milk in Bangladesh.

3.7 Summary

In this study nine analytical methods were established following Eurachem validation guideline for determination of chemical contaminants of food. All method validation performance criteria were fulfilled. These methods were simple, precise, selective and sensitive. Three methods utilizing GC-ECD, GC-MS and LC-MS/MS were used for analysis of 120 pesticides which includes organochlorine, organophosphorus and carbamate pesticides. Fruit (n=280) and vegetable (n=455) samples were analyzed for pesticide residues. Chlorpyrifos was detected by GC-MS in 2 samples of cabbage which were within MRL of 1.0 mg/kg set by BFSA. Dimethoate was also detected by GC-MS in 4 green chili samples which were within MRL of 1.0 mg/kg set by BFSA. Carbofuran was detected by LC-MS/MS in 2 tomato samples and in 2 eggplant samples. All samples were within the MRL of 0.01 mg/kg set by European Commission. Arsenic, lead and cadmium were analyzed by AAS-GFA in fruit (n=280) and vegetable (n=455) samples. Arsenic was detected in 13 potato samples, in 01 tomato samples, in 11 eggplant samples and in 1 carrot samples. Cadmium was detected in 6 potato samples, all sample were within the maximum limit of 0.1 mg/kg set by BFSA. Lead and chromium were analyzed by AAS-GFA in 17 turmeric powder samples. High amount of lead and chromium were found in 8 turmeric powder samples. Eight samples exceeded maximum limit of lead of 2.5 mg/kg set by BSTI. Aflatoxin B_1, B_2, G_1 and G_2 were analyzed in 25 wheat and 25 maize samples by HPLC-FLD with post column derivatization unit-coring cell. No targeted aflatoxins was detected in any sample. Benzoic acid and sorbic acid were analyzed by HPLC-UV in 25 fruit drink and 27 tomato ketchup samples. Benzoic acid was detected in 17 fruit drink samples and in 21 tomato ketchup samples. Eleven fruit drink sample exceeded maximum limit of benzoic acid (120 mg/kg) set by BSTI. One tomato ketchup sample exceeded maximum limit of benzoic acid (750 mg/kg) set by BSTI. Sudan I, II, III and IV were analyzed by HPLC-UV in 20 chili powder samples. Sudan III was detected in 1 sample out of 20 samples. Ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were analyzed by LC-MS/MS in 42 samples of pasteurized milk. No targeted antibiotic was detected in any pasteurized milk sample.

Pesticide residues detected in 1.36 % sample of fruits and vegetables (Figure 128). All of the detected sample were within MRL.

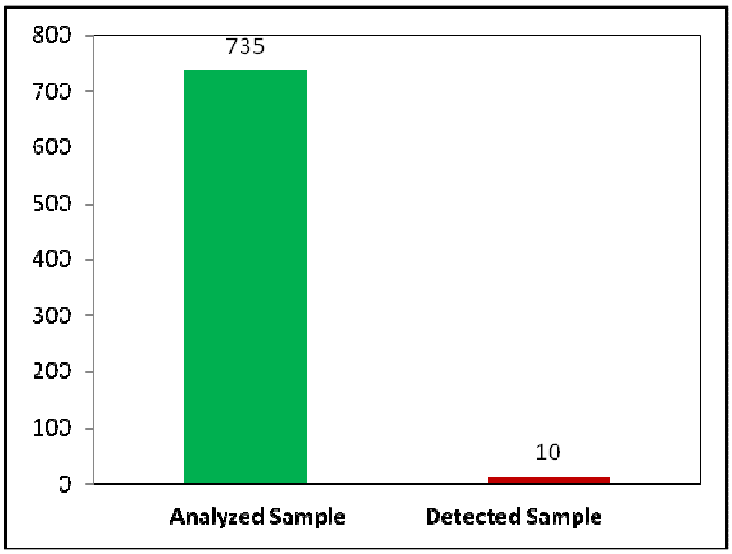


Figure 128: Analysis result of pesticide residues in fruits and vegetable

Arsenic was detected in 3.54 % sample of fruits and vegetables and Cadmium was detected in 0.82% sample of fruits and vegetables (Figure 129). All cadmium detected sample were within maximum limit.

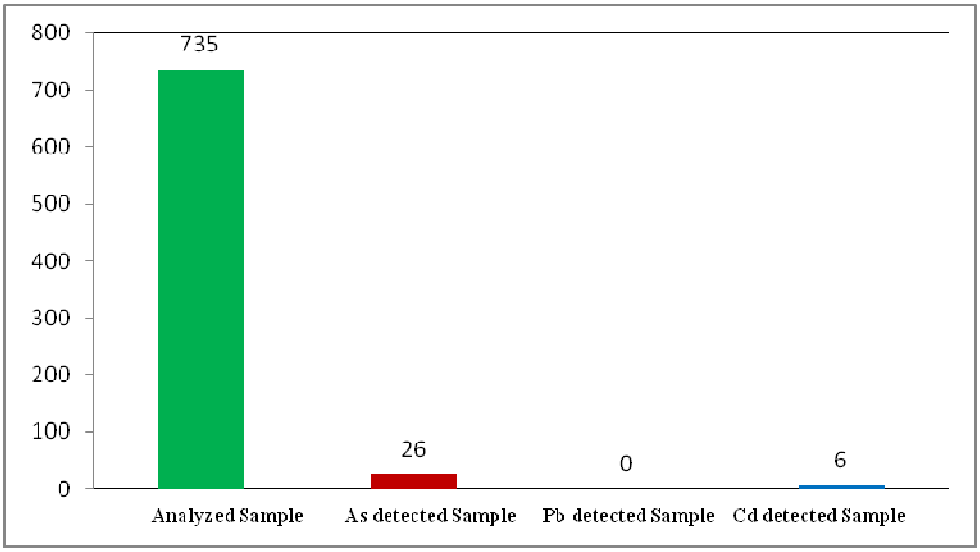
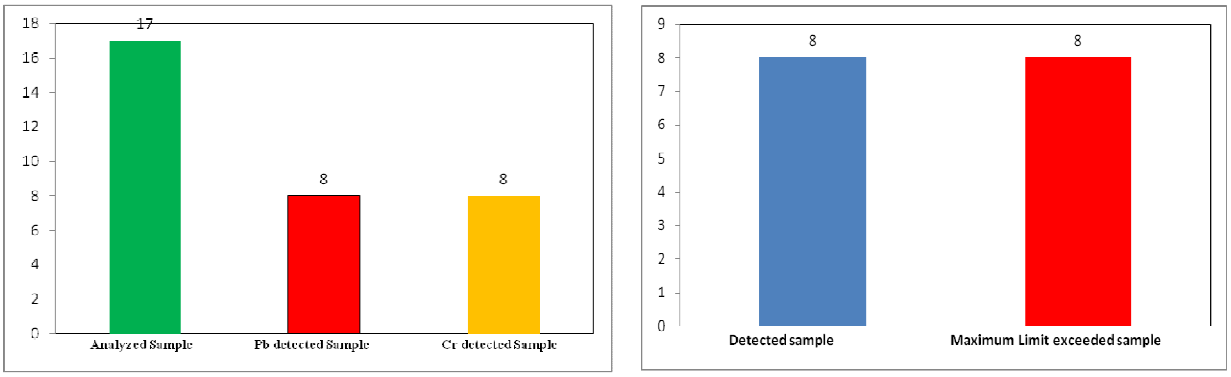


Figure 129: Analysis result of As, Pb and Cd in fruits and vegetable

Lead and chromium was detected in 47.06% of the turmeric powder sample (Figure 130).



(a) (b)

Figure 130: (a) Analysis result of Pb and Cr in turmeric powder (b) Maximum limit exceeded sample

Aflatoxin B_1, B_2, G_1 and G_2 were analyzed in wheat and maize sample. No targeted aflatoxin was detected in any wheat and maize sample (Figure 131).

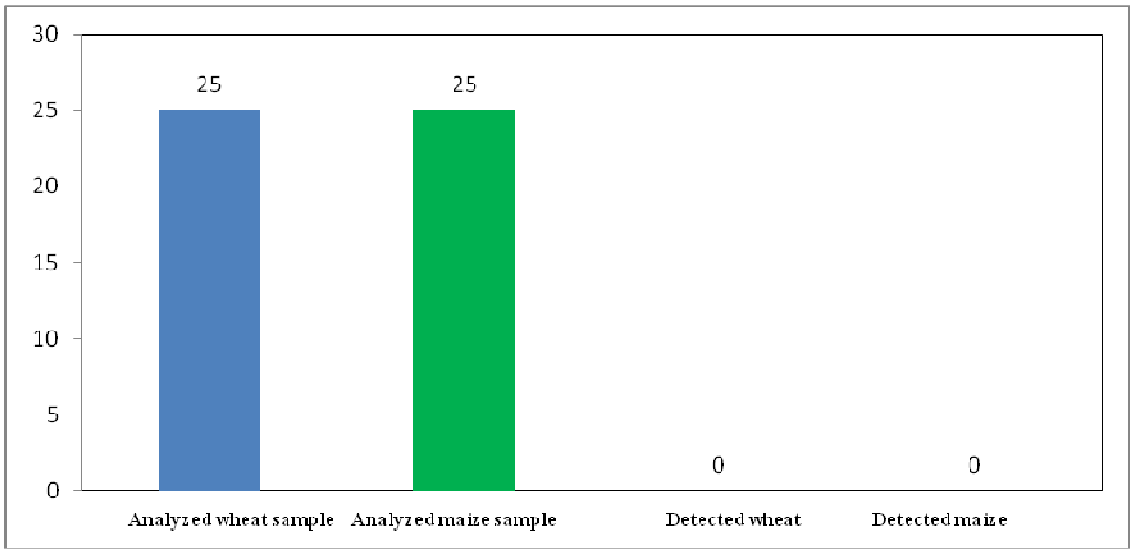


Figure 131: Analysis result of aflatoxin B_1, B_2, G_1 and G_2 in wheat and maize sample

Benzoic acid (BA) was detected in 68% fruit drink sample and in 77.78% tomato ketchup sample (Figure 132). Results show that 64.71% of benzoic acid detected fruit drink sample exceeded maximum limit and 6.76% of benzoic acid detected tomato ketchup sample exceeded maximum limit (Figure 133).

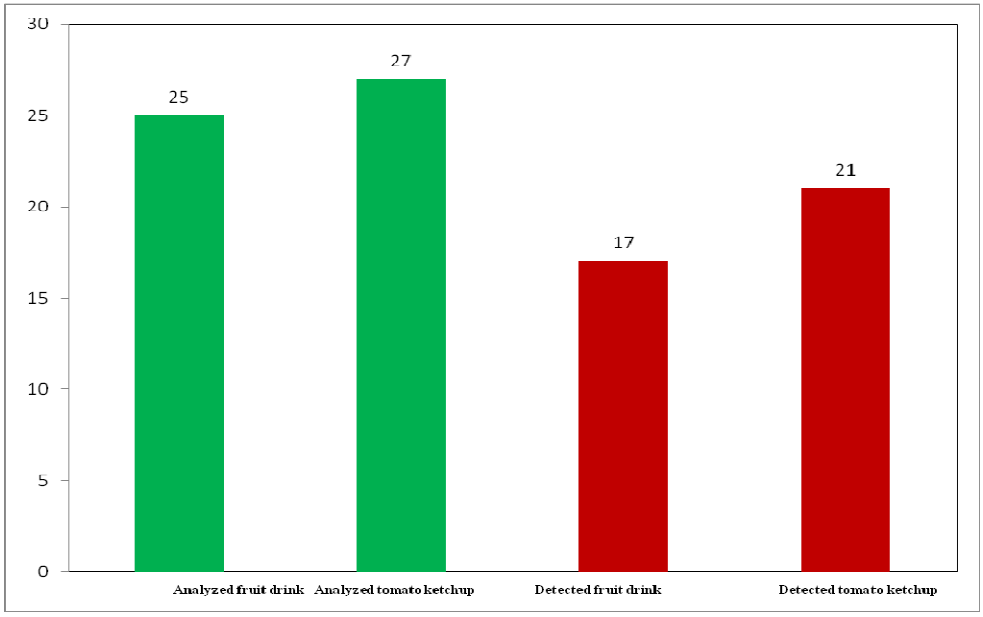


Figure 132: Analysis result of bezoic acid and sorbic acid in fruit drink and tomato ketchup sample

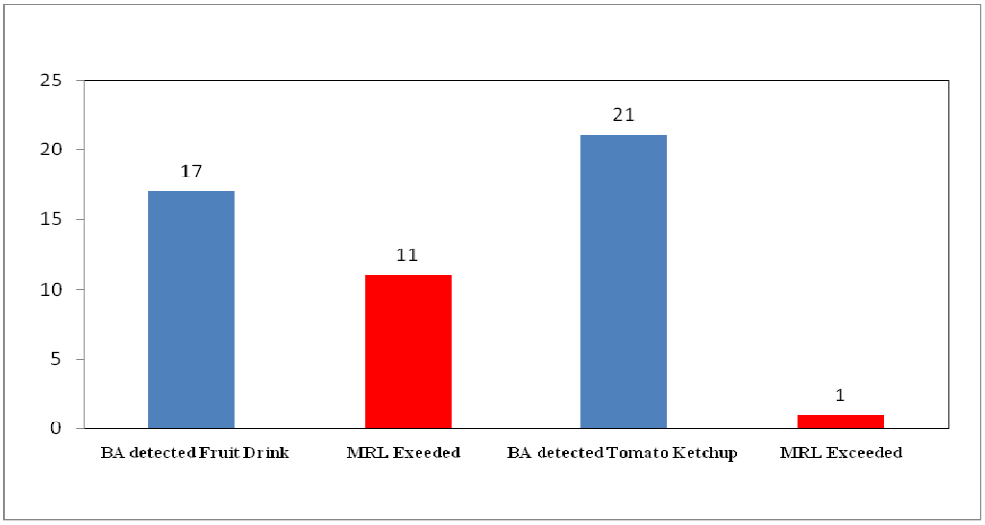


Figure 133: Analysis result of bezoic acid detected fruit drink and tomato ketchup sample

Sudan red I, II, III and IV were analyzed in chili powder sample. Sudan III was is detected in 5% chili powder sample (Figure 134).

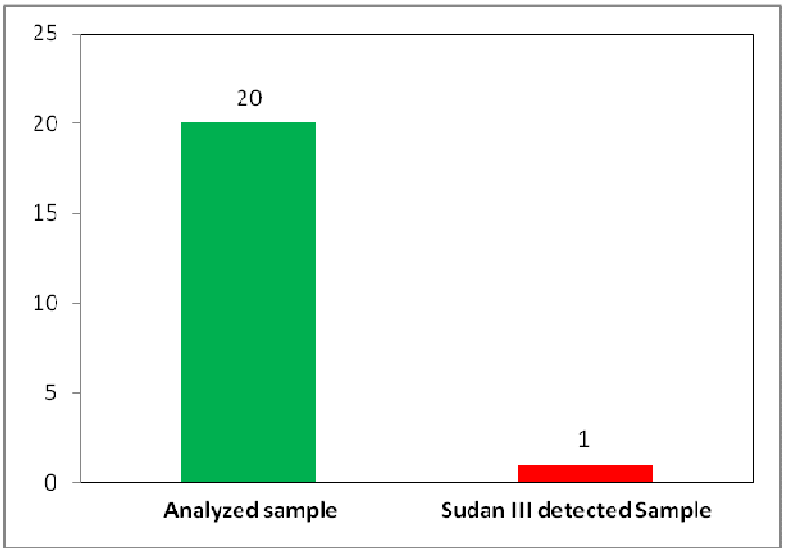


Figure 134: Analysis result of sudan red I, II, III and IV in chili powder sample

Six antibiotic residues were analyzed in pasteurized milk sample. No targeted antibiotic was detected in any pasteurized milk sample (Figure 135).

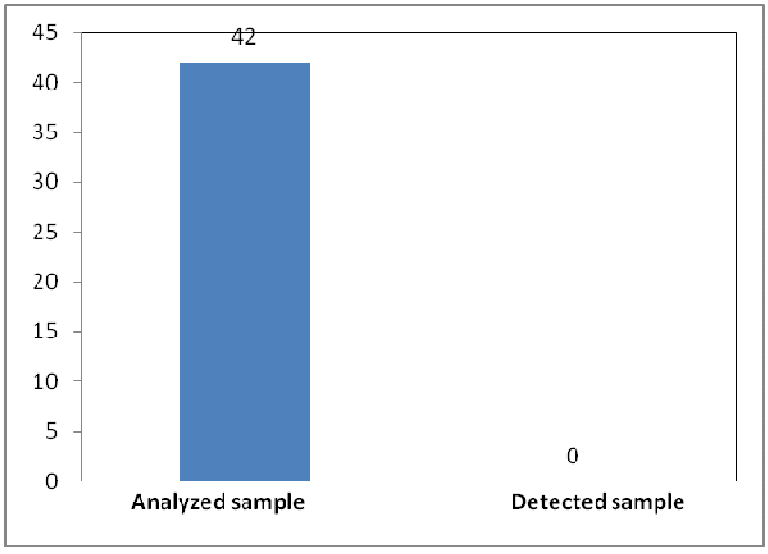


Figure 135: Analysis result of antibiotic residue in pasteurized milk sample

In this study analysis result shows that pesticide residues detected in 1.36 % sample of fruits and vegetables. All of the detected sample were within the MRL. Arsenic was detected in 3.54 % sample of fruits and vegetables and cadmium detected in 0.82% sample of fruits and vegetables. All sample of fruits and vegetables were within the maximum limit. Lead and chromium were detected in 47.06% of the turmeric powder sample. No targeted aflatoxin B_1, B_2, G_1 and G_2 was detected in any wheat and maize sample. Benzoic acid was detected in 68% fruit drink sample and in 77.78% tomato ketchup sample. 64.71% of benzoic acid detected fruit drink sample exceeded maximum limit and 6.76% of benzoic acid detected tomato ketchup sample exceeded maximum limit. Sudan III was is detected in 5% chili powder sample. No targeted antibiotic was detected in any sample of pasteurized milk.

All these food contaminants are harmful for human health and causes food borne diseases ranging from diarrhoea to cancer. According to WHO report approximately 600 million people in the world fall sick after consuming contaminated food and 4,20,000 people die every year. US$110 billion spend each year in productivity and medical expenses. It is resulting from unsafe food in low-and middle-income countries **[106].**

For an effective food safety control system in any country it is necessary to monitor all these food contaminants on regular basis. The analytical methods in this study are easy, effective, rugged and suitable for analysis of chemical contaminants in food. This present study will be very helpful for the policy maker to take sound scientific decisions.

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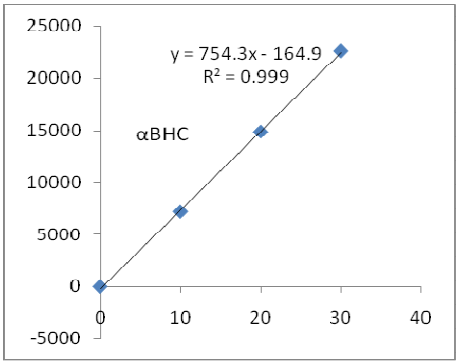
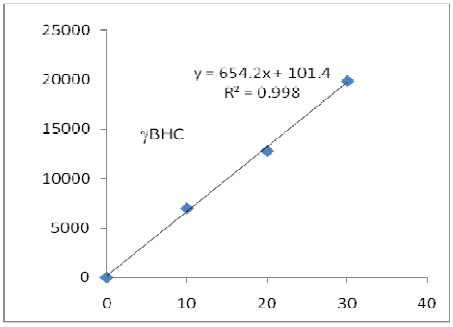
Annexure

Calibration Curve of 19 Organochlorine Pesticides Analyzed by GC-ECD

α BHC	
Conc (ppb)	Area
0	0
10	7170
20	14845
30	22587
STEYX	213.7618
Slope	754.36
Intercept	-164.9



γ BHC	
Conc (ppb)	Area
0	0
10	6989
20	12800
30	19871
STEYX	386.5703
Slope	654.24
Intercept	101.4



β BHC	
Conc (ppb)	Area
0	0
10	6550
20	13560
30	20214
STEYX	134.1581
Slope	676.52
Intercept	-66.8

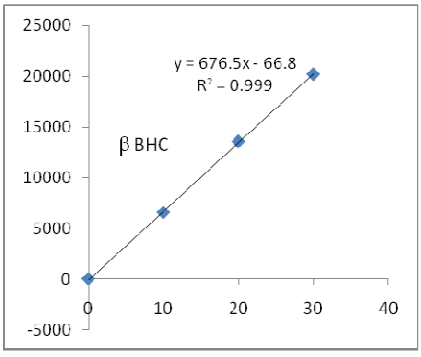
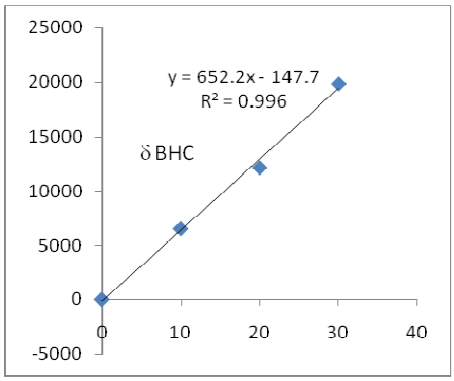
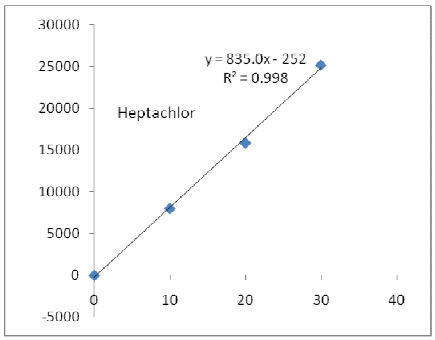


Figure-136: Calibration curve of α BHC, γ BHC and β BHC

δ BHC	
Conc (ppb)	Area
0	0
10	6530
20	12145
30	19871
STEYX	638.5287
Slope	652.28
Intercept	-147.7



Heptachlor	
Conc (ppb)	Area
0	0
10	8010
20	15870
30	25215
STEYX	538.1531
Slope	835.05
Intercept	-252



Aldrin	
Conc (ppb)	Area
0	0
10	7149
20	15010
30	21178
STEYX	514.680241
Slope	713.95
Intercept	125

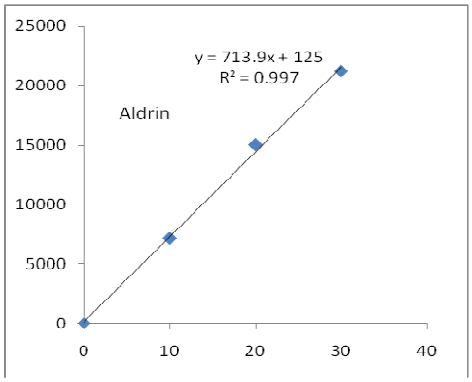
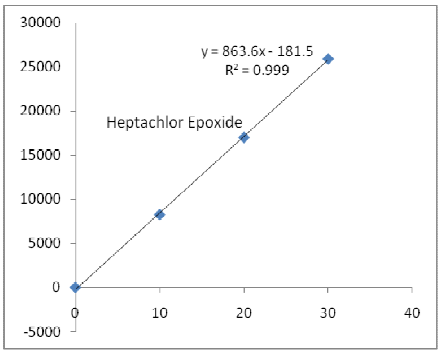
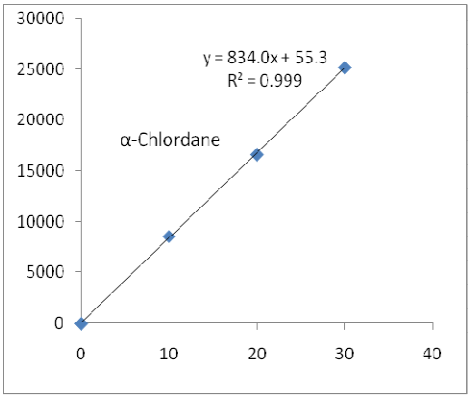


Figure-137: Calibration curve of δ BHC, Heptachlor and Aldrin

Heptachlor Epoxide	
Conc (ppb)	Area
0	0
10	8235
20	16987
30	25871
STEYX	237.3937
Slope	863.65
Intercept	-181.5



α-Chlordane	
Conc (ppb)	Area
0	0
10	8554
20	16587
30	25125
STEYX	162.3234
Slope	834.08
Intercept	55.3



γ-Chlordane	
Conc (ppb)	Area
0	0
10	9517
20	17890
30	26225
STEYX	453.0137
Slope	870.48
Intercept	350.8

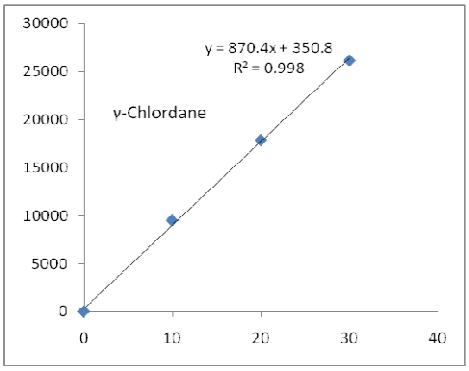
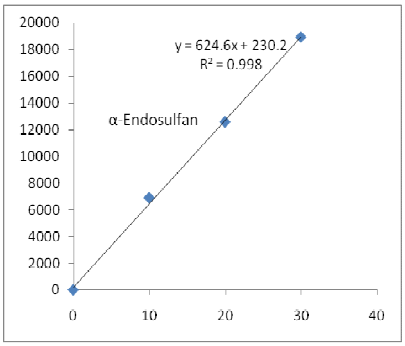
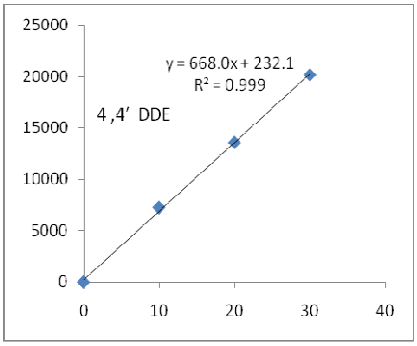


Figure-138: Calibration curve of Heptachlor Epoxide, α-Chlordane and γ- Chlordane

α-Endosulfan	
Conc (ppb)	Area
0	0
10	6893
20	12580
30	18925
STEYX	352.704409
Slope	624.62
Intercept	230.2



4 ,4′ DDE	
Conc (ppb)	Area
0	0
10	7263
20	13587
30	20159
STEYX	308.0736
Slope	668.01
Intercept	232.1



Dieldrin	
Conc (ppb)	Area
0	0
10	6657
20	12985
30	18250
STEYX	505.645
Slope	610.78
Intercept	311.3

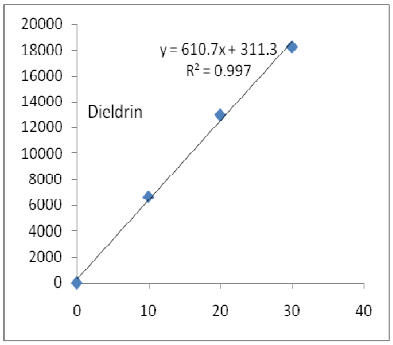
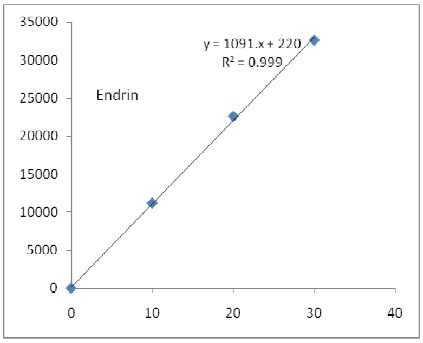
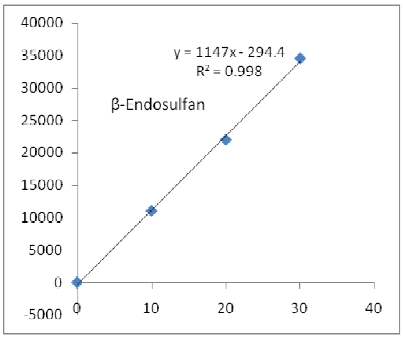


Figure-139: Calibration curve of α-Endosulfan, 4, 4′ DDE and Dieldrin

Endrin	
Conc (ppb)	Area
0	0
10	11195
20	22598
30	32589
STEYX	496.8018
Slope	1091.7
Intercept	220



β-Endosulfan	
Conc (ppb)	Area
0	0
10	11052
20	22010
30	34581
STEYX	601.0544
Slope	1147.01
Intercept	-294.4



4,4′ DDD	
Conc (ppb)	Area
0	0
10	5273
20	11205
30	16157
STEYX	282.9101447
Slope	544.03
Intercept	-1.7

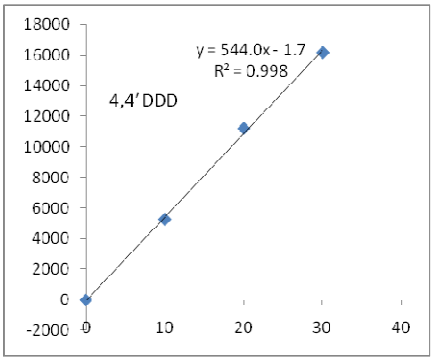
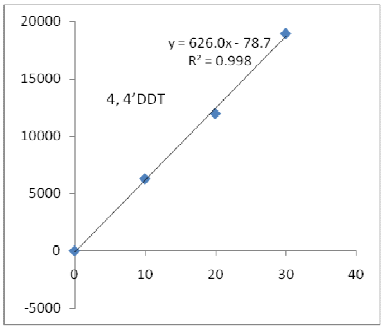
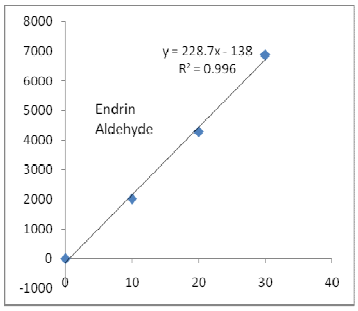


Figure-140: Calibration curve of Endrin, β-Endosulfan and 4, 4′ DDD

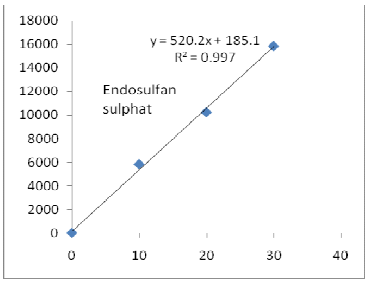
4, 4'DDT	
Conc (ppb)	Area
0	0
10	6292
20	11985
30	18970
STEYX	386.5594
Slope	626.03
Intercept	-78.7



Endrin Aldehyde	
Conc (ppb)	Area
0	0
10	2020
20	4280
30	6870
STEYX	202.0272
Slope	228.7
Intercept	-138



Endosulfan sulphate	
Conc (ppb)	Area
0	0
10	5835
20	10251
30	15870
STEYX	421.5496
Slope	520.26
Intercept	185.1



Methoxychlor	
Conc (ppb)	Area
0	0
10	6815
20	12500
30	18970
STEYX	326.4334
Slope	625.95
Intercept	182

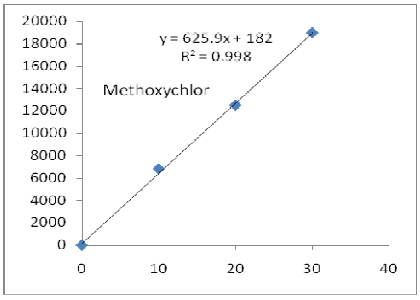
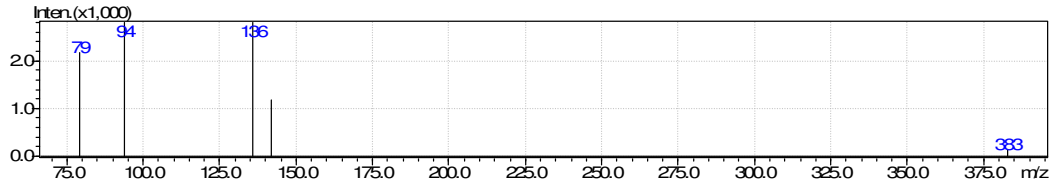
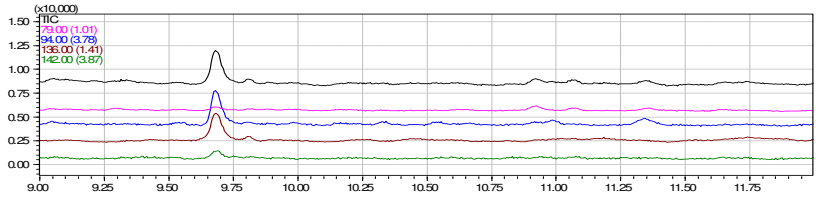


Figure-141: Calibration curve of Endrin Aldehyde, Endosulfan sulphat and Methoxychlor

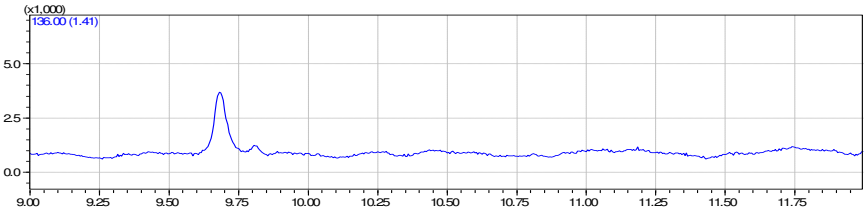
Mass Spectrum, total ion and target ion chromatogram of 16 organophosphorus pesticides



(a)

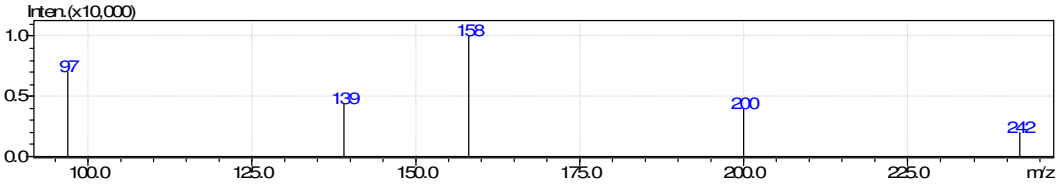


(b)

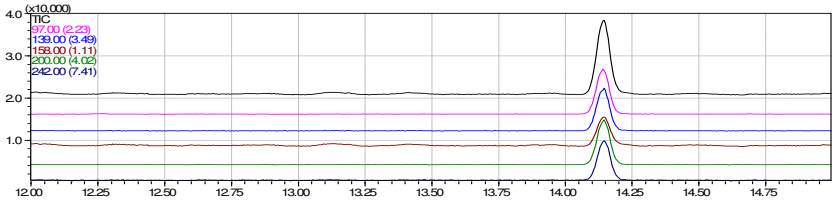


(c)

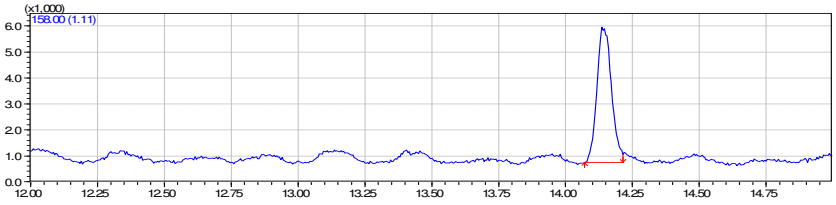
Figure-142: (a) Mass Spectrum, (b) total ion and (c) target ion (m/z 136.00) chromatogram of Acephate



(a)



(b)



(c)

Figure-143: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 158.00) chromatogram of ethoprophos

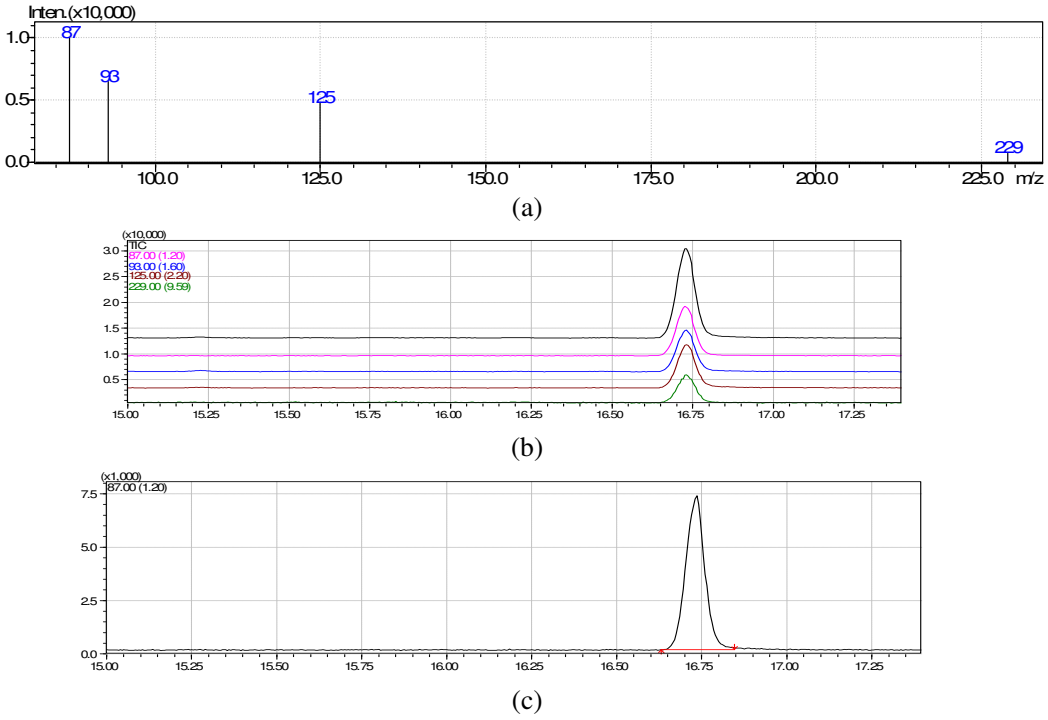


Figure-144: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 87.00) chromatogram of dimethoate

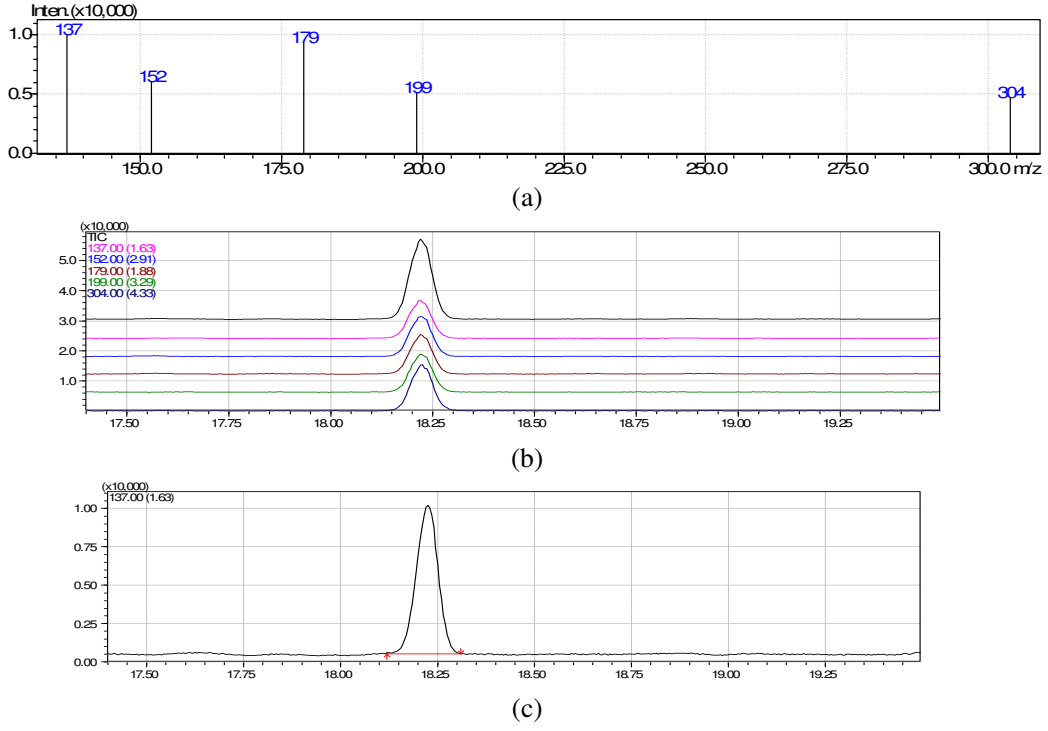


Figure-145: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 137.00) chromatogram of diazinone

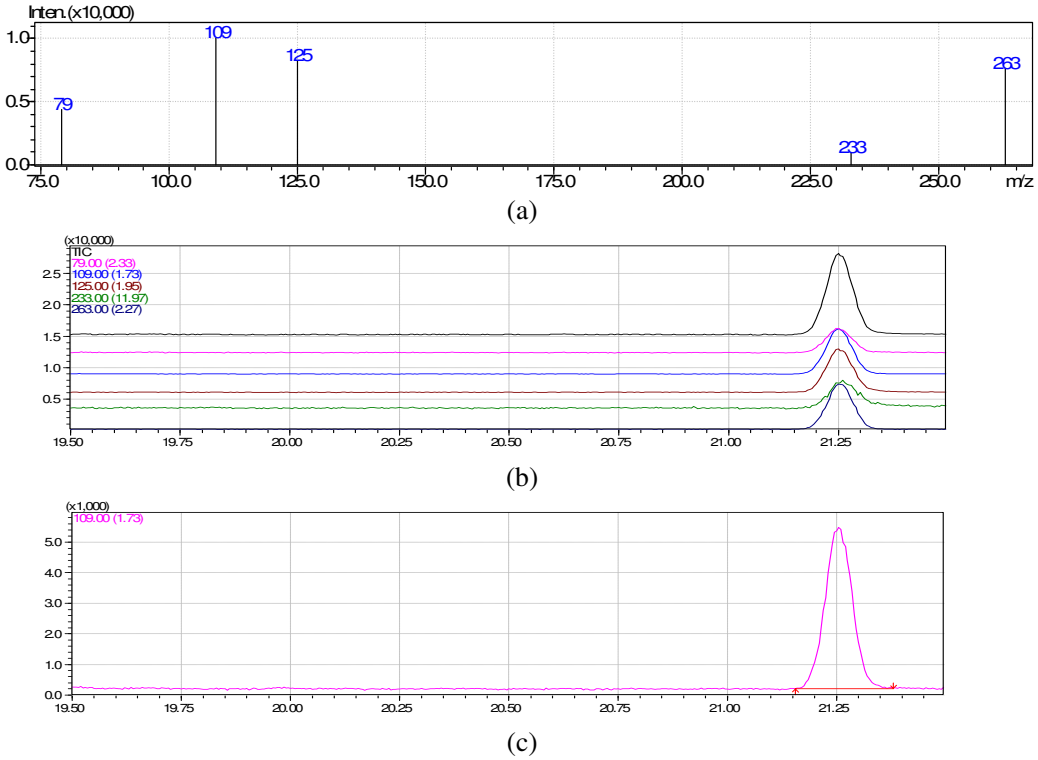


Figure-146: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 109.00) chromatogram of Methyl parathion

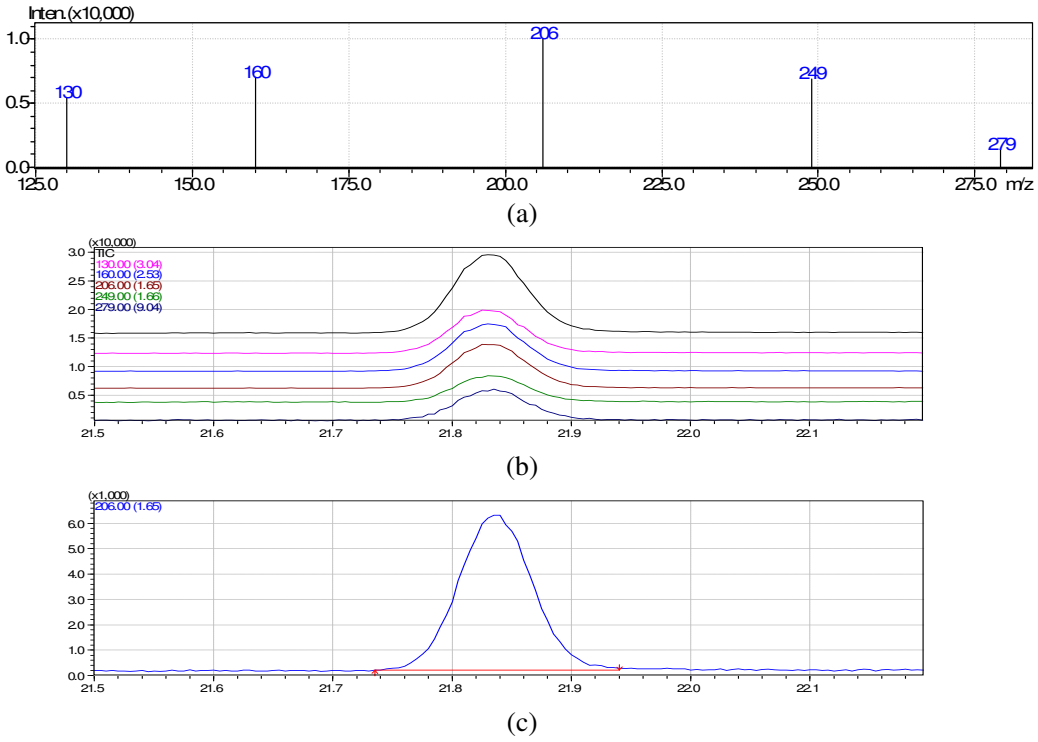
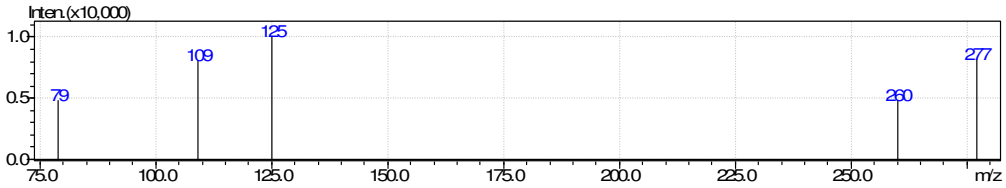
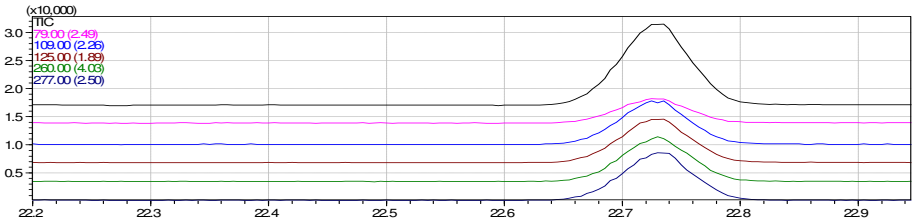


Figure-147: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 206.00) chromatogram of metalaxyl



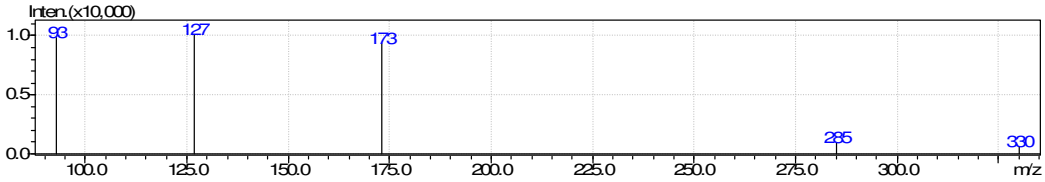
(a)



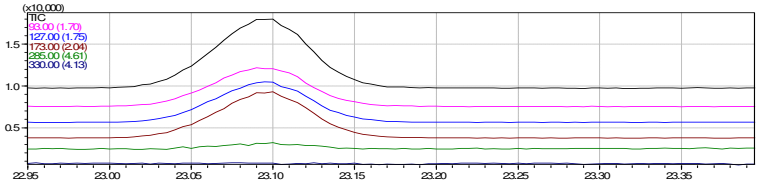
(b)

(c)

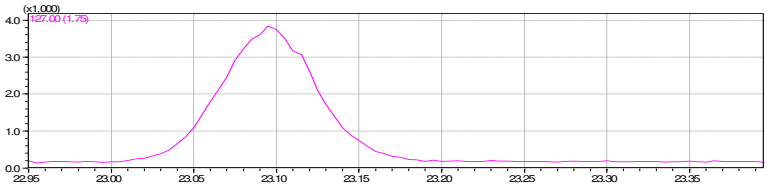
Figure-148: Mass Spectrum, total ion and target ion(m/z 125.00) chromatogram of Fenitrothion



(a)



(b)



(c)

Figure-149: Mass Spectrum, total ion and target ion(m/z 127.00) chromatogram of Malathion

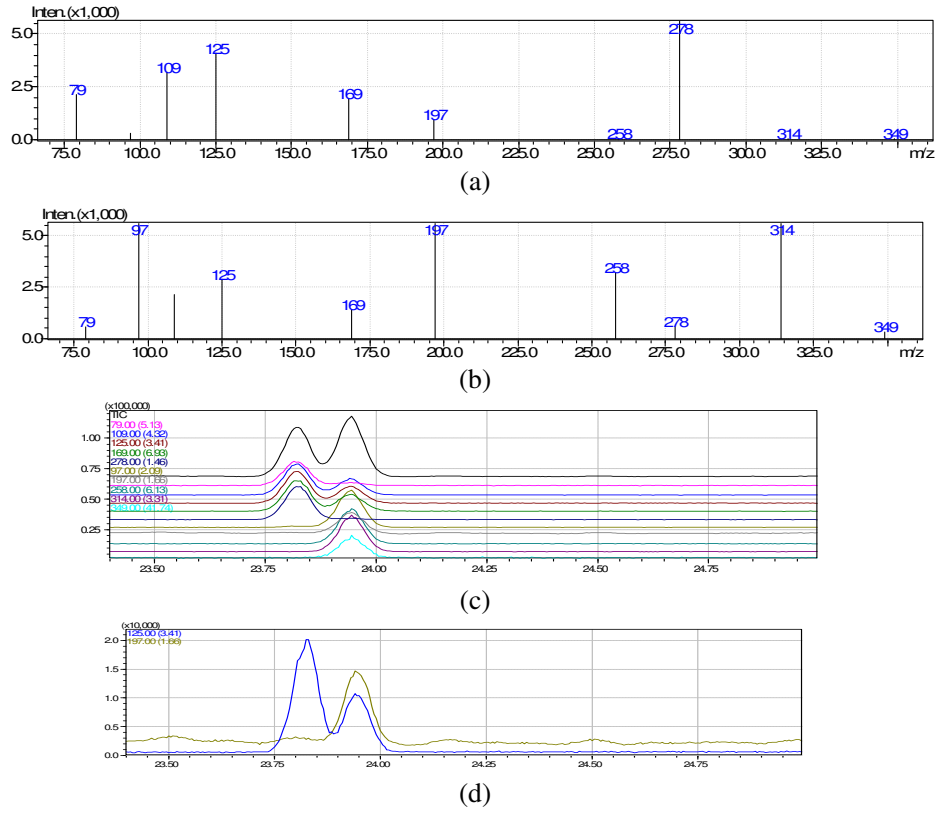


Figure-150: (a) Mass Spectrum of fenthion (b) Mass Spectrum of chlorpyrifos, (c) total ion and (d) target ion(m/z 125.00 and m/z 197.00) chromatogram of fenthion and chlorpyrifos

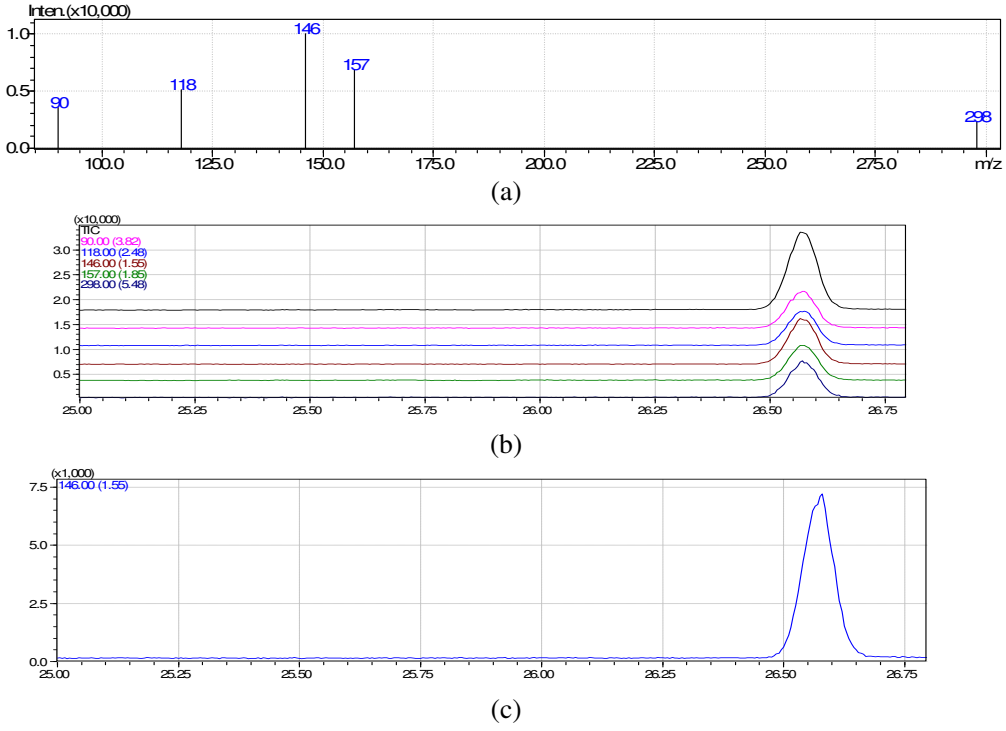


Figure-151: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 146.00) chromatogram of quinalphos

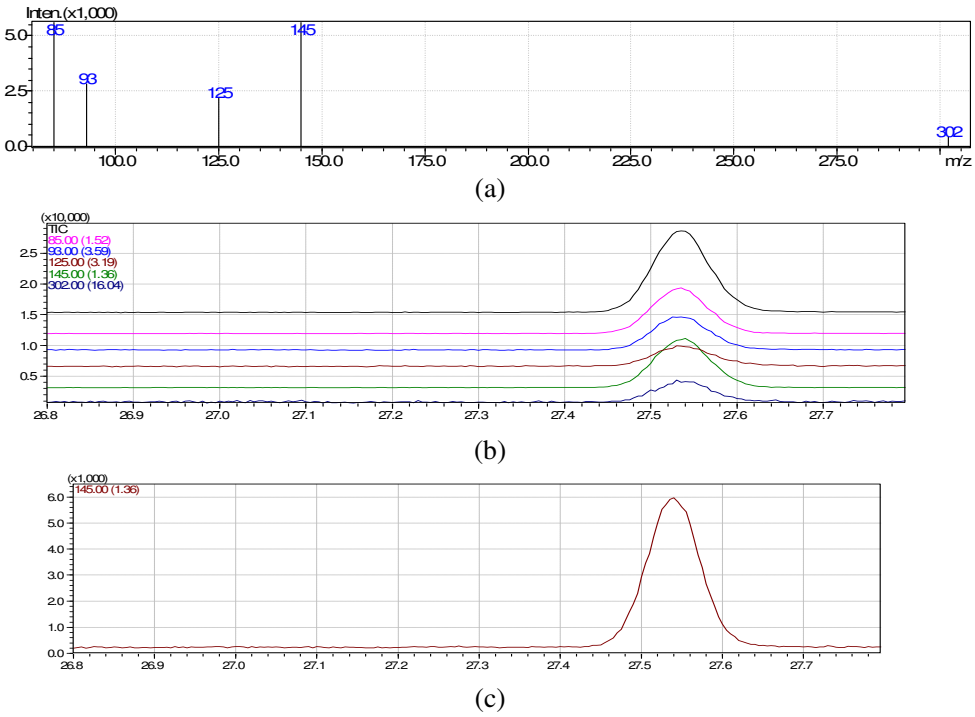


Figure-152: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 145.00) chromatogram of methidathion

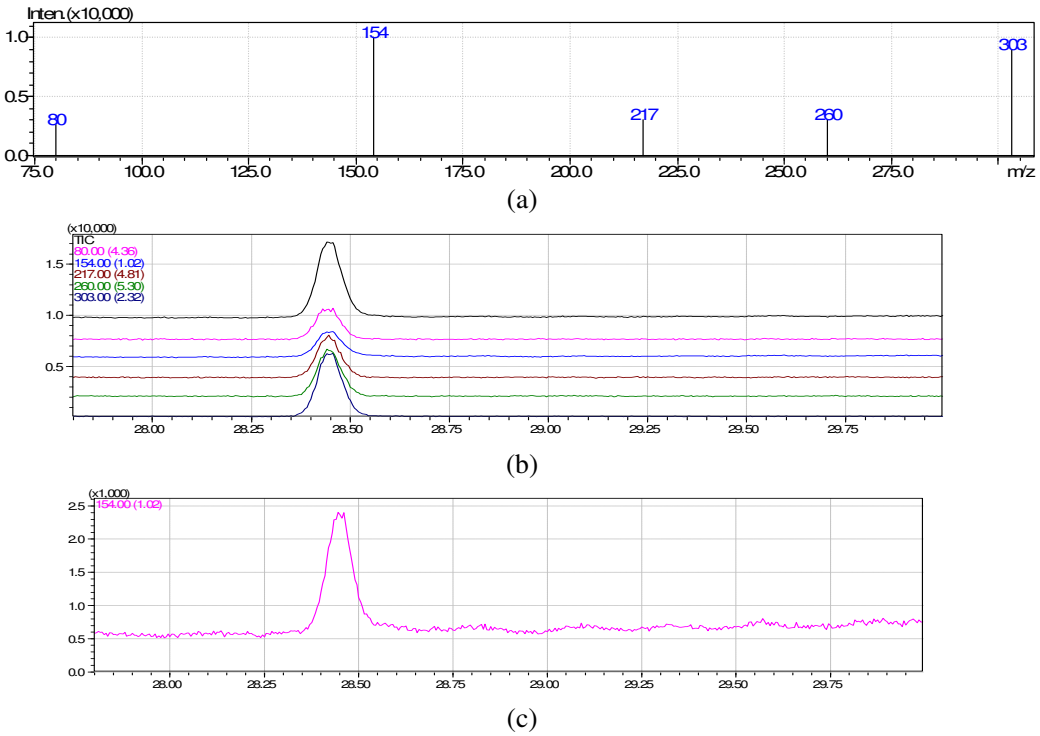


Figure-153: (a) Mass Spectrum, (b) total ion and (c) target ion(m/z 154.00) chromatogram of fenamiphos

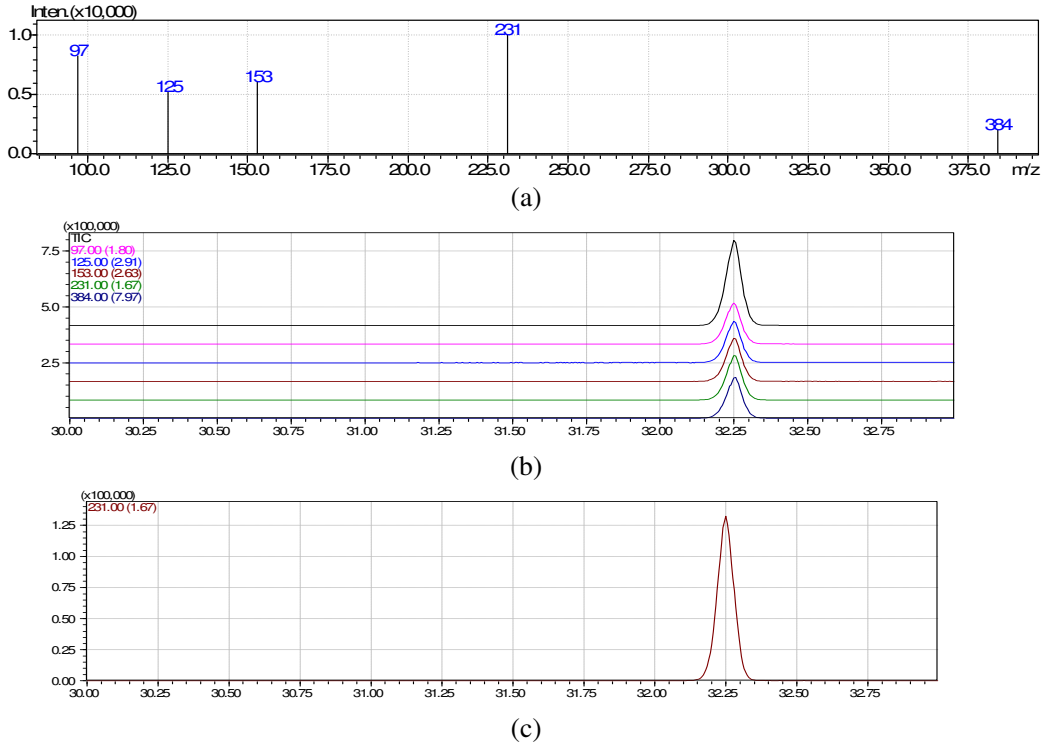


Figure-154: Mass Spectrum, total ion and target ion(m/z 231.00) chromatogram of ethion

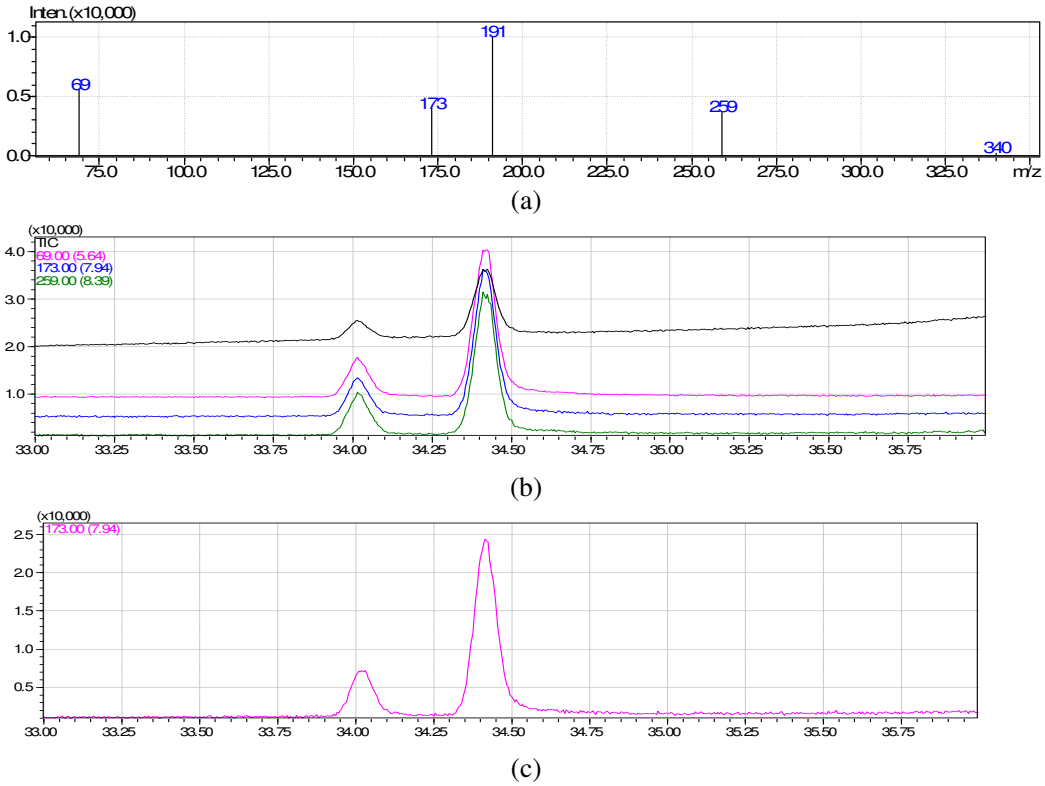
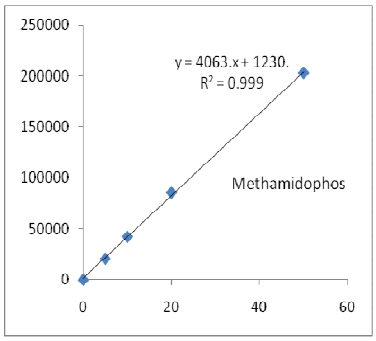


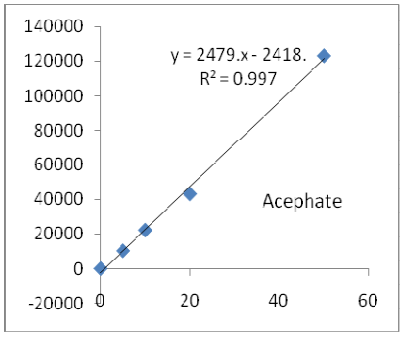
Figure-155: (a) Mass spectrum, (b) total ion and (c) target ion(m/z 173.00) chromatogram of propiconazole

Calibration Curve of 16 Organophosphorus Pesticides Analyzed by GC-MS

Conc(µg/L)	Area
0	0
5	20588
10	42214
20	85514
50	203210
Intercept	1230.13291
Slope	4063.23924



Conc(µg/L)	Area
0	0
5	10244
10	22154
20	43025
50	123210
Intercept	-2418.33
Slope	2479.113



Conc(µg/L)	Area
0	0
5	40547
10	82101
20	164570
50	402210
Intercept	1182.263
Slope	8041.373

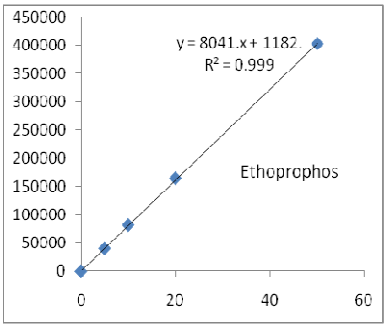
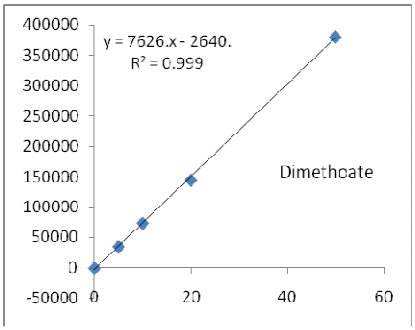
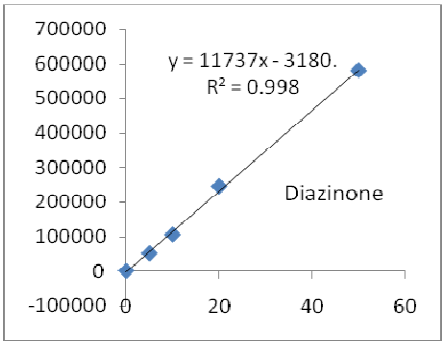


Figure-156: Calibration Curve of methamidophos, acephate, ethoprophos

Conc(µg/L)	Area
0	0
5	35447
10	74025
20	145007
50	380540
Intercept	-2640.59
Slope	7626.141



Conc(µg/L)	Area
0	0
5	50788
10	105450
20	245007
50	580540
Intercept	-3180.34
Slope	11737.49



Conc(µg/L)	Area
0	0
5	12250
10	25987
20	52368
50	123025
Intercept	892.8734
Slope	2460.772

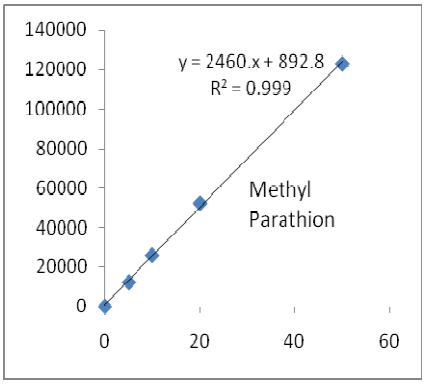
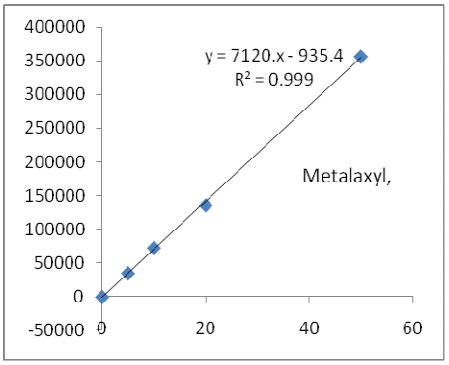
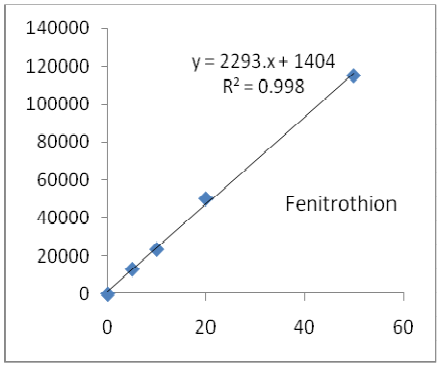


Figure-157: Calivbration curve of dimethoate, diazinone, methyl parathion

Conc(µg/L)	Area
0	0
5	35125
10	72698
20	135980
50	356740
Intercept	-935.424
Slope	7120.237



Conc(µg/L)	Area
0	0
5	13122
10	23568
20	50256
50	115000
Intercept	1404.025
Slope	2293.246



Conc(µg/L)	Area
0	0
5	16122
10	33568
20	64256
50	165080
Intercept	-272.937
Slope	3298.714

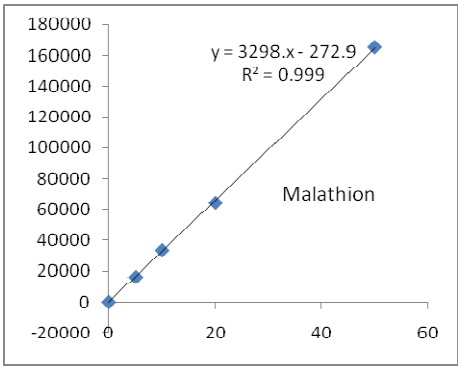
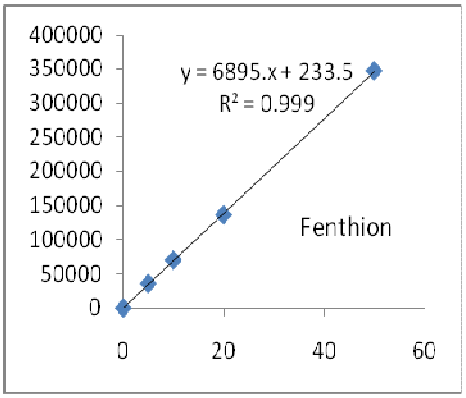
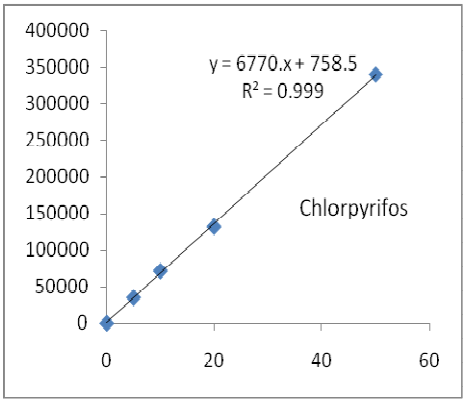


Figure-158: Calibration curve of metalaxyl, fanitrothion and malathion

Conc(µg/L)	Area
0	0
5	35780
10	70024
20	135801
50	345650
Intercept	233.5791
Slope	6895.142



Conc(µg/L)	Area
0	0
5	35250
10	71623
20	132252
50	340140
Intercept	758.5348
Slope	6770.263



Conc(µg/L)	Area
0	0
5	10450
10	21365
20	46025
50	118500
Intercept	-1334.24
Slope	2388.367

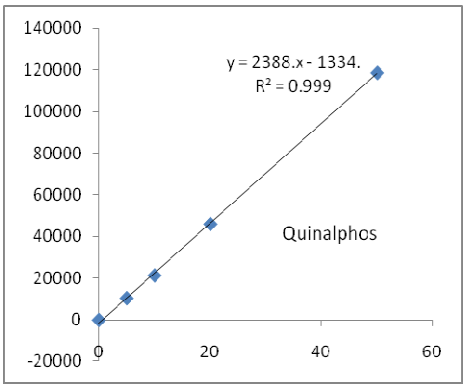
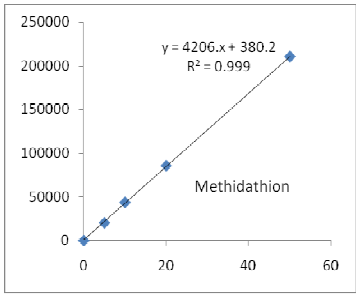
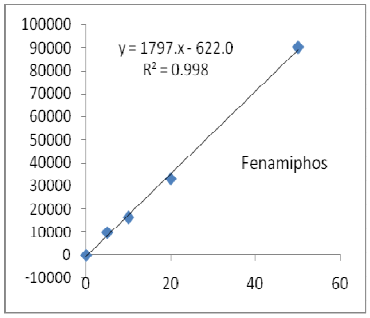


Figure-159: Calibration curve of fenthion, chlorpyrifos, quinalphos

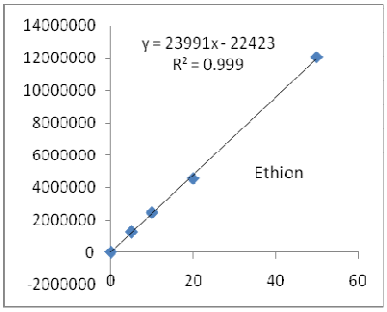
Conc(µg/L)	Area
0	0
5	20151
10	43589
20	85521
50	210212
Intercept	380.2911
Slope	4206.724



Conc(µg/L)	Area
0	0
5	9874
10	16477
20	33210
50	90145
Intercept	-622.089
Slope	1797.841



Conc(µg/L)	Area
0	0
5	1245202
10	2445202
20	4545202
50	12045200
Intercept	-22422.5
Slope	239916.7



Conc(µg/L)	Area
0	0
5	32021
10	60102
20	130020
50	300215
Intercept	2340.453
Slope	6007.715

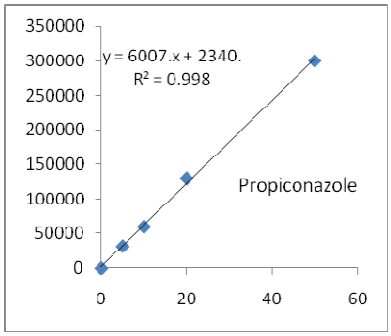
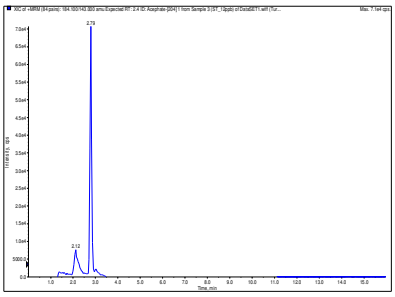
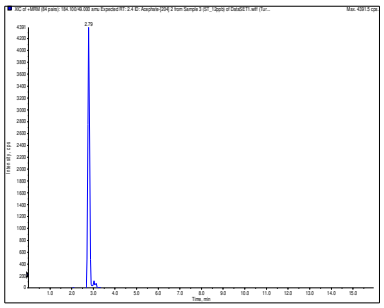


Figure-160: Calibration curve of methidathion, fanamiphos, ethion and propiconazole

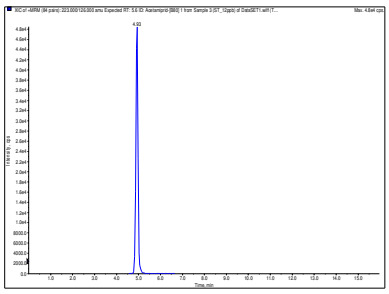
MRM Transition chromatogram of Mix-1(31 Pesticides) analyzed by LC-MS/MS



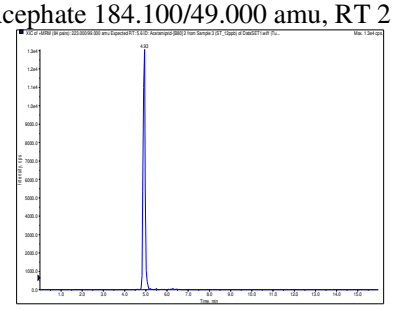
Acephate 184.100/143.000 amu, RT 2.79



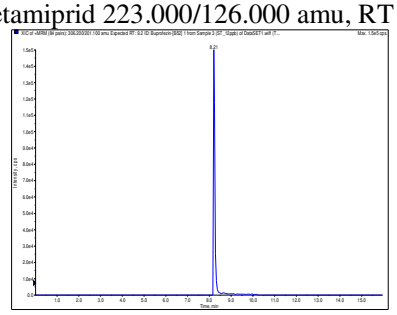
Acephate 184.100/49.000 amu, RT 2.79



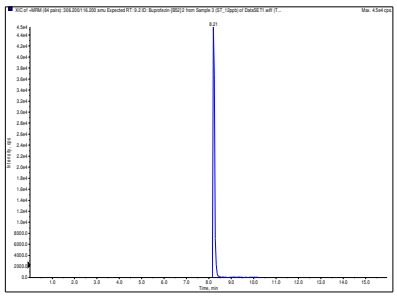
Acetamiprid 223.000/126.000 amu, RT 4.93



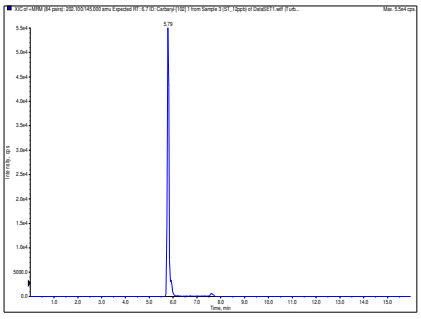
Acetamiprid 223.000/99.000 amu, RT 4.93



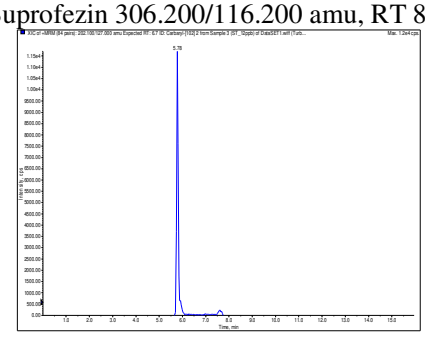
Buprofezin 306.200/201.100 amu, RT 8.21



Buprofezin 306.200/116.200 amu, RT 8.21

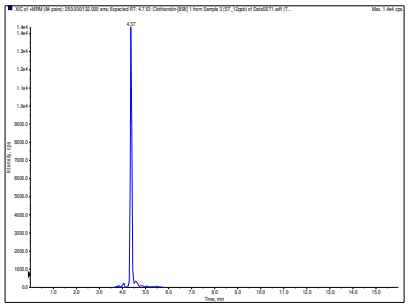


Carbaryl 202.100/145.000 amu, RT 5.79

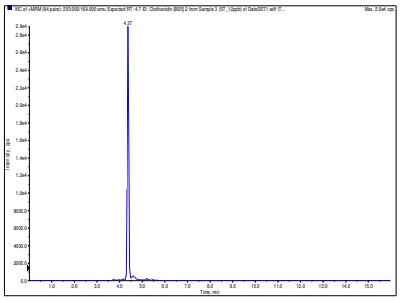


Carbaryl 202.100/127.000 amu, RT 5.78

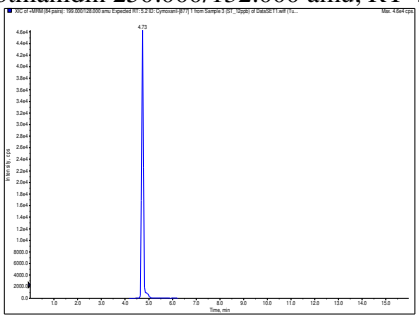
Figure-161: MRM transition of Acephate, Acetamiprid, Buprofezin and Carbaryl



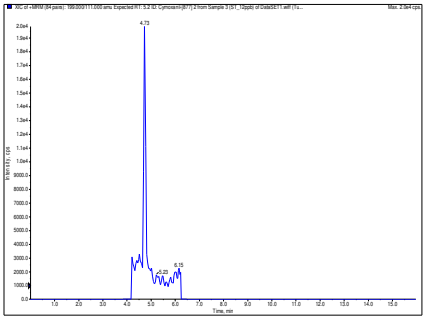
Clothianidin 250.000/132.000 amu, RT 4.37



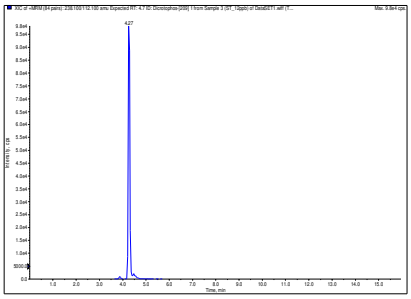
Clothianidin 250.000/169.000 amu, RT 4.37



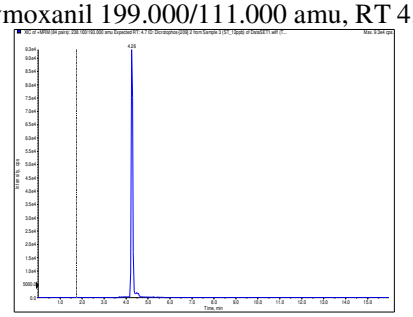
Cymoxanil 199.000/128.000 amu, RT 4.73



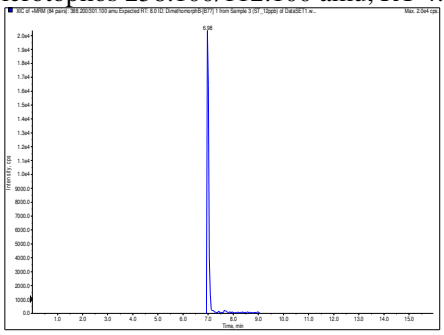
Cymoxanil 199.000/111.000 amu, RT 4.73



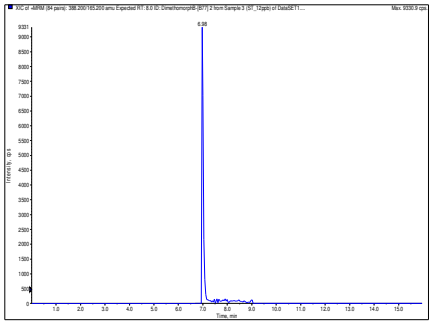
Dicrotophos 238.100/112.100 amu, RT 4.27



Dicrotophos 238.100/193.000 amu, RT 4.26

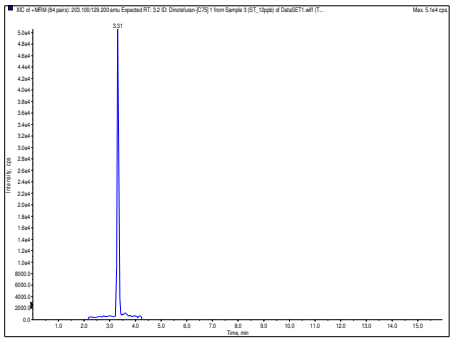


Dimethomorph 388.2/301.1 amu, RT 6.98

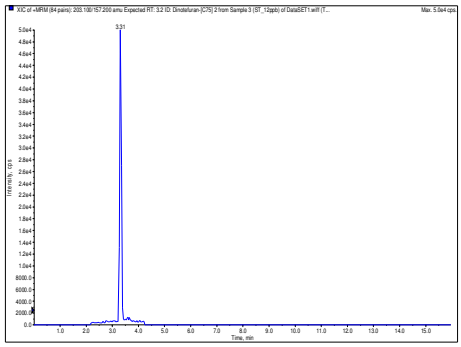


Dimethomorph 388.2/165.2 amu, RT 6.98

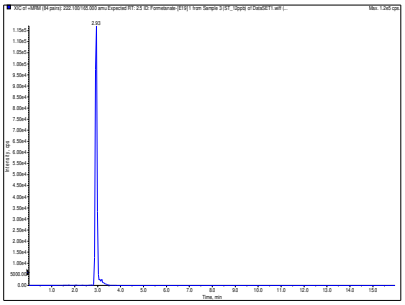
Figure-162: MRM transition of Clothianidin, Cymoxanil, Dicrotophos and Dimethomorph



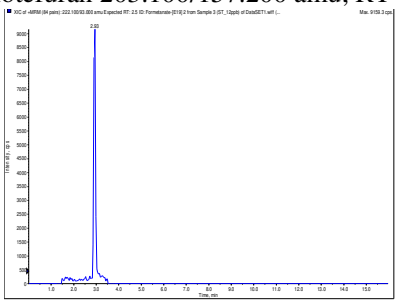
Dinotefuran 203.100/129.200 amu, RT 3.31



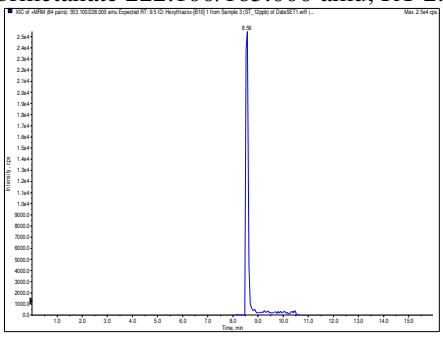
Dinotefuran 203.100/157.200 amu, RT 3.31



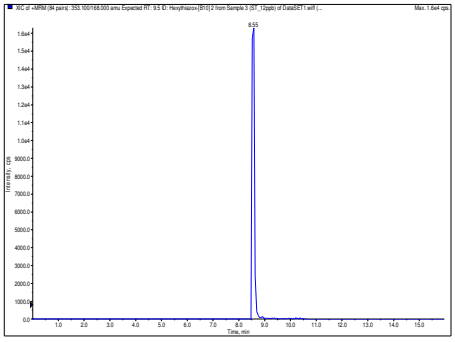
Formetanate 222.100/165.000 amu, RT 2.93



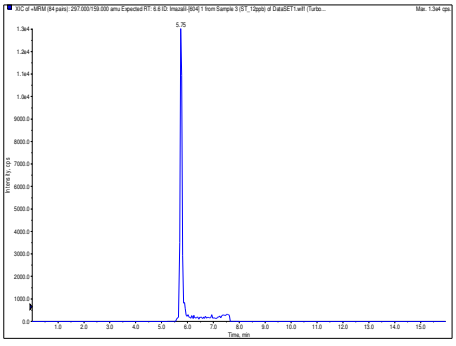
Formetanate 222.100/93.000 amu, RT 2.93



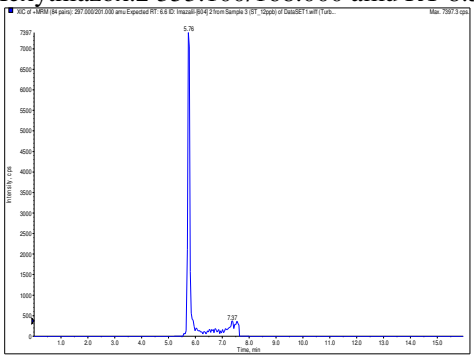
Hexythiazox.1 353.100/228.000 amu RT 8.56



Hexythiazox.2 353.100/168.000 amu RT 8.55

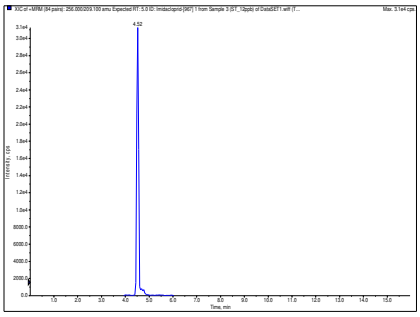


Imazalil 297.000/159.000 amu, RT 5.75

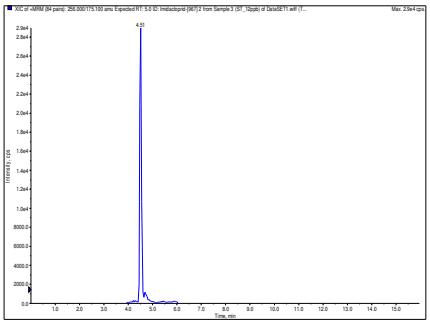


Imazalil 297.000/201.000 amu, RT 5.75

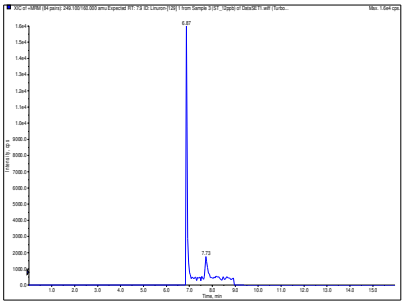
Figure-163: MRM transition of Dinotefuran, Formetanate, Hexythiazox and Imazalil



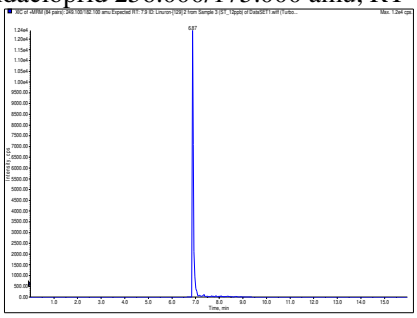
Imidacloprid 256.000/209.000 amu, RT 4.52



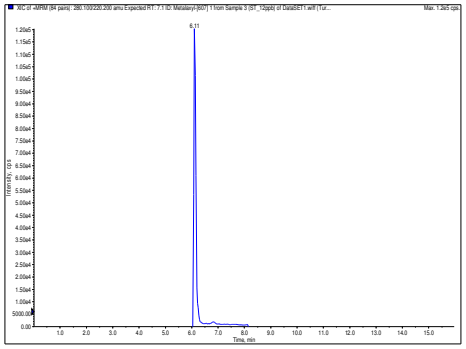
Imidacloprid 256.000/175.000 amu, RT 4.51



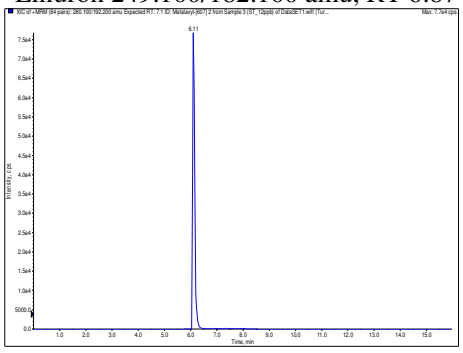
Linuron 249.100/160.000 amu, RT 6.87



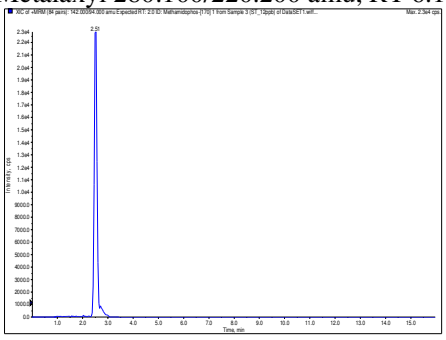
Linuron 249.100/182.100 amu, RT 6.87



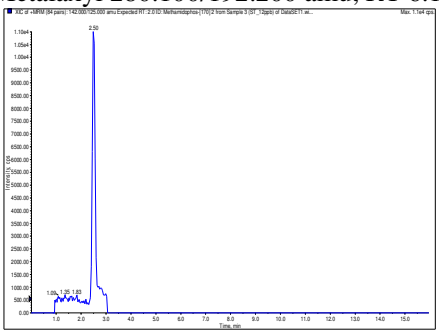
Metalaxyl 280.100/220.200 amu, RT 6.11



Metalaxyl 280.100/192.200 amu, RT 6.11

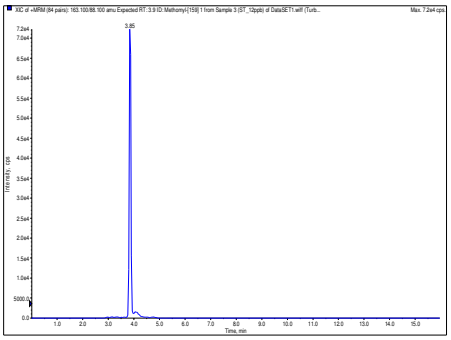


Methamidophos 142.000/94.000 amu, RT 2.51

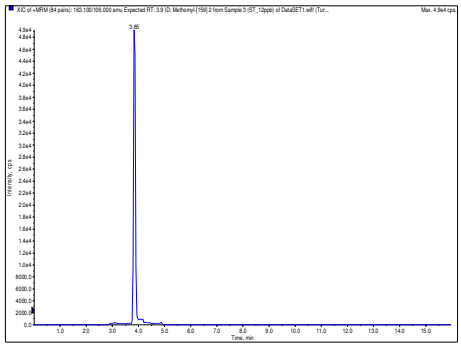


Methamidophos 142.000/125.000 amu, RT 2.50

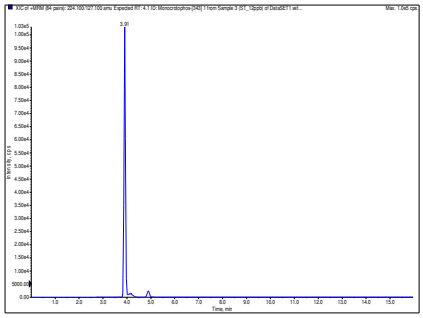
Figure-164: MRM transition of Imidacloprid, Linuron, Metalaxyl and Methamidophos



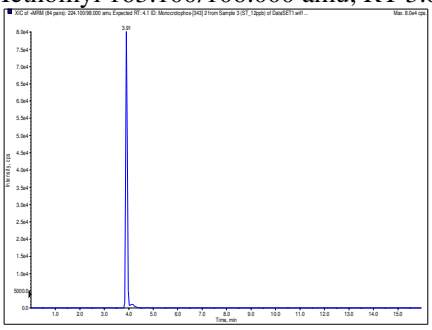
Methomyl 163.100/88.100 amu, RT 3.85



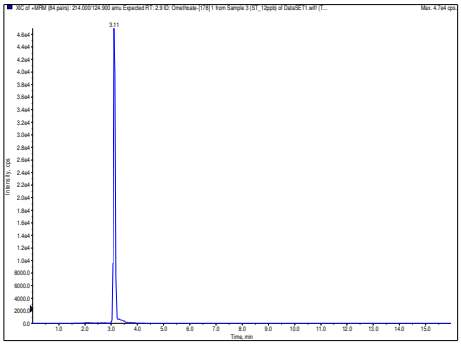
Methomyl 163.100/106.000 amu, RT 3.85



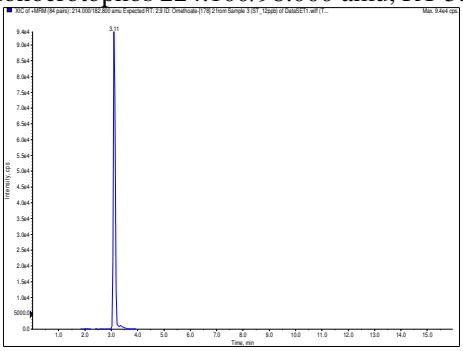
Monocrotophos 224.100/127.100 amu, RT 3.91



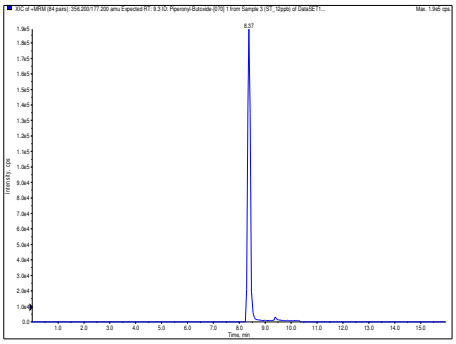
Monocrotophos 224.100/98.000 amu, RT 3.91



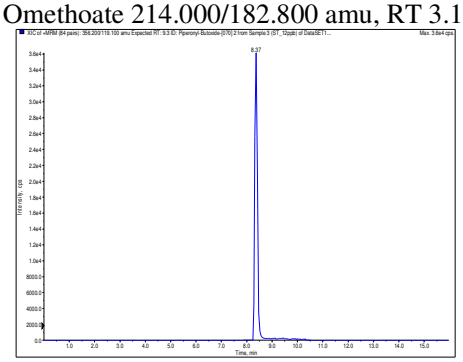
Omethoate 214.000/124.900 amu, RT 3.11



Omethoate 214.000/182.800 amu, RT 3.11

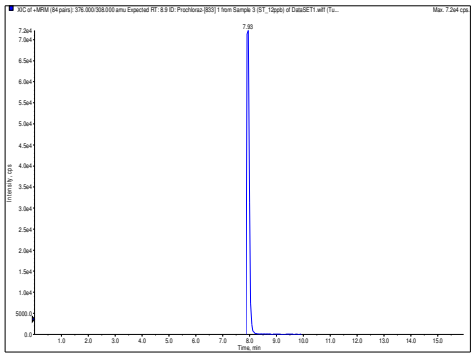


Piperonyl butoxide 356.2/177.2 amu, RT 8.37

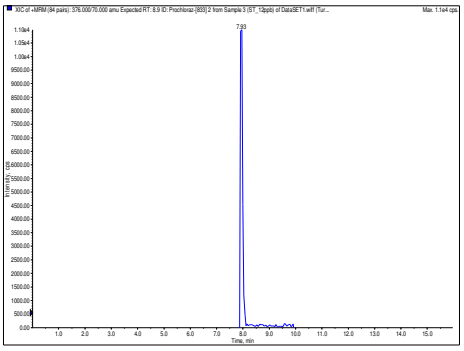


Piperonyl butoxide 356.2/119.1 amu, RT 8.37

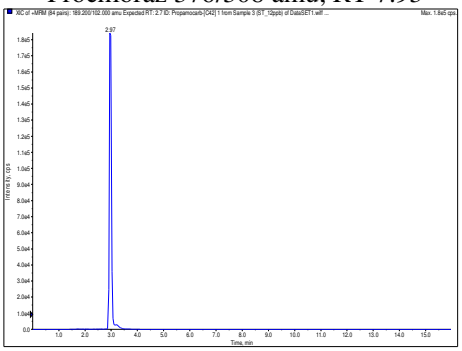
Figure-165: MRM transition of Methomyl, Monocrotophos, Omethoate and Piperonyl butoxide



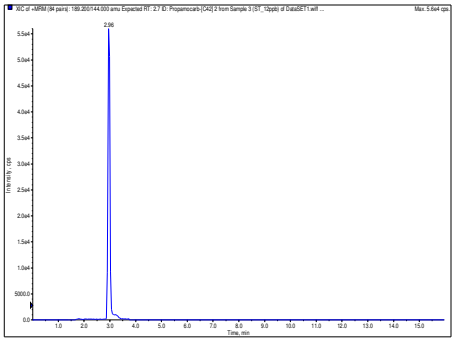
Prochloraz 376/308 amu, RT 7.93



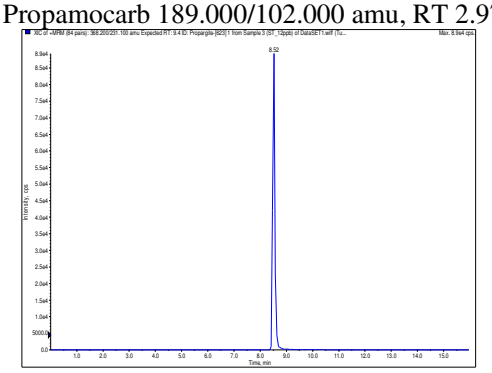
Prochloraz 376/70 amu, RT 7.93



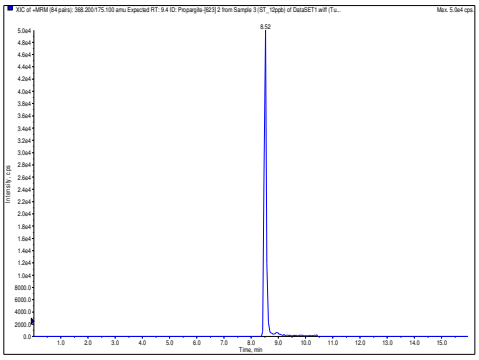
Propamocarb 189.000/102.000 amu, RT 2.97



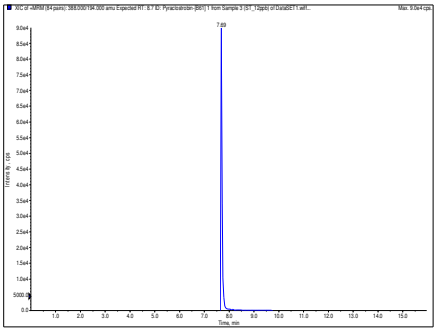
Propamocarb 189.000/144.000 amu, RT 2.96



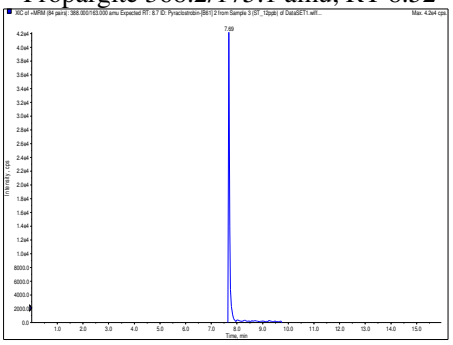
Propargite 368.2/231.1 amu, RT 8.52



Propargite 368.2/175.1 amu, RT 8.52

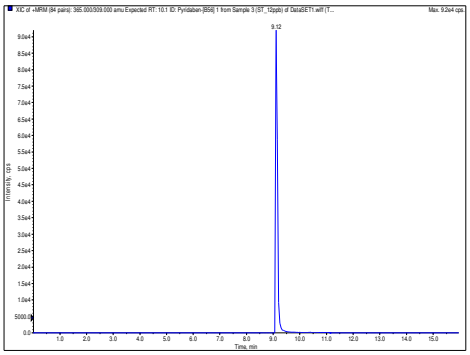


Pyraclostrobin 388.0/194.0 amu, RT 7.69

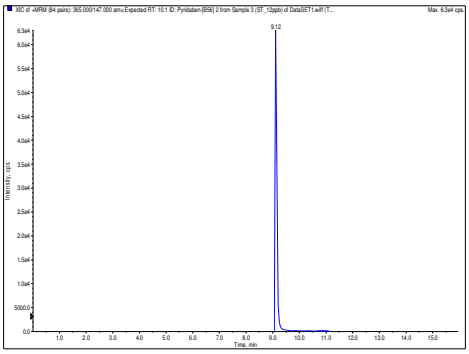


Pyraclostrobin 388.0/163.0 amu, RT 7.69

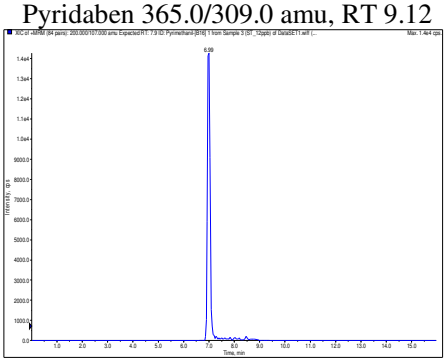
Figure-166: MRM transition of Prochloraz, Propamocarb, Propargite and Pyraclostrobin



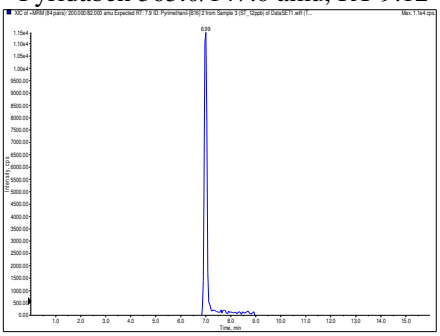
Pyridaben 365.0/309.0 amu, RT 9.12



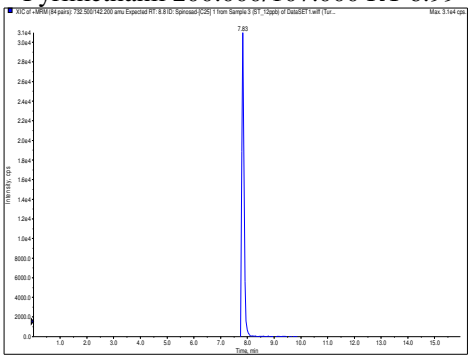
Pyridaben 365.0/147.0 amu, RT 9.12



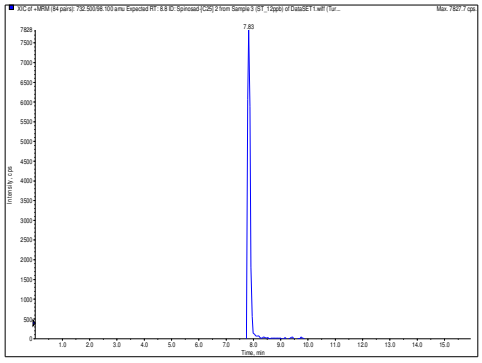
Pyrimethanil 200.000/107.000 RT 6.99



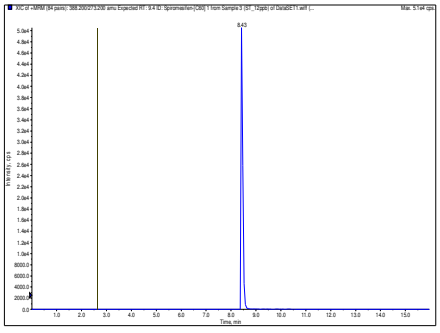
Pyrimethanil 200.000/82.000 RT 6.99



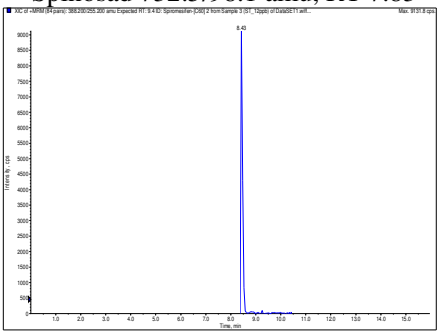
Spinosad 732.5/142.2 amu, RT 7.83



Spinosad 732.5/98.1 amu, RT 7.83

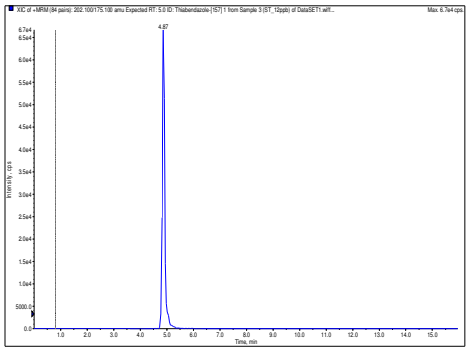


Spiromesifen 388.2/273.2 amu, RT 8.43

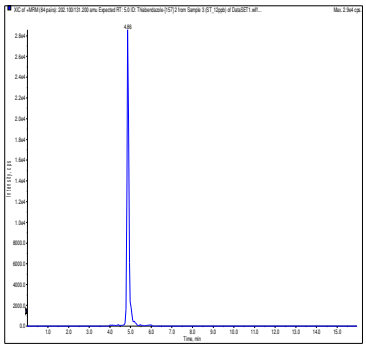


Spiromesifen 388.2/255.2 amu, RT 8.43

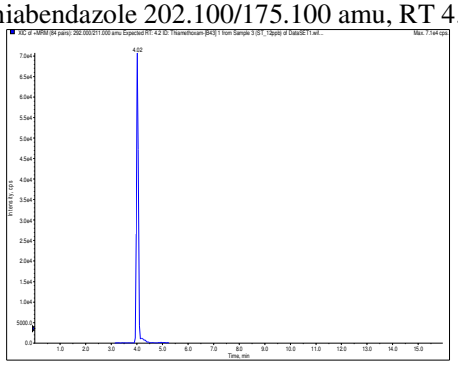
Figure-167: MRM transition of Pyridaben, Pyrimethanil, Spinosad and Spiromesifen



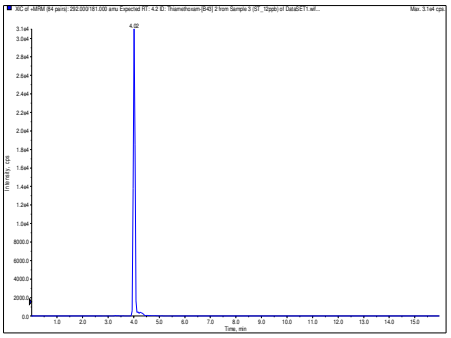
Thiabendazole 202.100/175.100 amu, RT 4.87



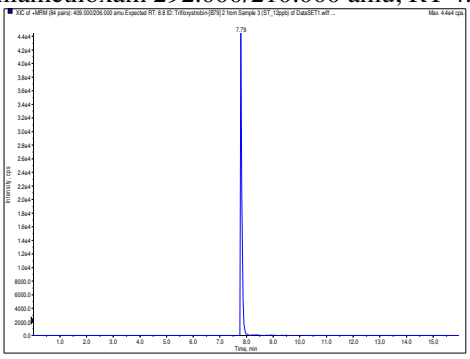
Thiabendazole 202.100/131.200 amu, RT 4.86



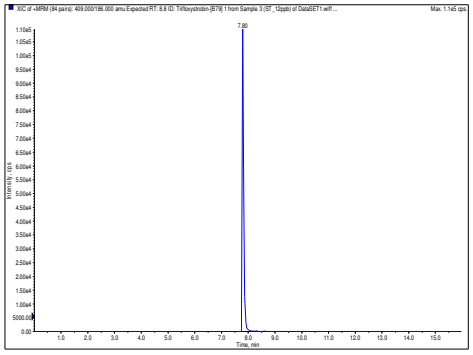
Thiamethoxam 292.000/210.000 amu, RT 4.02



Thiamethoxam 292.000/181.000 amu, RT 4.02



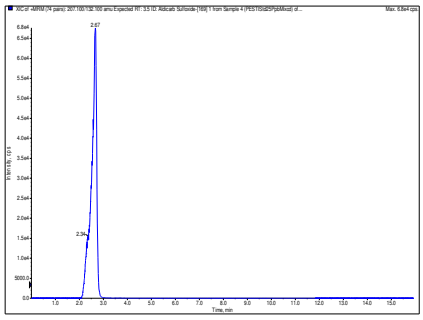
Trifloxystrobin 409.0/206.0 amu, RT 7.80



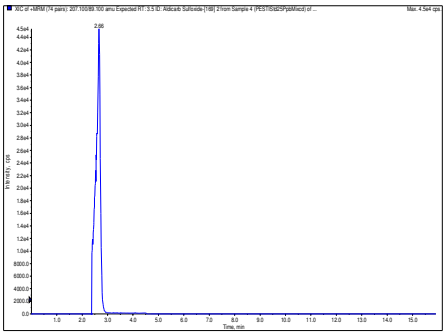
Trifloxystrobin 409.0/186.0 amu, RT 7.80

Figure-168: MRM transition of Thiabendazole, Thiamethoxam and Trifloxystrobin

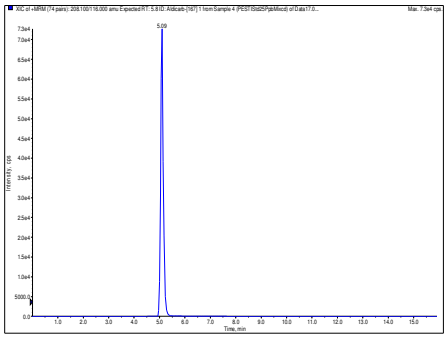
MRM Transition of Mix-2 (27 Pesticides) analyzed by LC-MS/MS



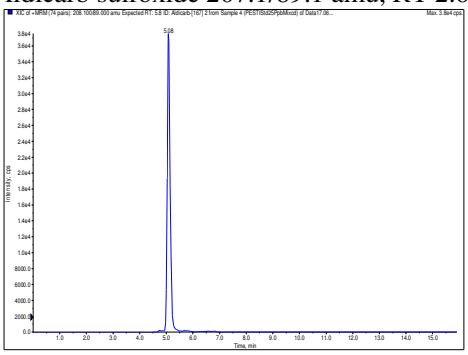
Aldicarb sulfoxide 207.1/132.1 amu, RT 2.67



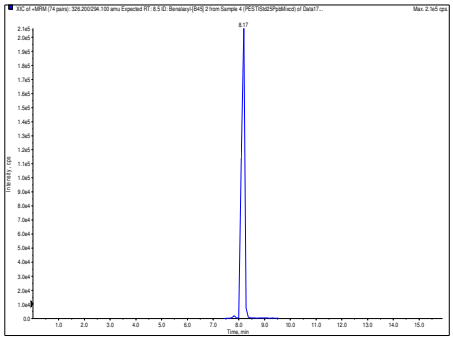
Aldicarb sulfoxide 207.1/89.1 amu, RT 2.66



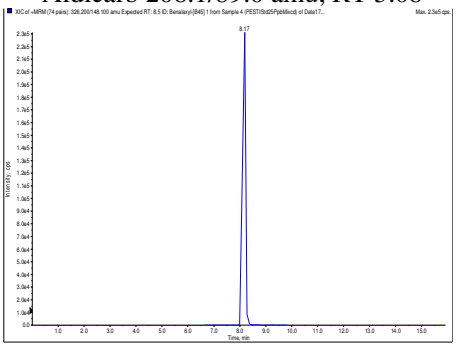
Aldicarb 208.1/116.0 amu, RT 5.09



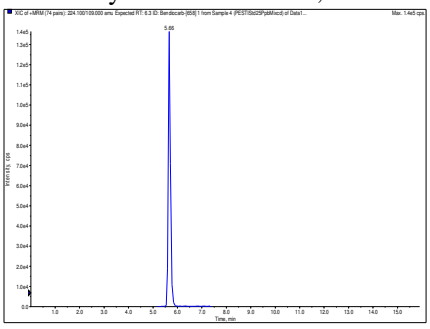
Aldicarb 208.1/89.0 amu, RT 5.08



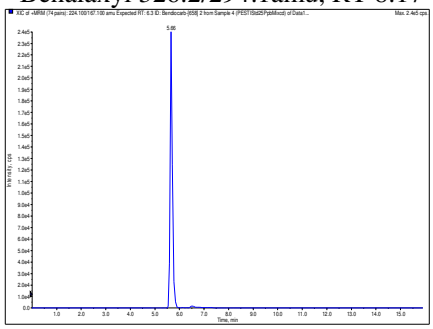
Benalaxyl 326.2/148.1amu, RT 8.17



Benalaxyl 326.2/294.1amu, RT 8.17

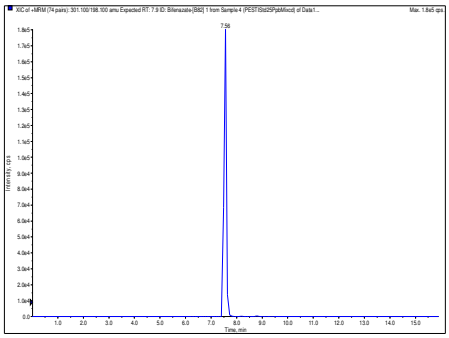


Bendiocarb 224.1/109.0 amu, RT 5.66

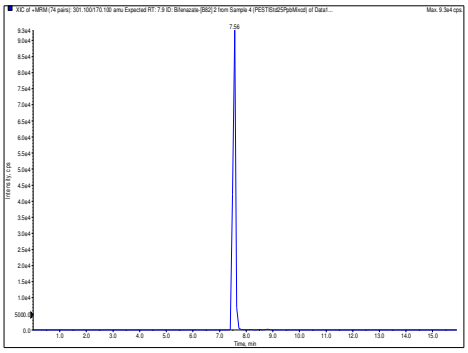


Bendiocarb 224.1/167.1 amu, RT 5.66

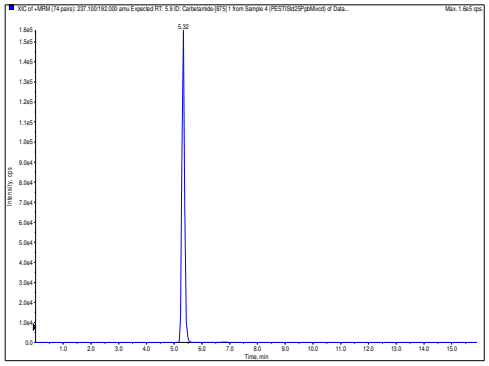
Figure-169: MRM transition of Aldicarb sulfoxide, Aldicarb, Benalaxyl and Bendiocarb



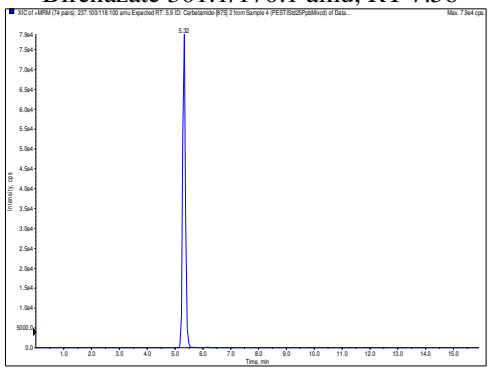
Bifenazate 301.1/198.1 amu, RT 7.56



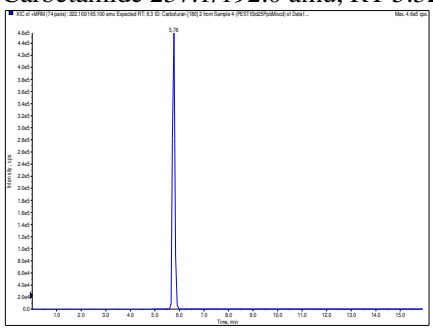
Bifenazate 301.1/170.1 amu, RT 7.56



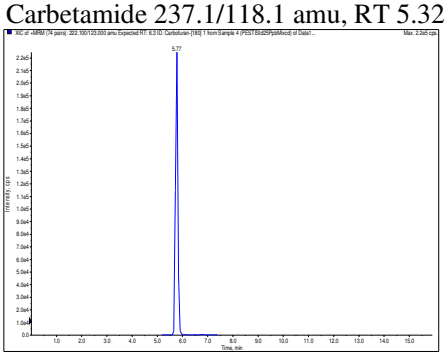
Carbetamide 237.1/192.0 amu, RT 5.32



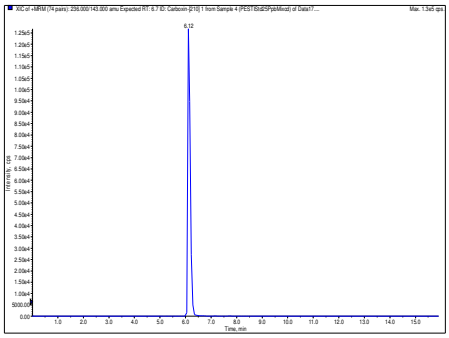
Carbetamide 237.1/118.1 amu, RT 5.32



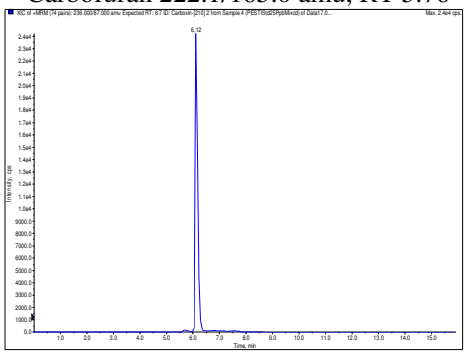
Carbofuran 222.1/123.0 amu, RT 5.77



Carbofuran 222.1/165.0 amu, RT 5.76

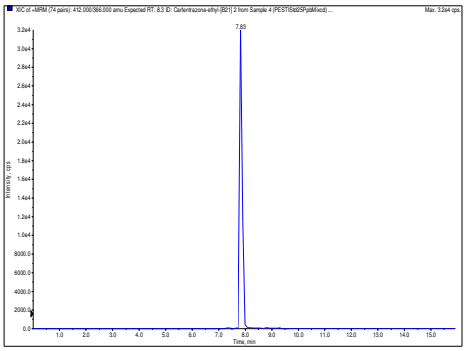


Carboxin 236.0/143.0 amu, RT 6.12

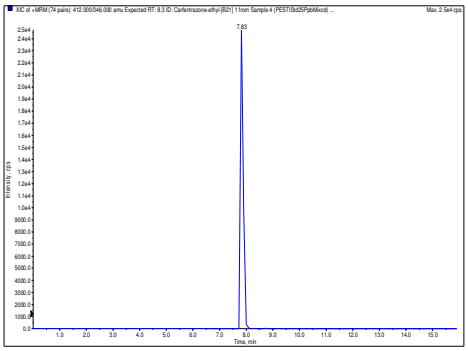


Carboxin 236.0/87.0 amu, RT 6.12

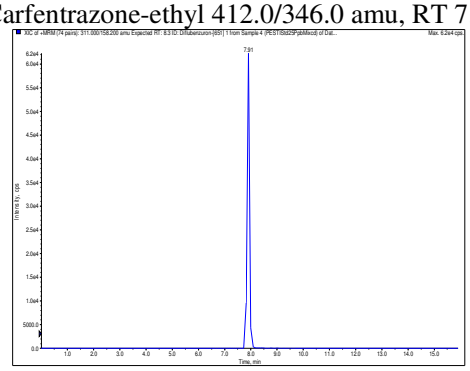
Figure-170: MRM transition of Bifenazate, Carbetamide, Carbofuran and Carboxin



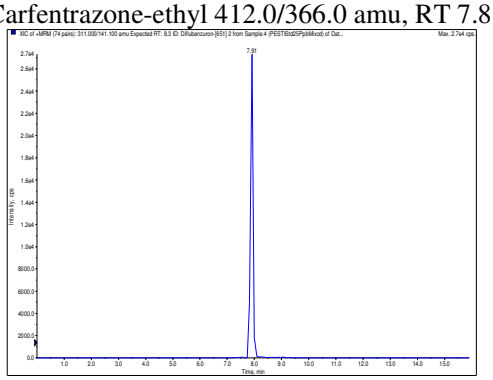
Carfentrazone-ethyl 412.0/346.0 amu, RT 7.83



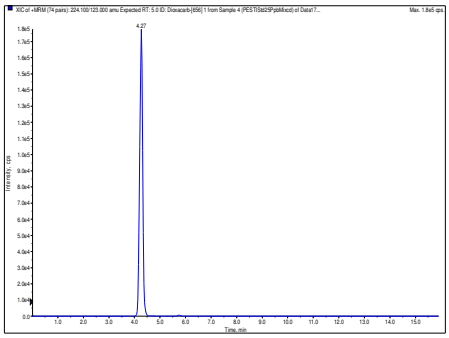
Carfentrazone-ethyl 412.0/366.0 amu, RT 7.83



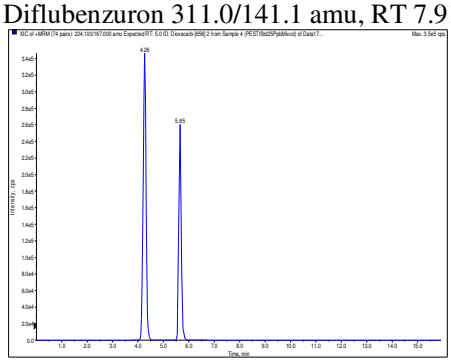
Diflubenzuron 311.0/158.2 amu, RT 7.91



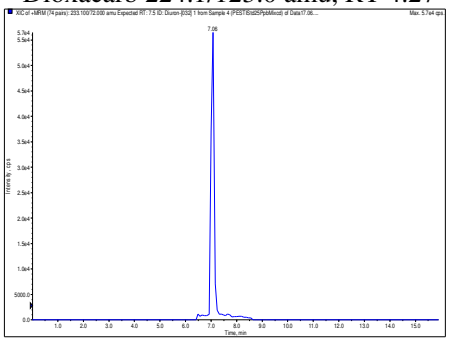
Diflubenzuron 311.0/141.1 amu, RT 7.91



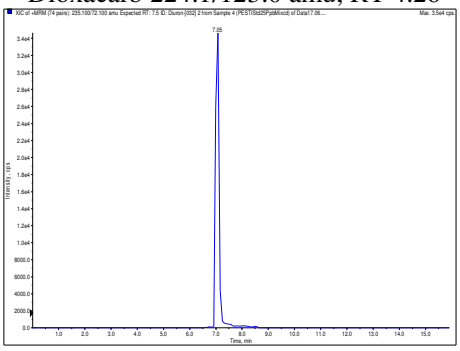
Dioxacarb 224.1/123.0 amu, RT 4.27



Dioxacarb 224.1/123.0 amu, RT 4.26

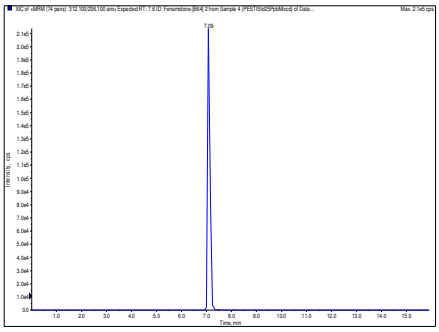


Diuron 233.1/72.0 amu, RT 7.06

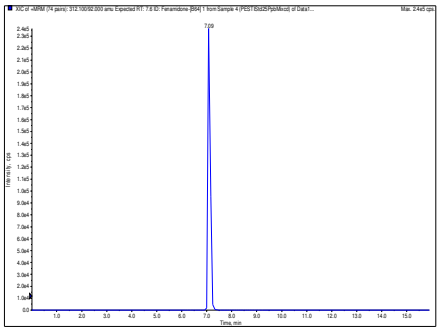


Diuron 235.1/72.0 amu, RT 7.06

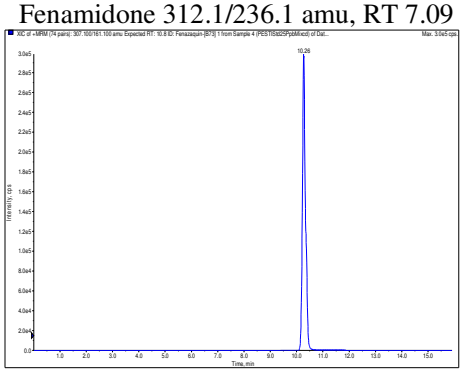
Figure-171: MRM transition of Carfentrazone-ethyl, Diflubenzuron, Dioxacarb and Diuron



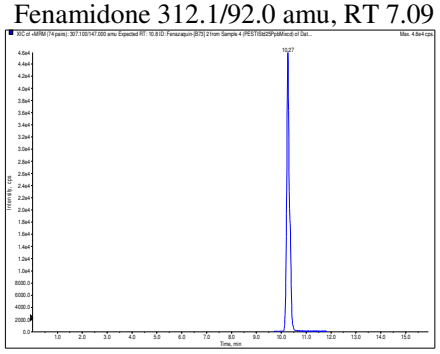
Fenamidone 312.1/236.1 amu, RT 7.09



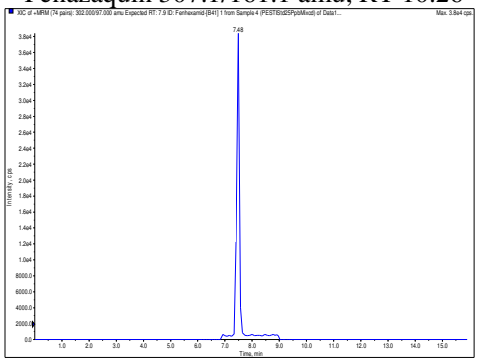
Fenamidone 312.1/92.0 amu, RT 7.09



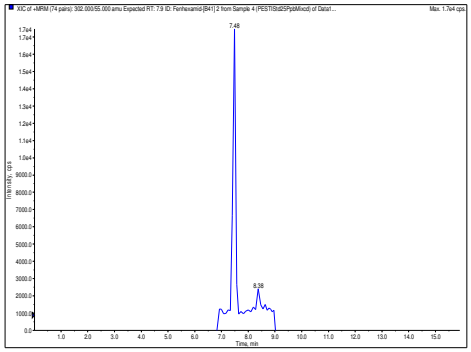
Fenazaquin 307.1/161.1 amu, RT 10.26



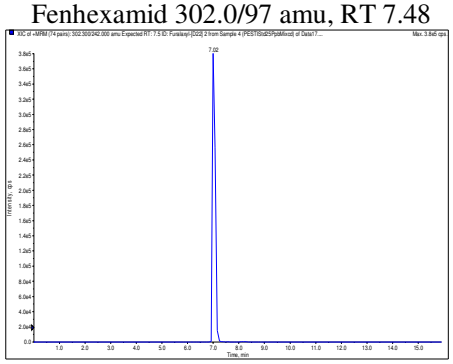
Fenazaquin 307.1/147.0 amu, RT 10.27



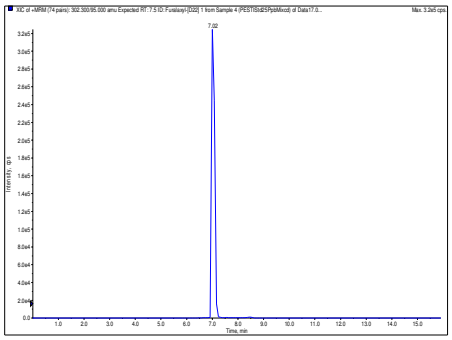
Fenhexamid 302.0/97 amu, RT 7.48



Fenhexamid 302.0/55 amu, RT 7.48

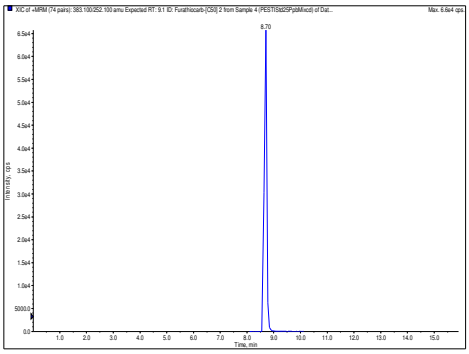


Furalaxyl 302.3/242.0 amu, RT 7.02

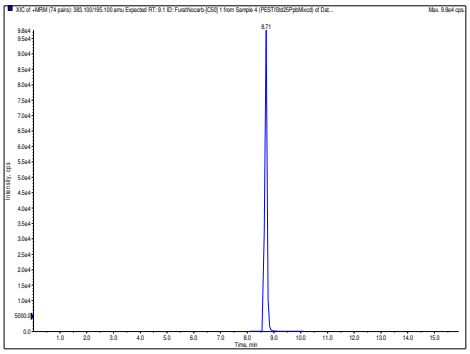


Furalaxyl 302.3/95.0 amu, RT 7.02

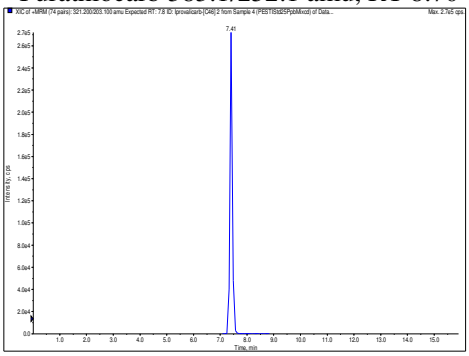
Figure-172: MRM transition of Fenamidone, Fenazaquin, Fenhexamid and Furalaxyl



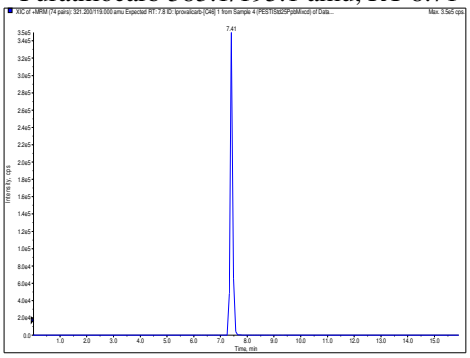
Furathiocarb 383.1/252.1 amu, RT 8.70



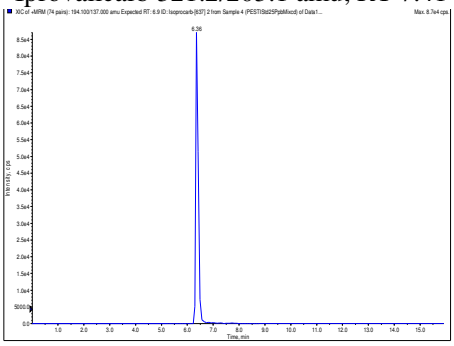
Furathiocarb 383.1/195.1 amu, RT 8.71



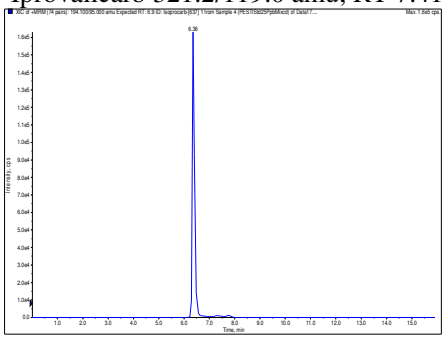
Iprovalicarb 321.2/203.1 amu, RT 7.41



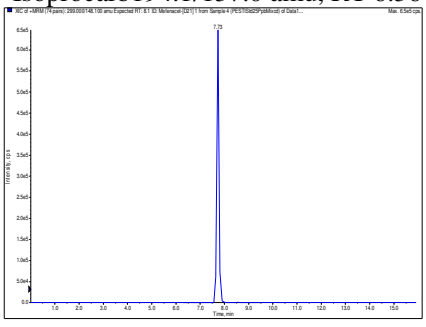
Iprovalicarb 321.2/119.0 amu, RT 7.41



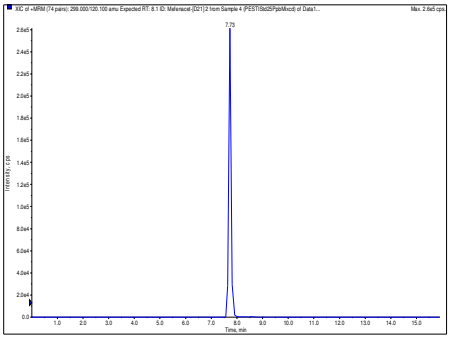
Isoprocarb194.1/137.0 amu, RT 6.36



Isoprocarb194.1/95.0 amu, RT 6.36

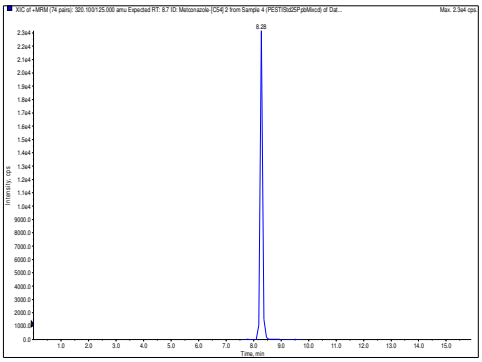


Mefenacet 299.0/148.1 amu, RT 7.73

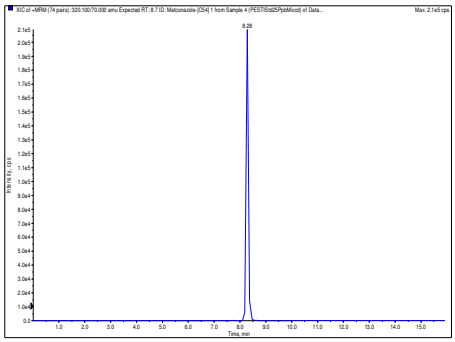


Mefenacet 299.0/120.1 amu, RT 7.73

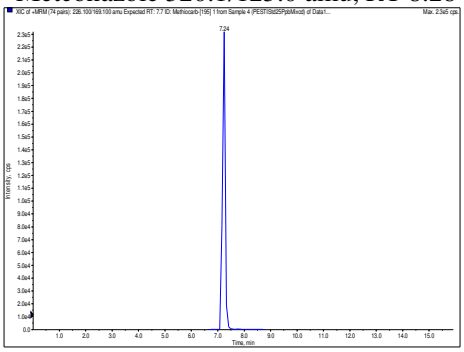
Figure-173: MRM transition of Furathiocarb, Iprovalicarb, Isoprocarb and Mefenacet



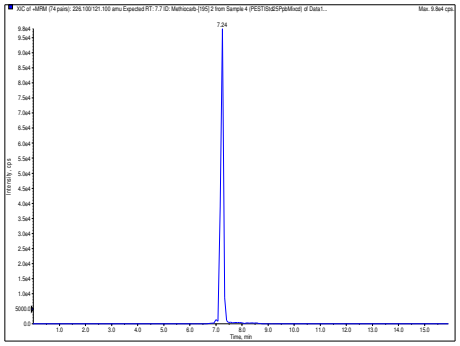
Metconazole 320.1/125.0 amu, RT 8.28



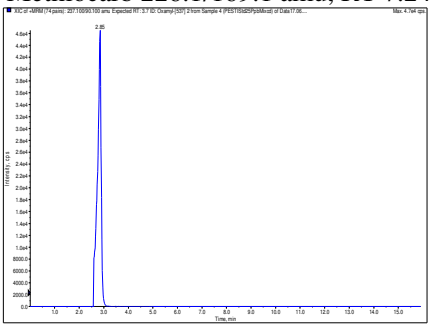
Metconazole 320.1/70.0 amu, RT 8.28



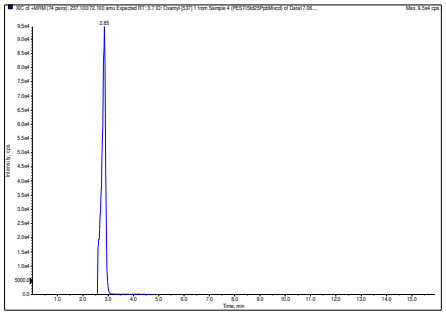
Methiocarb 226.1/169.1 amu, RT 7.24



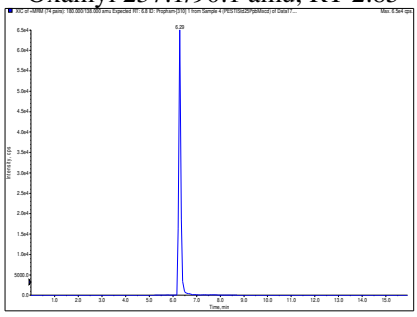
Methiocarb 226.1/121.1 amu, RT 7.24



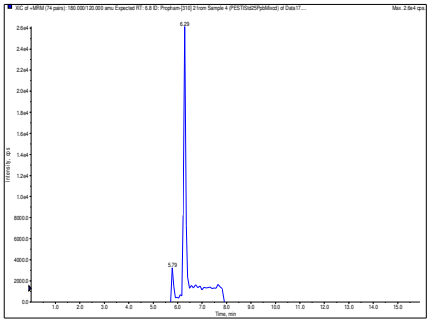
Oxamyl 237.1/90.1 amu, RT 2.85



Oxamyl 237.1/72.1 amu, RT 2.85

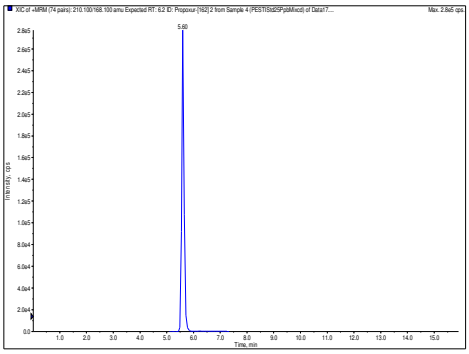


Propham 180.0/138.0 amu, RT 6.29

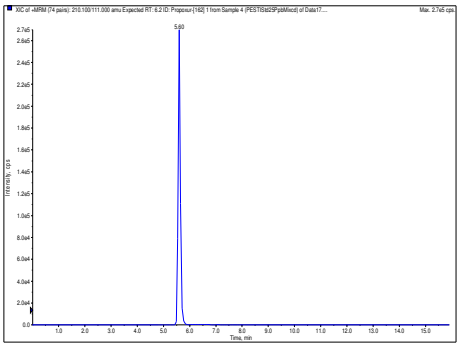


Propham 180.0/138.0 amu, RT 6.29

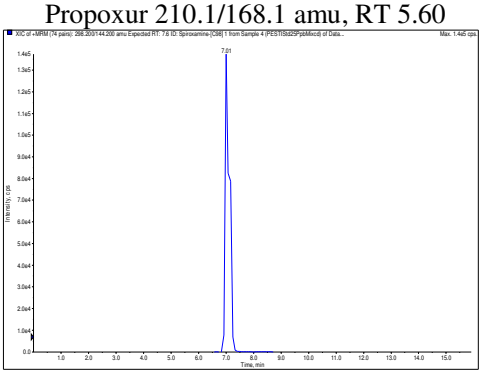
Figure-174: MRM transition of Metconazole, Methiocarb, Oxamyl and Propham



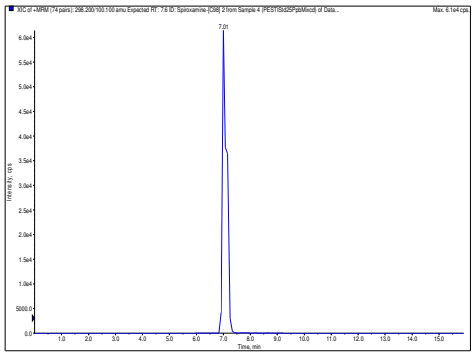
Propoxur 210.1/168.1 amu, RT 5.60



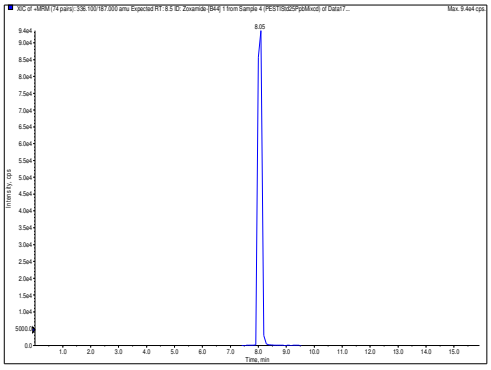
Propoxur 210.1/111.0 amu, RT 5.60



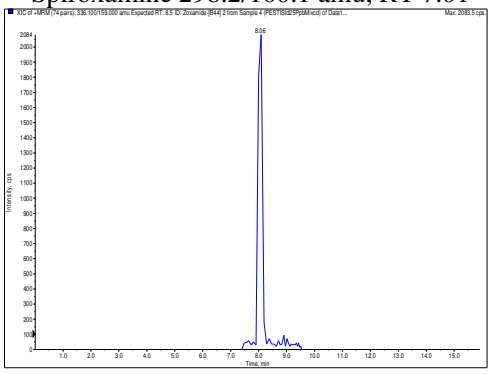
Spiroxamine 298.2/144.2 amu, RT 7.01



Spiroxamine 298.2/100.1 amu, RT 7.01



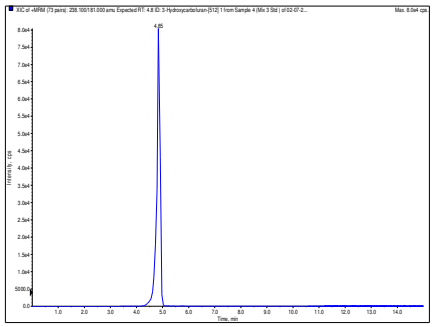
Zoxamide 336.1/187.0 amu, RT 8.05



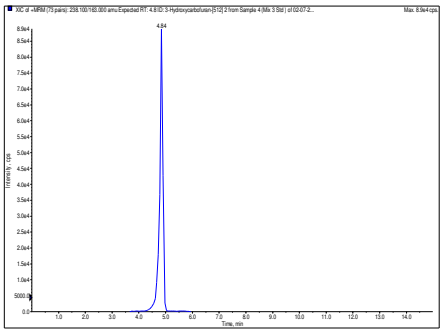
Zoxamide 336.1/159.0 amu, RT 8.06

Figure-175: MRM transition of Propoxur, Spiroxamine and Zoxamide

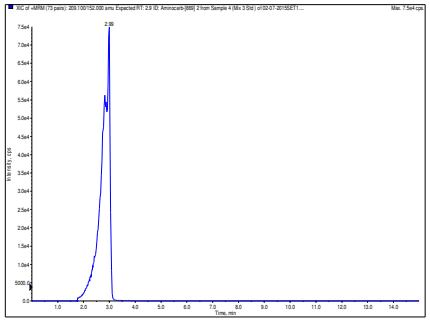
MRM Transition of Mix-3 (27 Pesticides) analyzed by LC-MS/MS



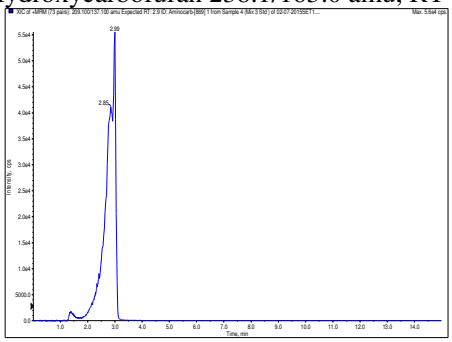
3-Hydroxycarbofuran 238.1/181.0 amu, RT 4.85



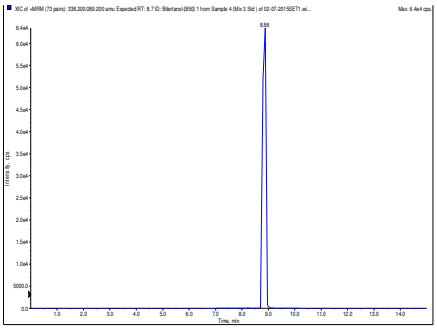
3-Hydroxycarbofuran 238.1/163.0 amu, RT 4.84



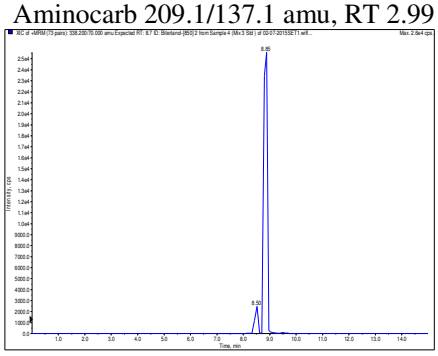
Aminocarb 209.1/152.0 amu, RT 2.99



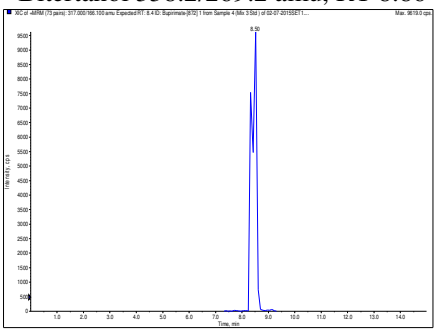
Aminocarb 209.1/137.1 amu, RT 2.99



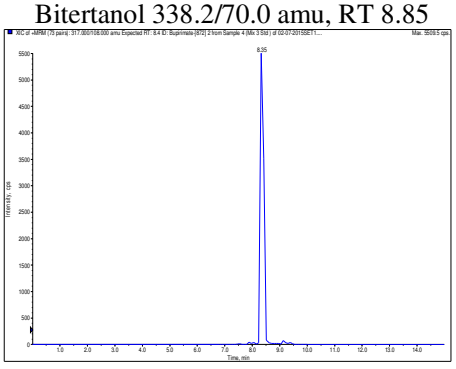
Bitertanol 338.2/269.2 amu, RT 8.86



Bitertanol 338.2/70.0 amu, RT 8.85

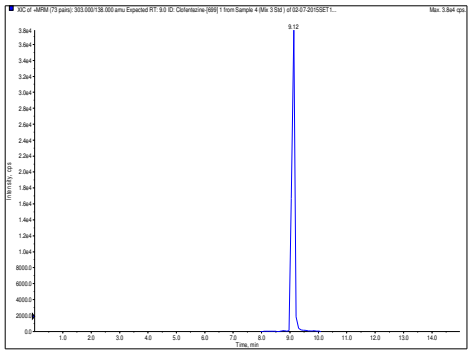


Bupirimate 317.0/166.1 amu, RT 8.50

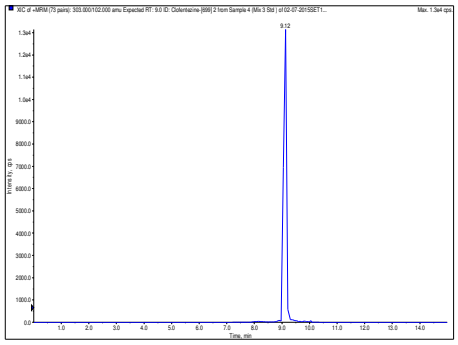


Bupirimate 317.0/108.0 amu, RT 8.35

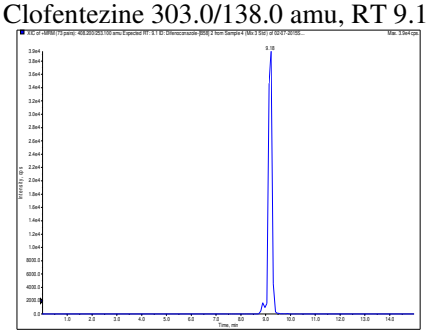
Figure-176: MRM transition of 3-Hydroxycarbofuran, Aminocarb, Bitertanol and Bupirimate



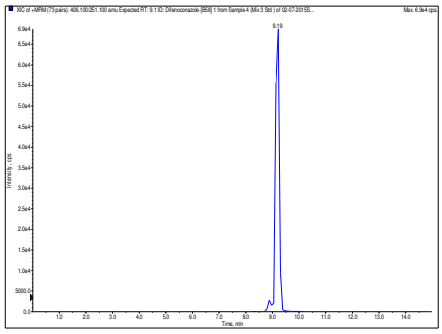
Clofentezine 303.0/138.0 amu, RT 9.12



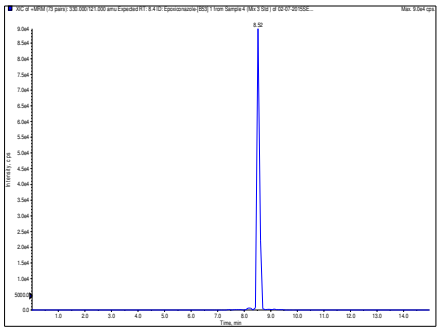
Clofentezine 303.0/102.0 amu, RT 9.12



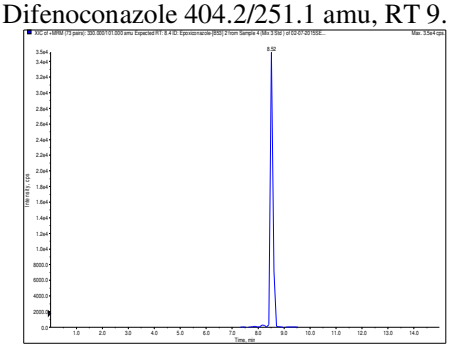
Difenoconazole 404.2/253.1 amu, RT 9.18



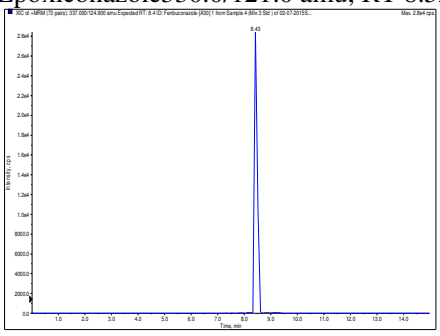
Difenoconazole 404.2/251.1 amu, RT 9.19



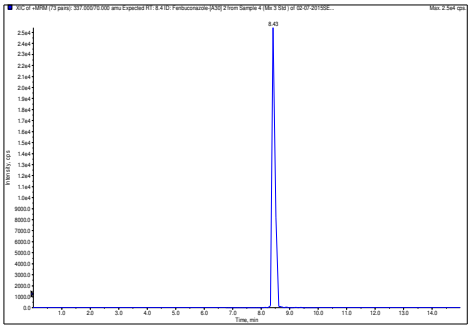
Epoxiconazole330.0/121.0 amu, RT 8.52



Epoxiconazole330.0/101.0 amu, RT 8.52

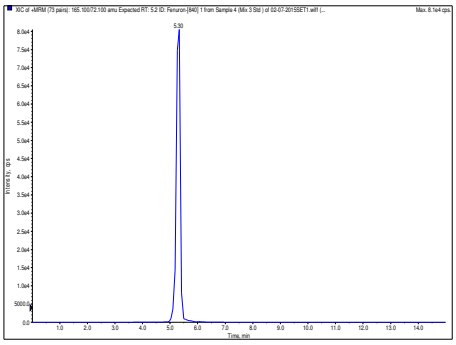


Fenbuconazole 337.0/124.9 amu, RT 8.43

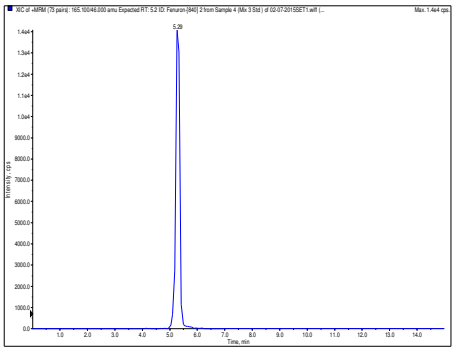


Fenbuconazole 337.0/70.0 amu, RT 8.43

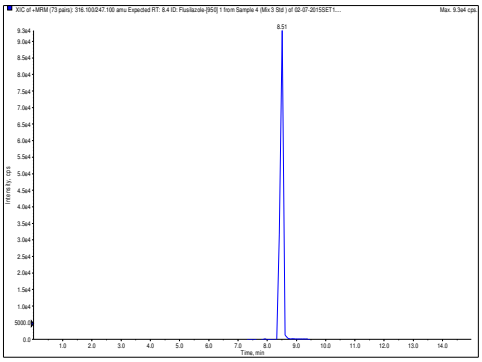
Figure-177: MRM transition of Clofentezine, Difenoconazole, Epoxiconazole and Fenbuconazole



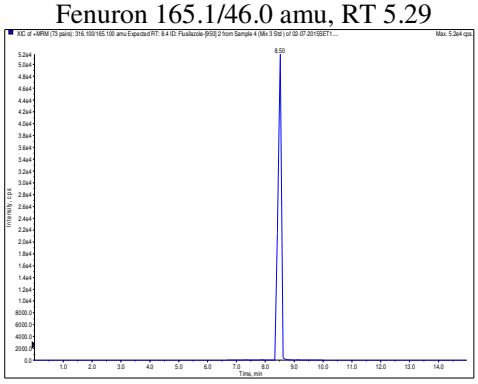
Fenuron 165.1/72.1 amu, RT 5.30



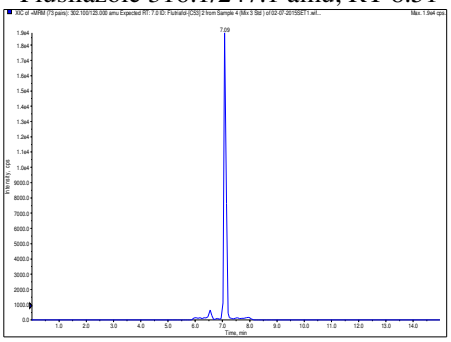
Fenuron 165.1/46.0 amu, RT 5.29



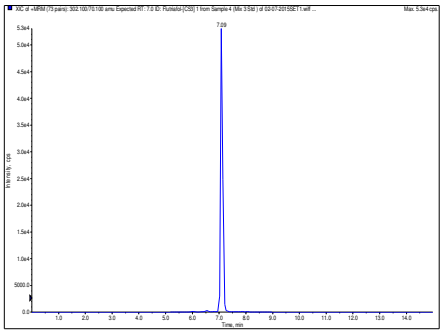
Flusilazole 316.1/247.1 amu, RT 8.51



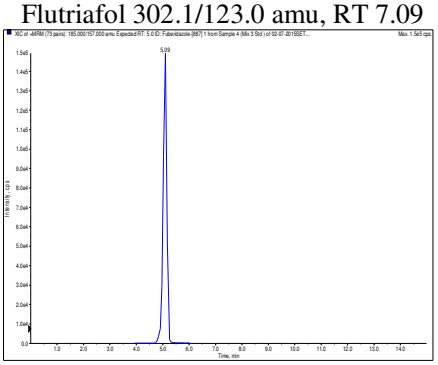
Flusilazole 316.1/165.1 amu, RT 8.50



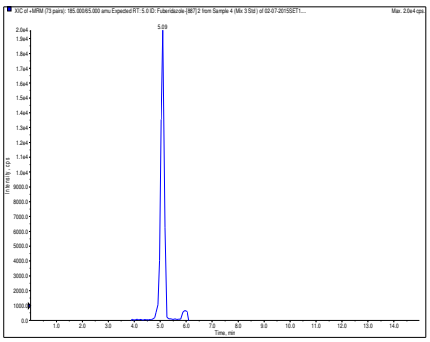
Flutriafol 302.1/123.0 amu, RT 7.09



Flutriafol 302.1/123.0 amu, RT 7.09

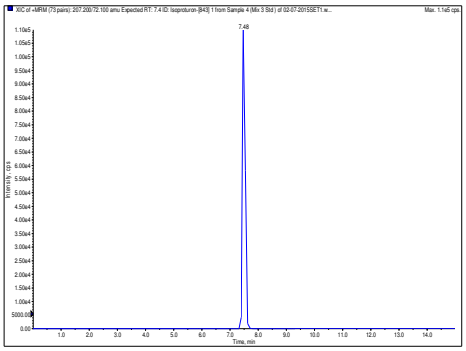


Fuberidazole 185.0/157.0 amu, RT 5.09

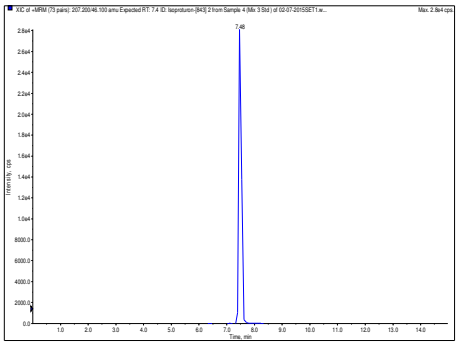


Fuberidazole 185.0/65.0 amu, RT 5.09

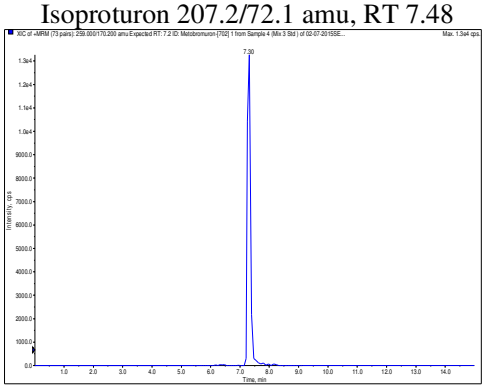
Figure-178: MRM transition of Fenuron, Flusilazole, Flutriafol and Fuberidazole



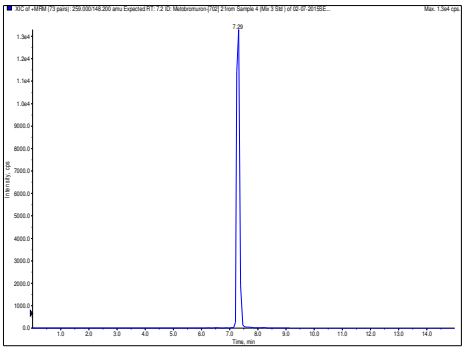
Isoproturon 207.2/72.1 amu, RT 7.48



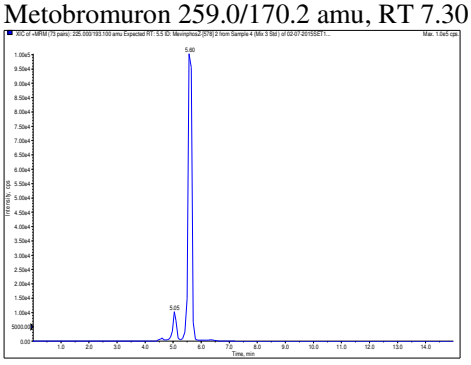
Isoproturon 207.2/46.1 amu, RT 7.48



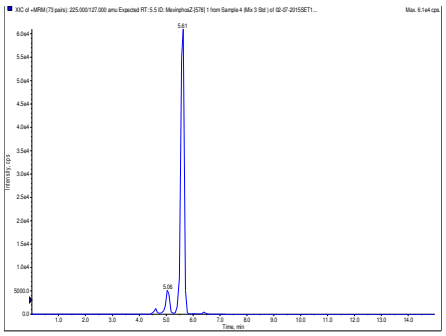
Metobromuron 259.0/170.2 amu, RT 7.30



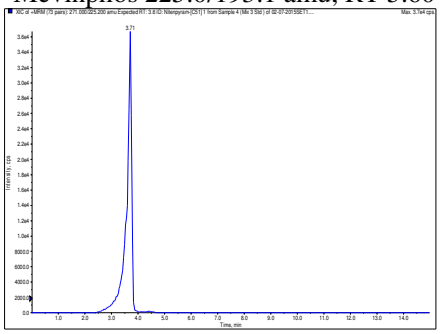
Metobromuron 259.0/148.2 amu, RT 7.29



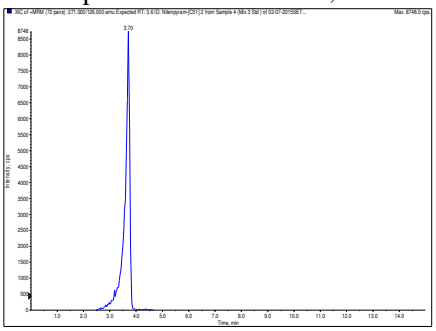
Mevinphos 225.0/193.1 amu, RT 5.60



Mevinphos 225.0/127.0 amu, RT 5.61

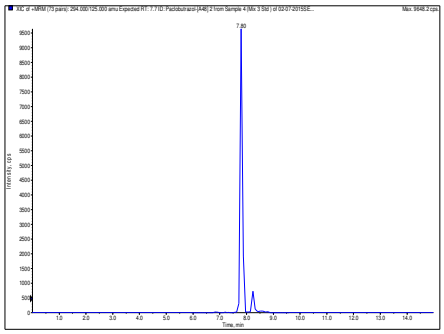


Nitenpyram 271.0/225.2 amu, RT 3.71

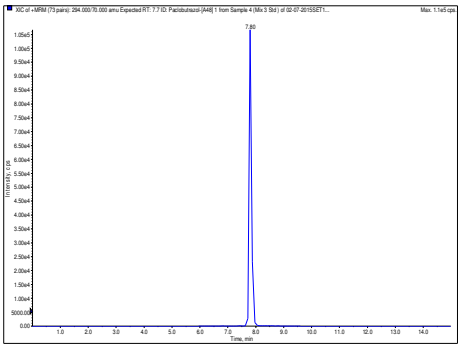


Nitenpyram 271.0/126.0 amu, RT 3.70

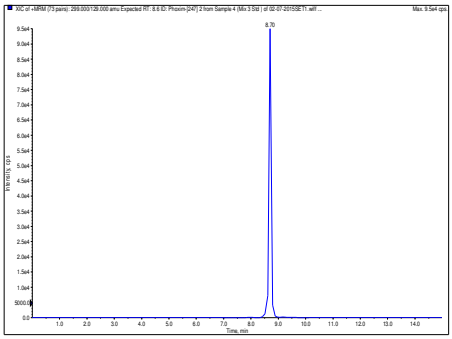
Figure-179: MRM transition of Isoproturon, Metobromuron, Mevinphos and Nitenpyram



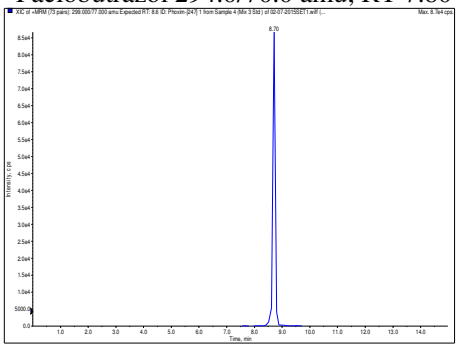
Paclobutrazol 294.0/125.0 amu, RT 7.80



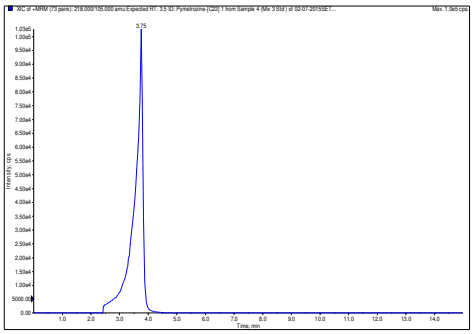
Paclobutrazol 294.0/70.0 amu, RT 7.80



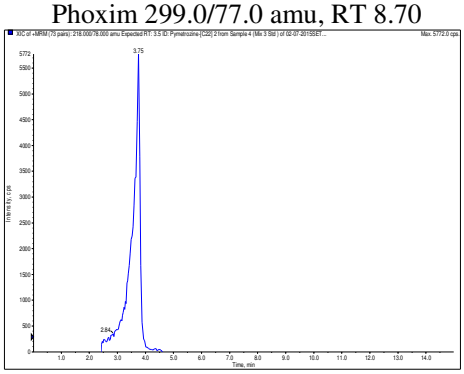
Phoxim 299.0/119.0 amu, RT 8.70



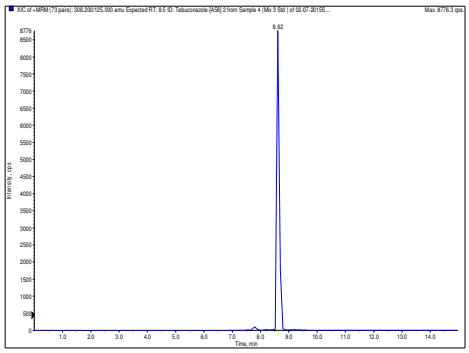
Phoxim 299.0/77.0 amu, RT 8.70



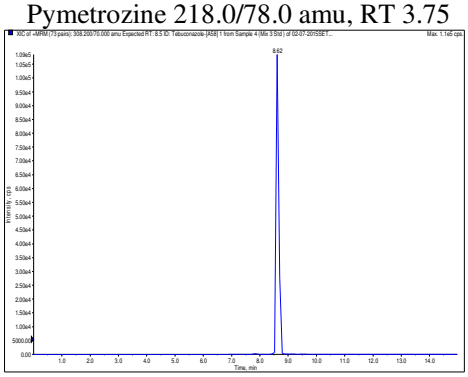
Pymetrozine 218.0/105.0 amu, RT 3.75



Pymetrozine 218.0/78.0 amu, RT 3.75

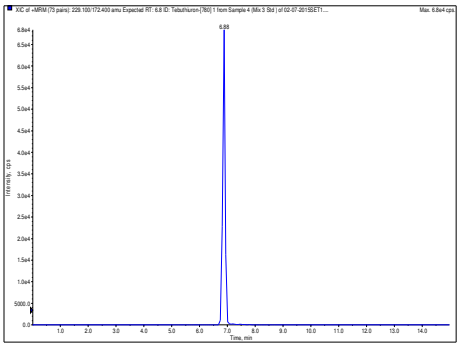


Tebuconazole 308.2/125.0 amu, RT 8.62

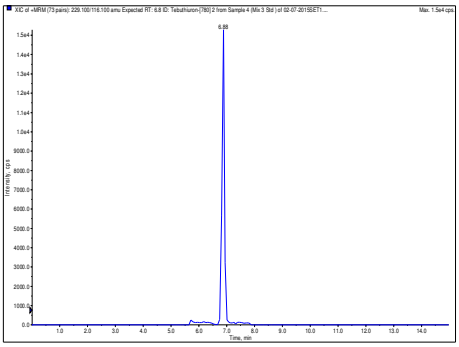


Tebuconazole 308.2/70.0 amu, RT 8.62

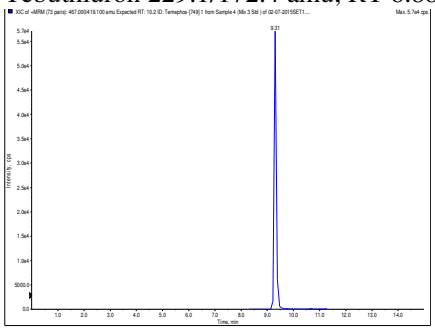
Figure-180: MRM transition of Paclobutrazol, Phoxim, Pymetrozine and Tebuconazole



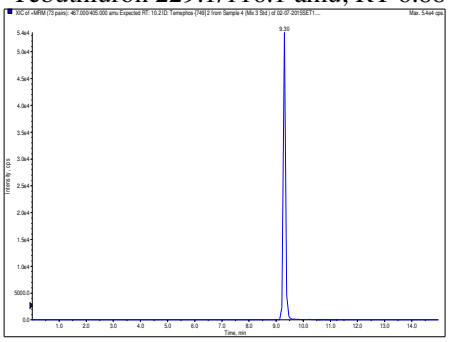
Tebuthiuron 229.1/172.4 amu, RT 6.88



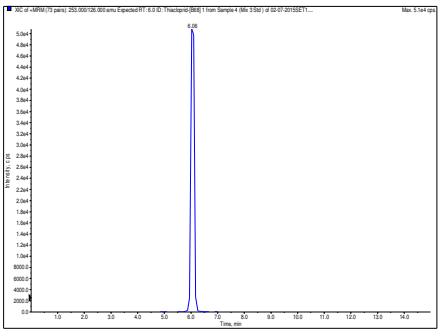
Tebuthiuron 229.1/116.1 amu, RT 6.88



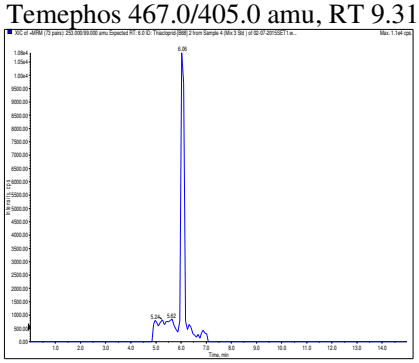
Temephos 467.0/419.1 amu, RT 9.31



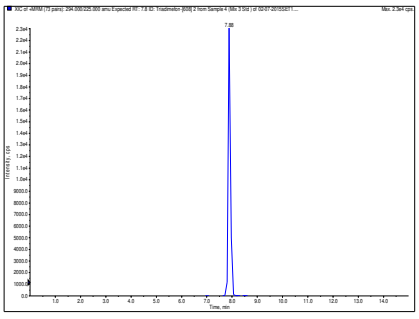
Temephos 467.0/405.0 amu, RT 9.31



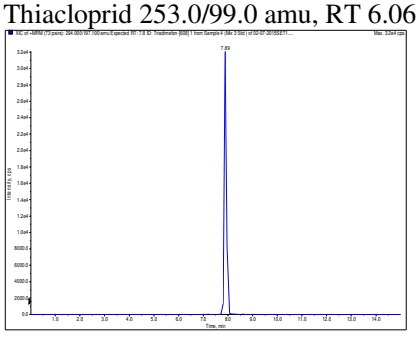
Thiacloprid 253.0/126.0 amu, RT 6.06



Thiacloprid 253.0/99.0 amu, RT 6.06

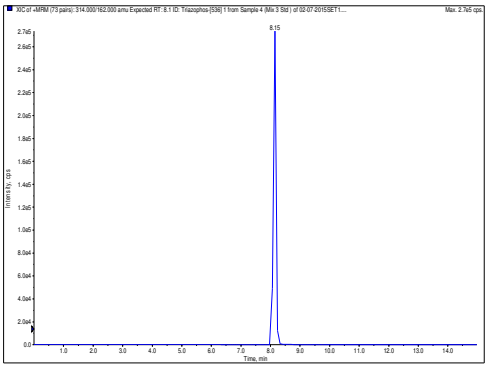


Triadimefon 294.0/225.0 amu, RT 7.88

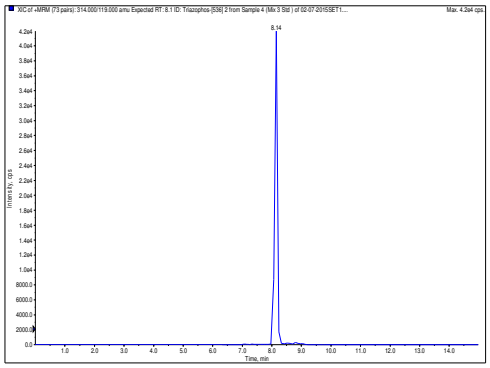


Triadimefon 294.0/197.1 amu, RT 7.89

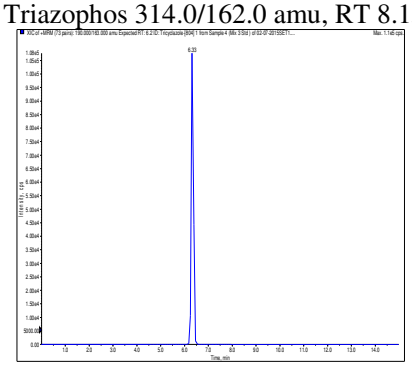
Figure-181: MRM transition of Tebuthiuron, Temephos, Thiacloprid and Triadimefon



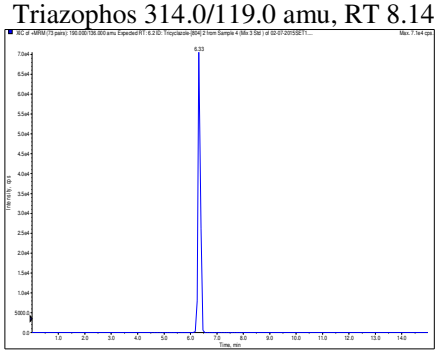
Triazophos 314.0/162.0 amu, RT 8.15



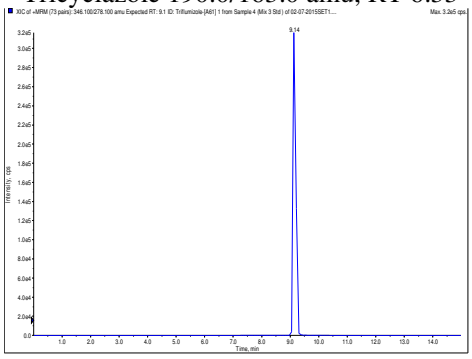
Triazophos 314.0/119.0 amu, RT 8.14



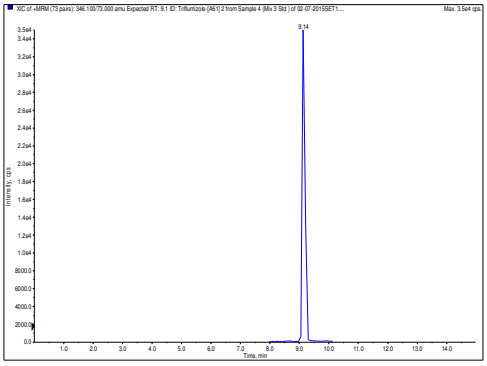
Tricyclazole 190.0/163.0 amu, RT 6.33



Tricyclazole 190.0/136.0 amu, RT 6.33



Triflumizole 346.1/278.1 amu, RT 9.14



Triflumizole 346.1/73.1 amu, RT 9.14

Figure-182: MRM transition of Triazophos, Tricyclazole and Triflumizole

Absorption spectrum of arsenic and cadmium detected sample of fruits and vegetables

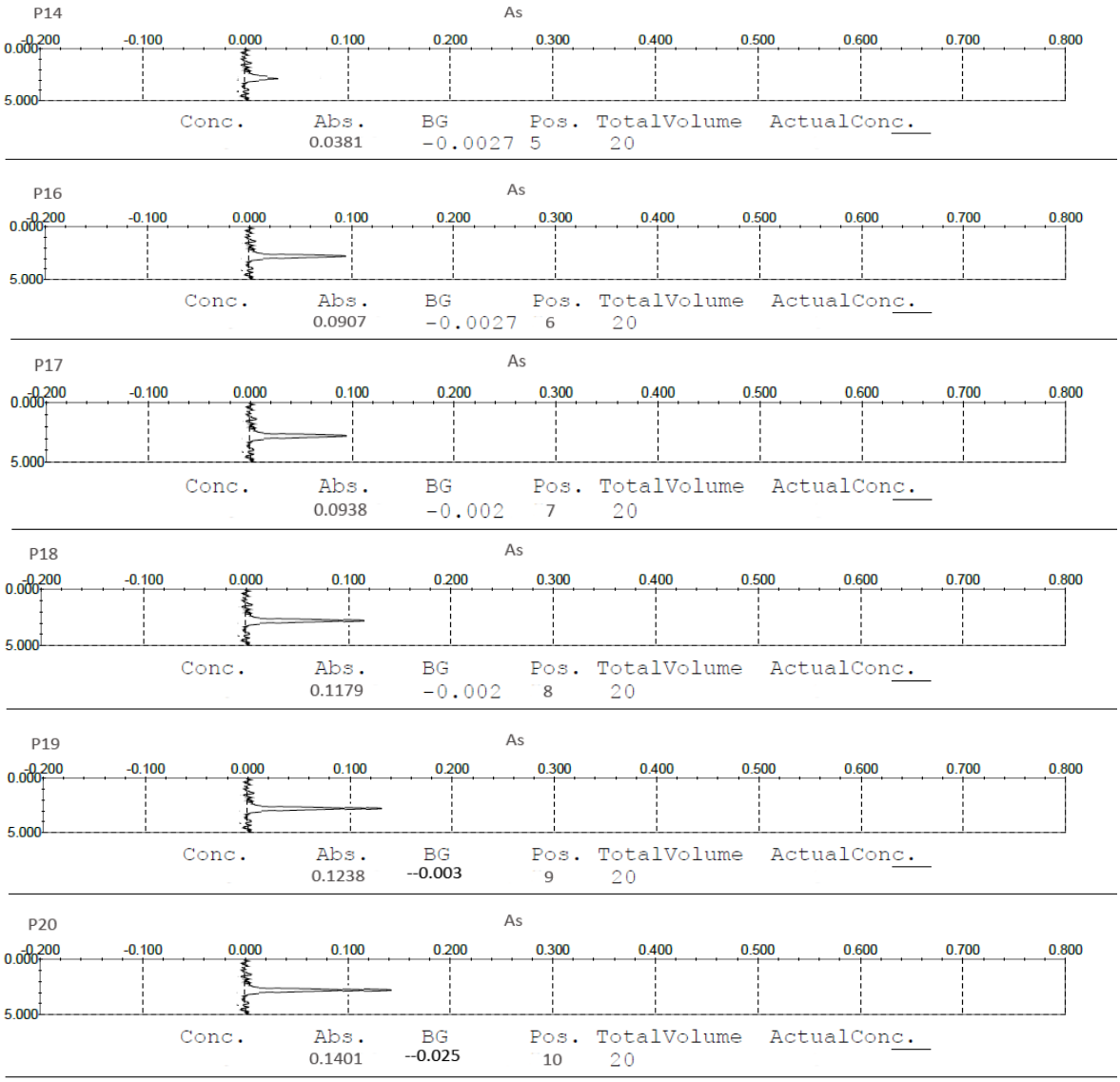


Figure-183: Absorption spectrum of Sample ID P14, P16, P17, P18, P19, P20 for Arsenic

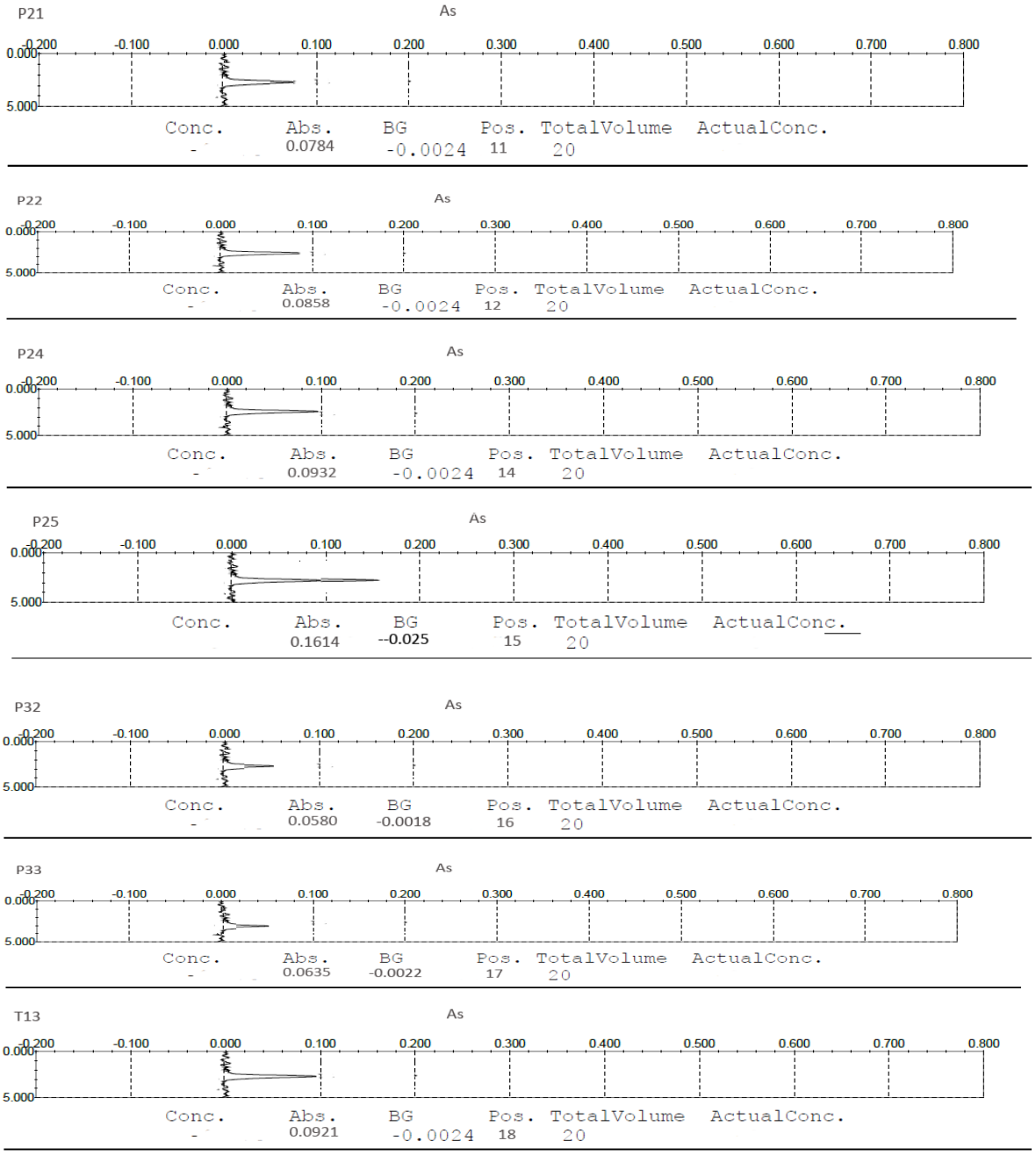


Figure-184: Absorption spectrum of Sample ID P21, P22, P24, P25, P32, P33, T13 for Arsenic

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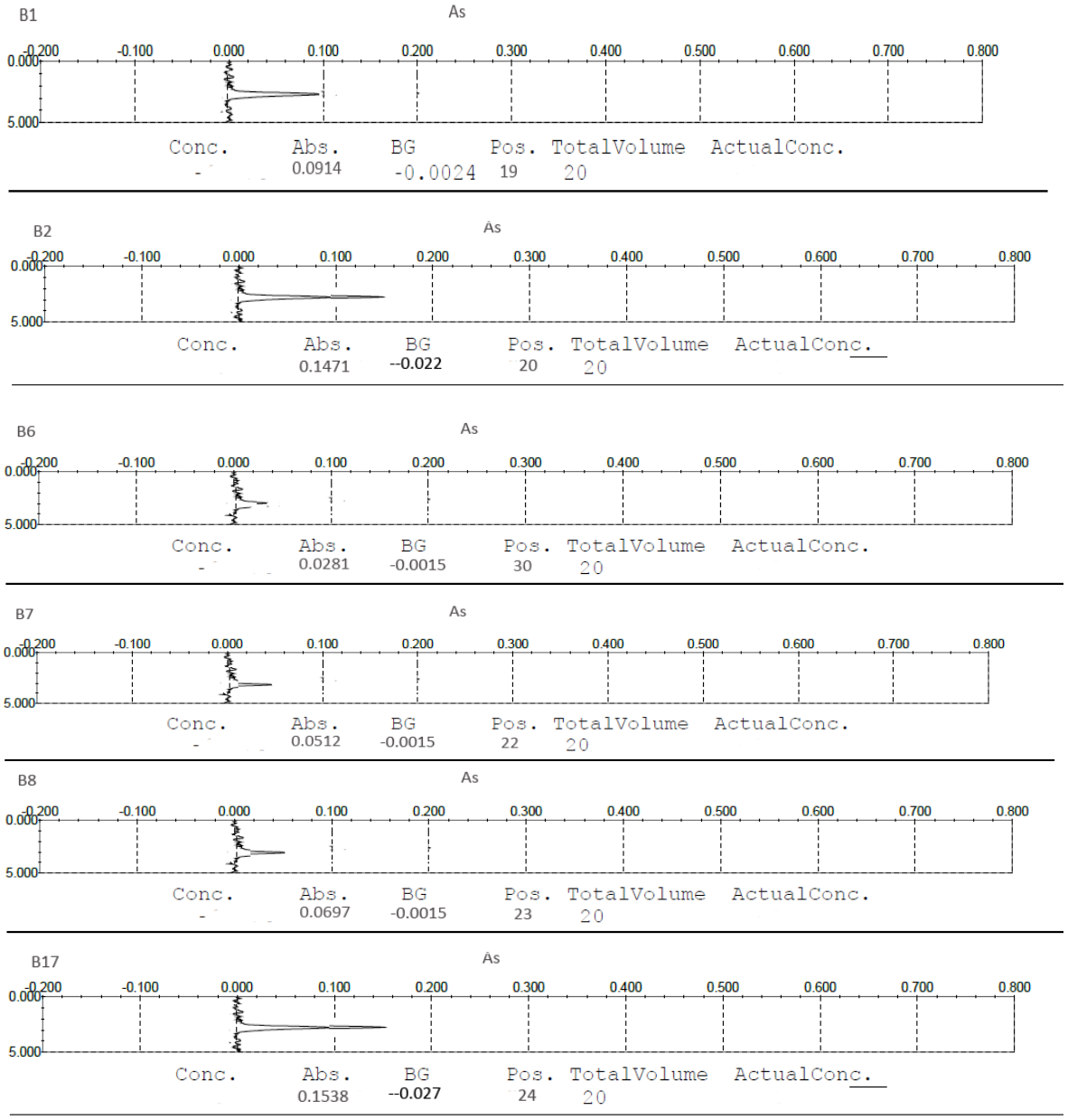

Figure-185: Absorption spectrum of Sample ID B1, B2, B6, B7, B8, B17 for Arsenic

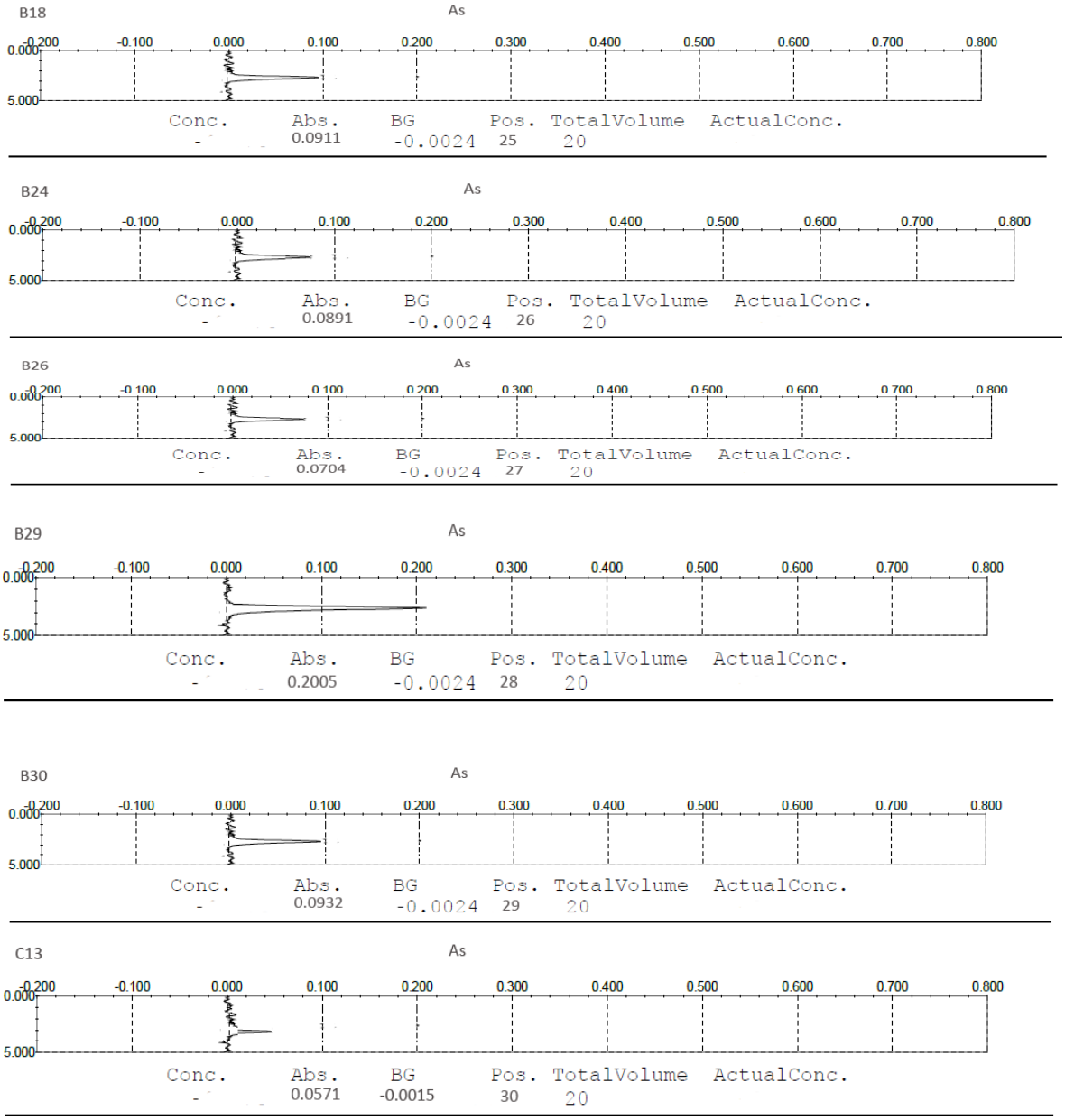

Figure-186: Absorption spectrum of Sample ID B18, B24, B26, B29, B30, C13 for Arsenic

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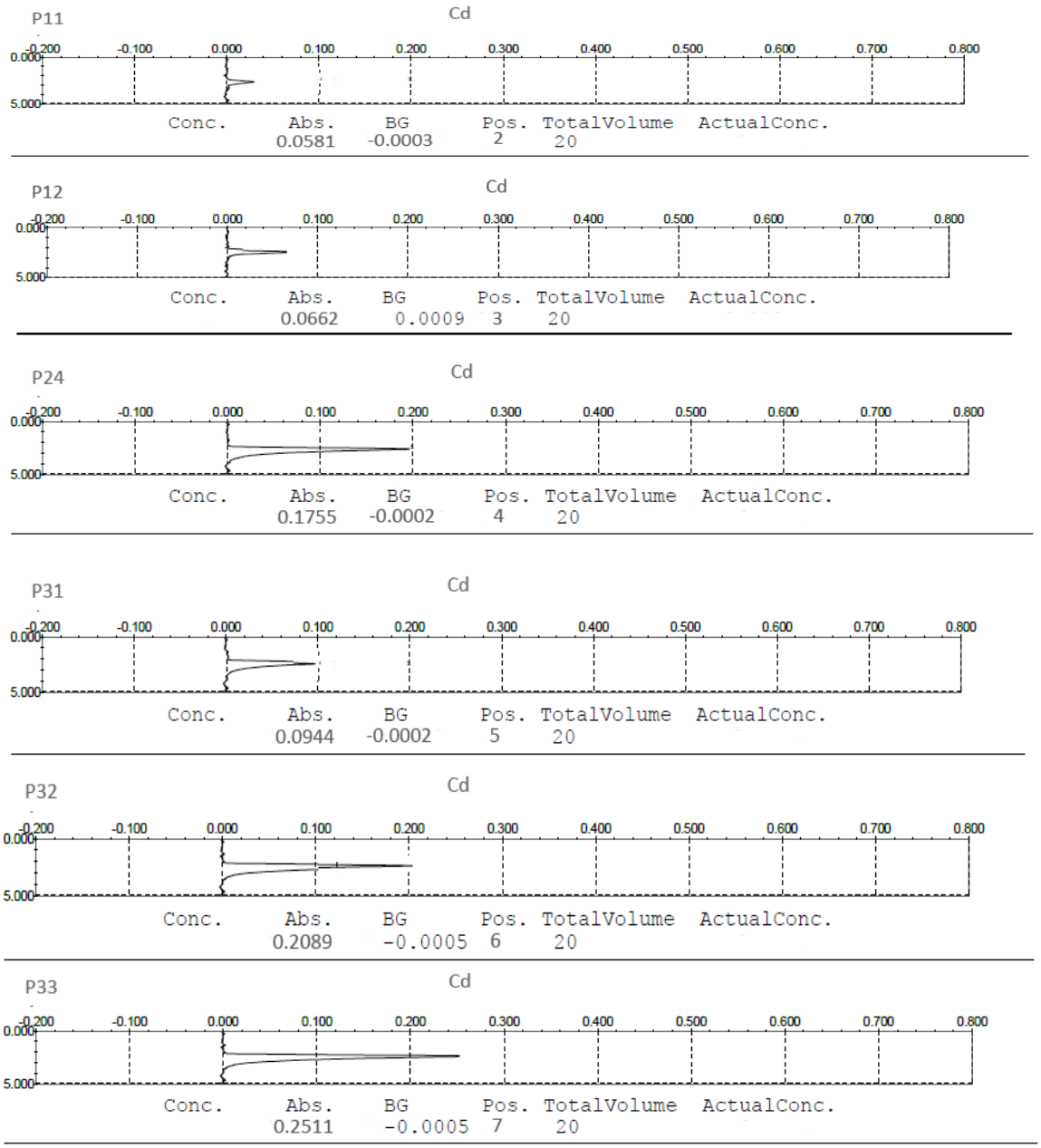


Figure-187: Absorption spectrum of Sample ID P11, P12, P24, P31, P32, P33 for Cadmium

Absorption spectrum of lead and chromium detected sample of Turmeric Powder

For Lead (Pb)

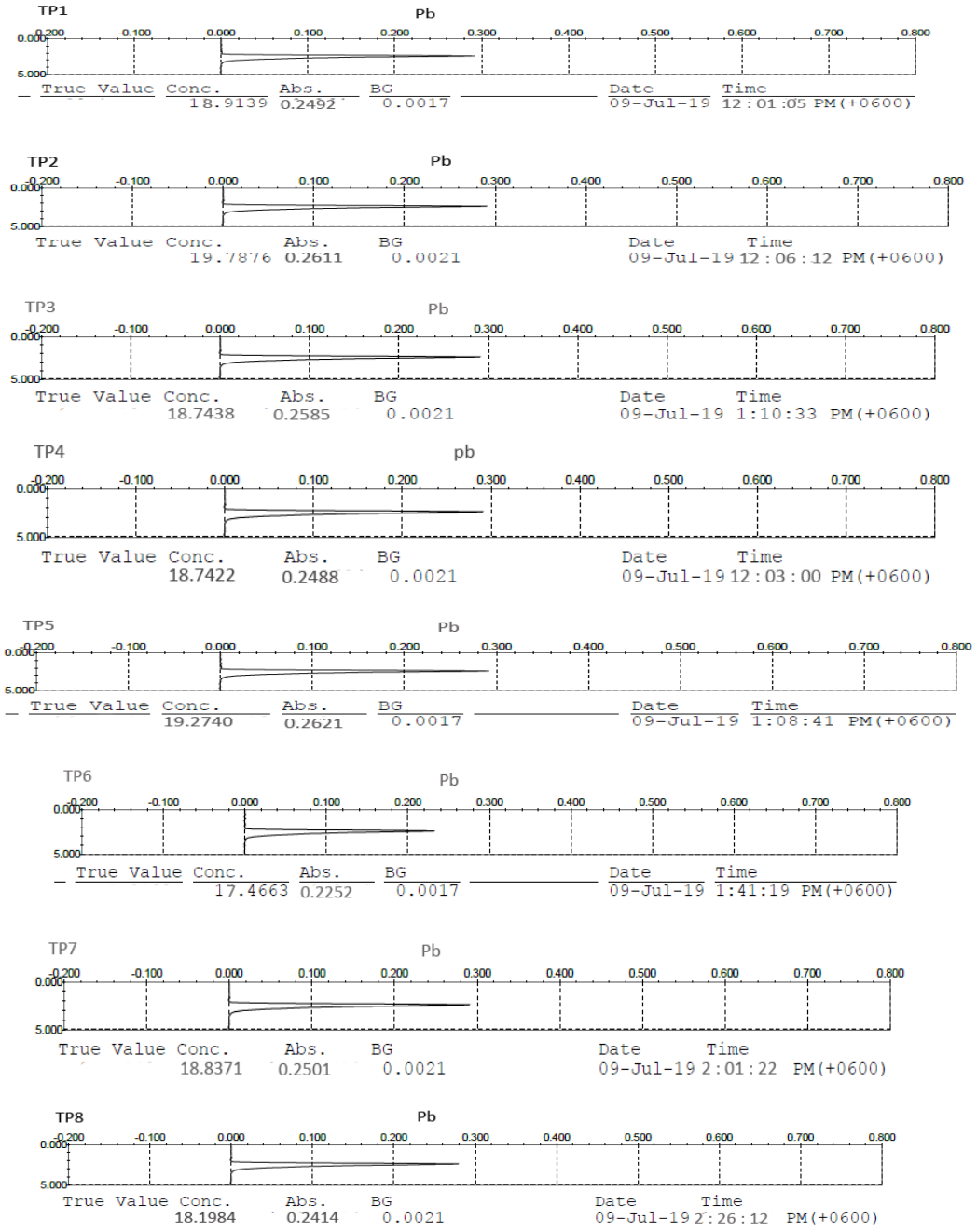


Figure-188: Absorption spectrum of Sample TP1, TP2, TP3, TP4, TP5, TP6, TP7 and TP8 for Lead

For Chromium (Cr)

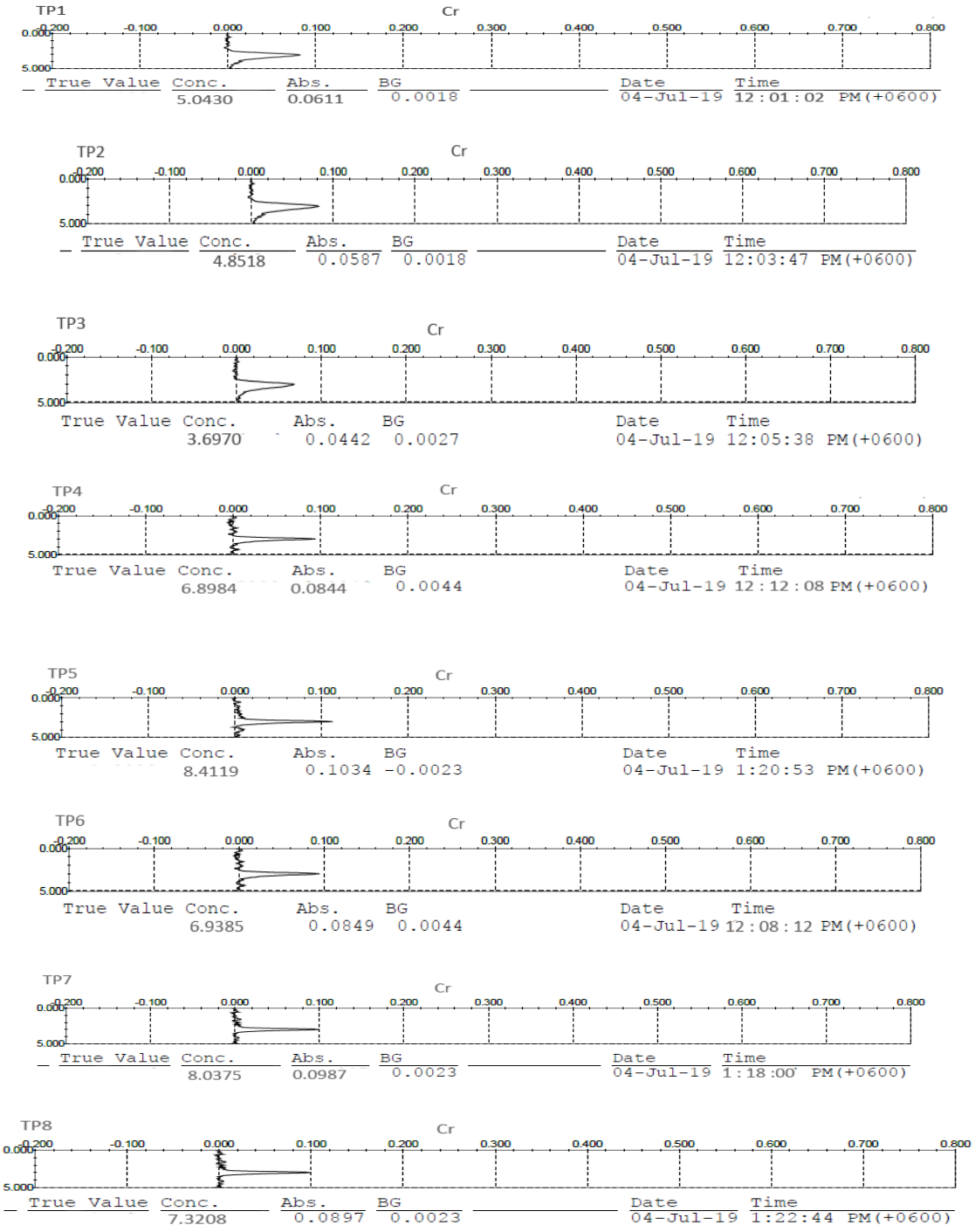
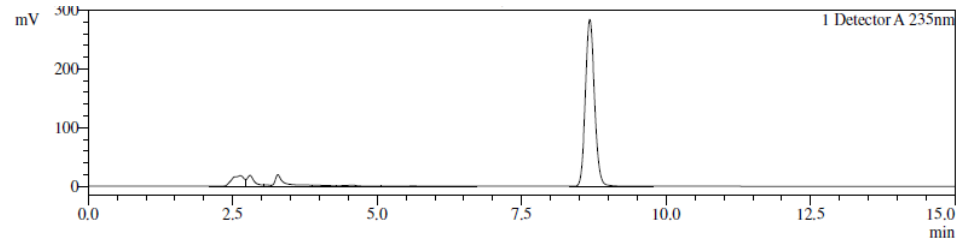
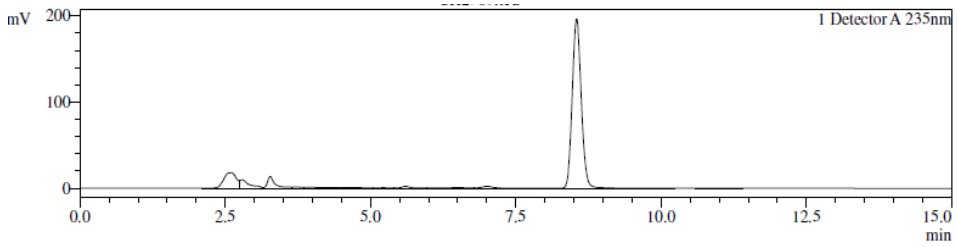


Figure-189: Absorption spectrum of Sample TP1, TP2, TP3, TP4, TP5, TP6, TP7 and TP8 for Chromium

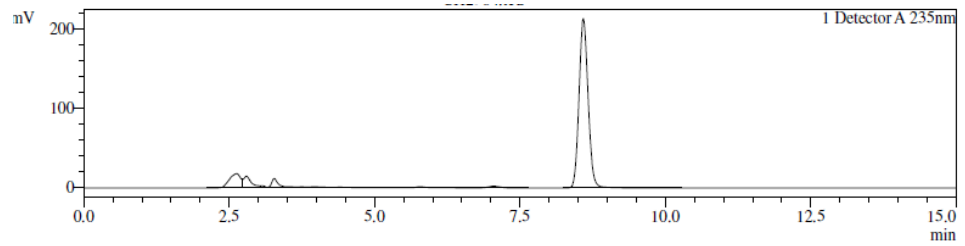
Chromatogram of benzoic acid detected sample of fruit drink and tomato ketchup



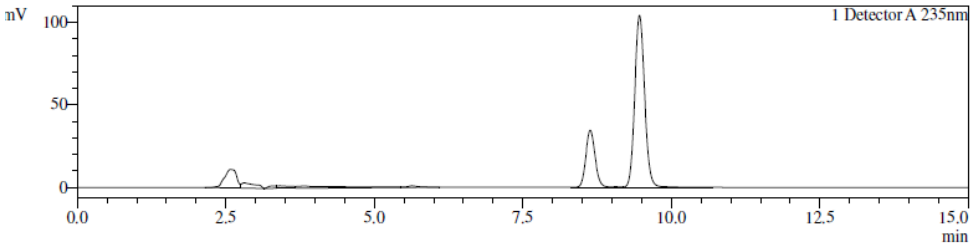
(a)



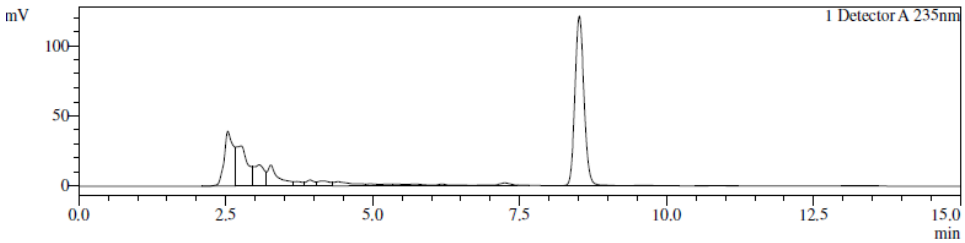
(b)



(c)



(d)



(e)

Figure-190: Chromatograms of fruit drink sample (a) FD 01 , (b) FD 02, (c) FD 03, (d) FD 04 and (e) FD 05

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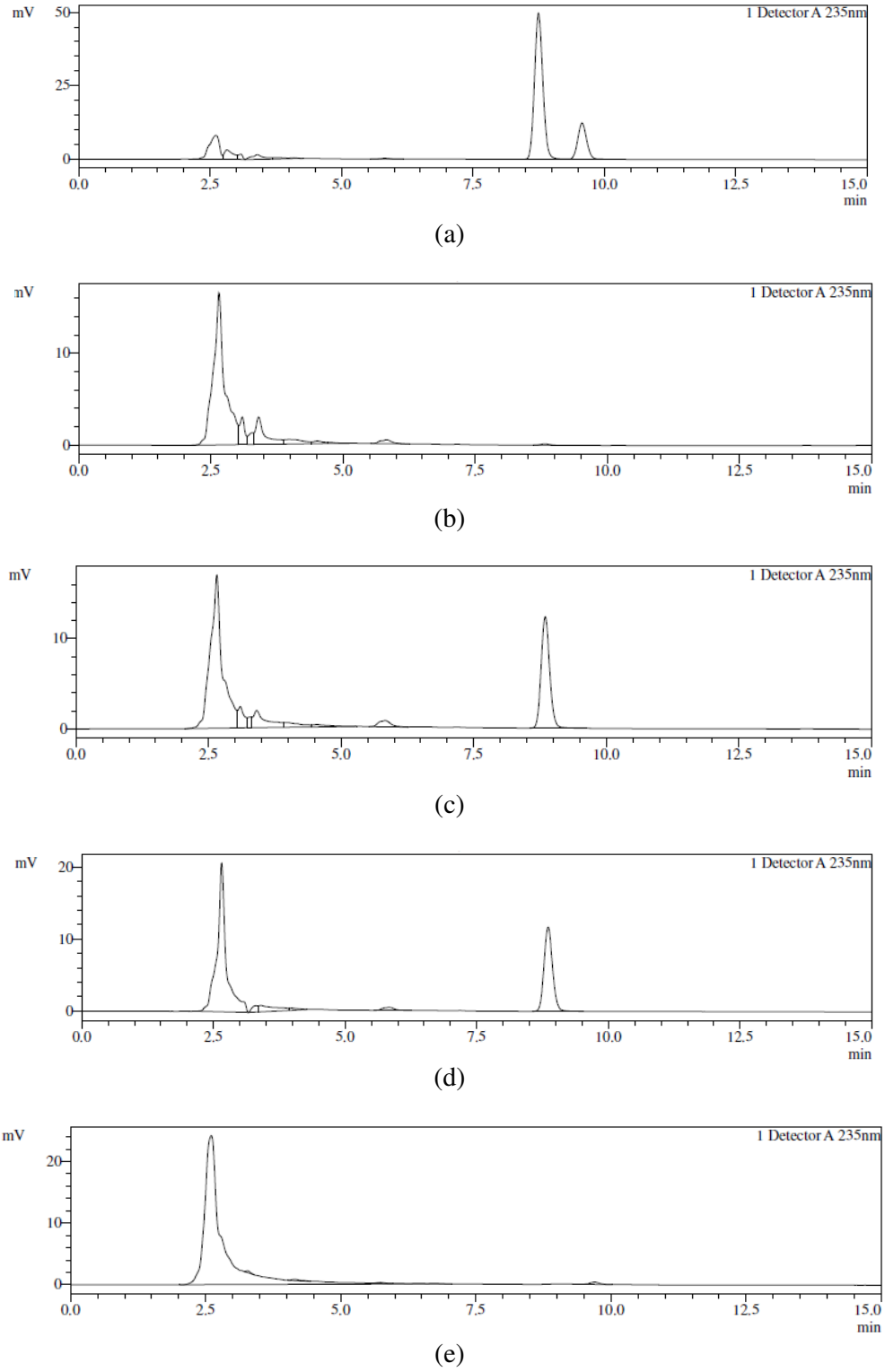

Figure-191: Chromatograms of fruit drink sample (a) FD 06, (b) FD 07, (c) FD 08, (d) FD 09 and (e) FD 10

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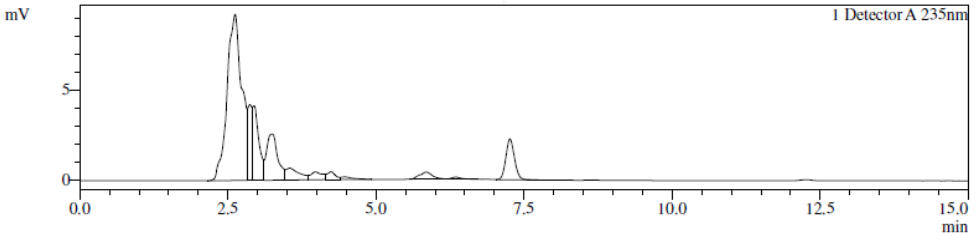
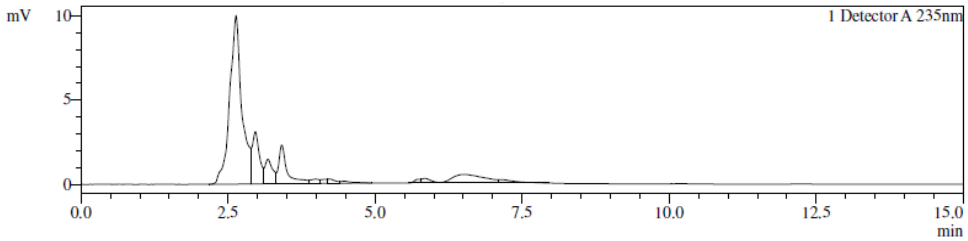
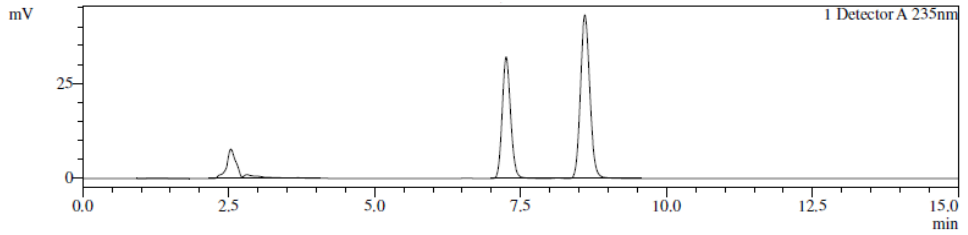


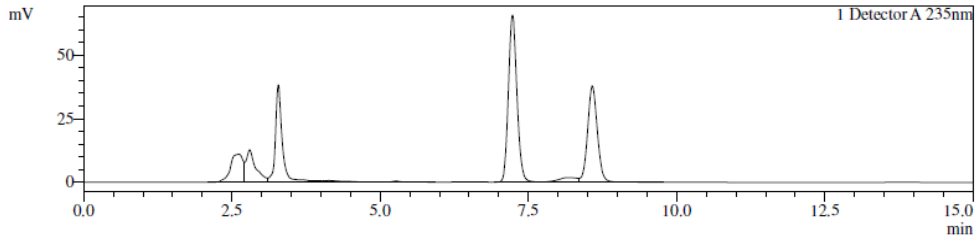
(a)



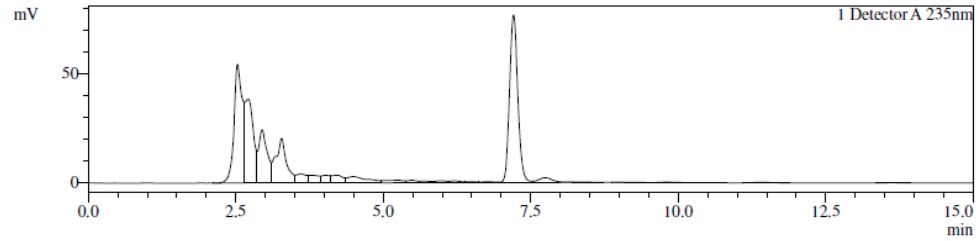
(b)



(c)



(d)



(e)

Figure-192: Chromatograms of fruit drink sample (a) FD 11, (b) FD 12, (c) FD 13, (d) FD 14 and (e) FD 15

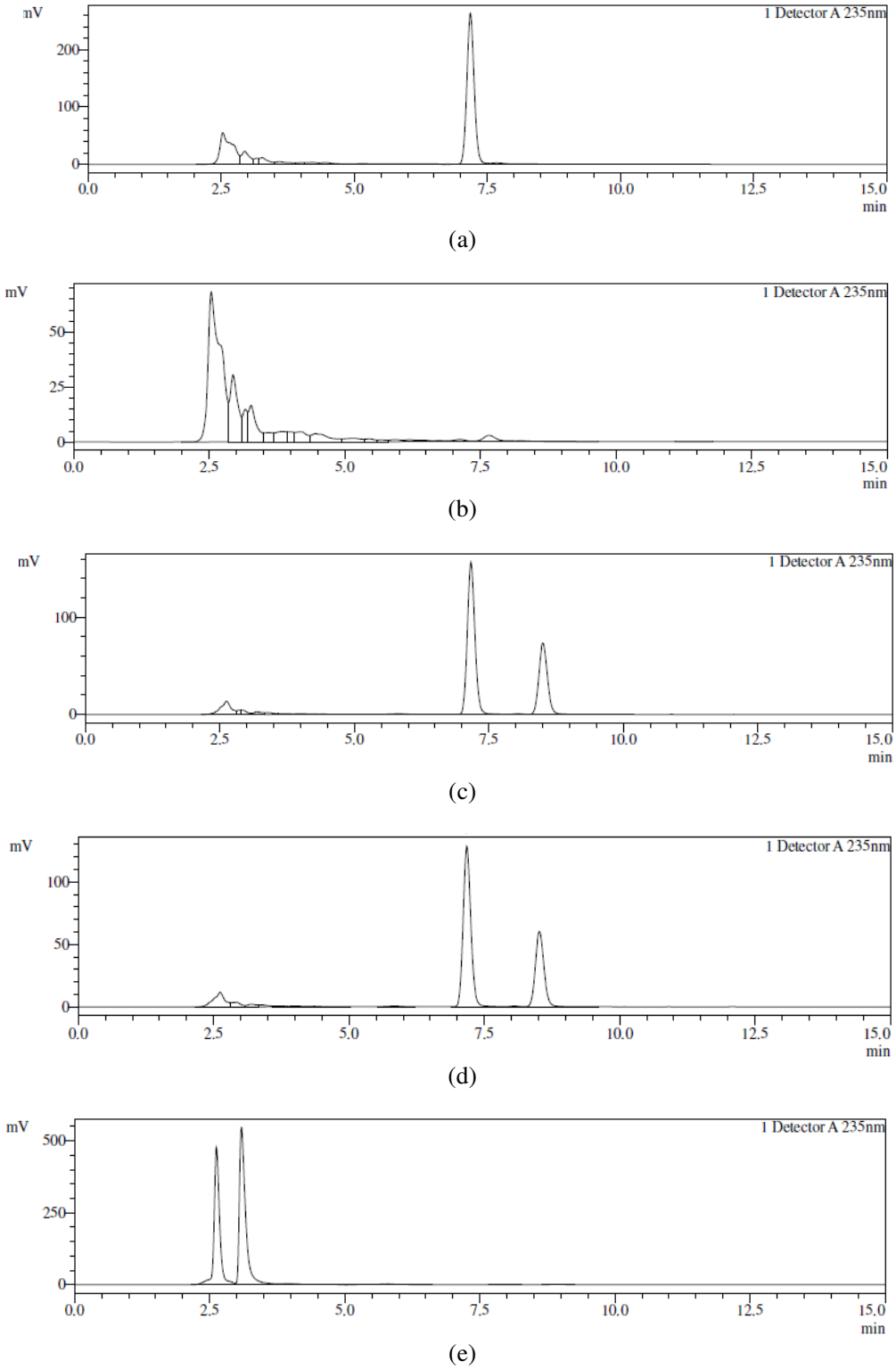


Figure-193: Chromatograms of fruit drink sample (a) FD 16, (b) FD 17, (c) FD 18, (d) FD 19 and (e) FD 20

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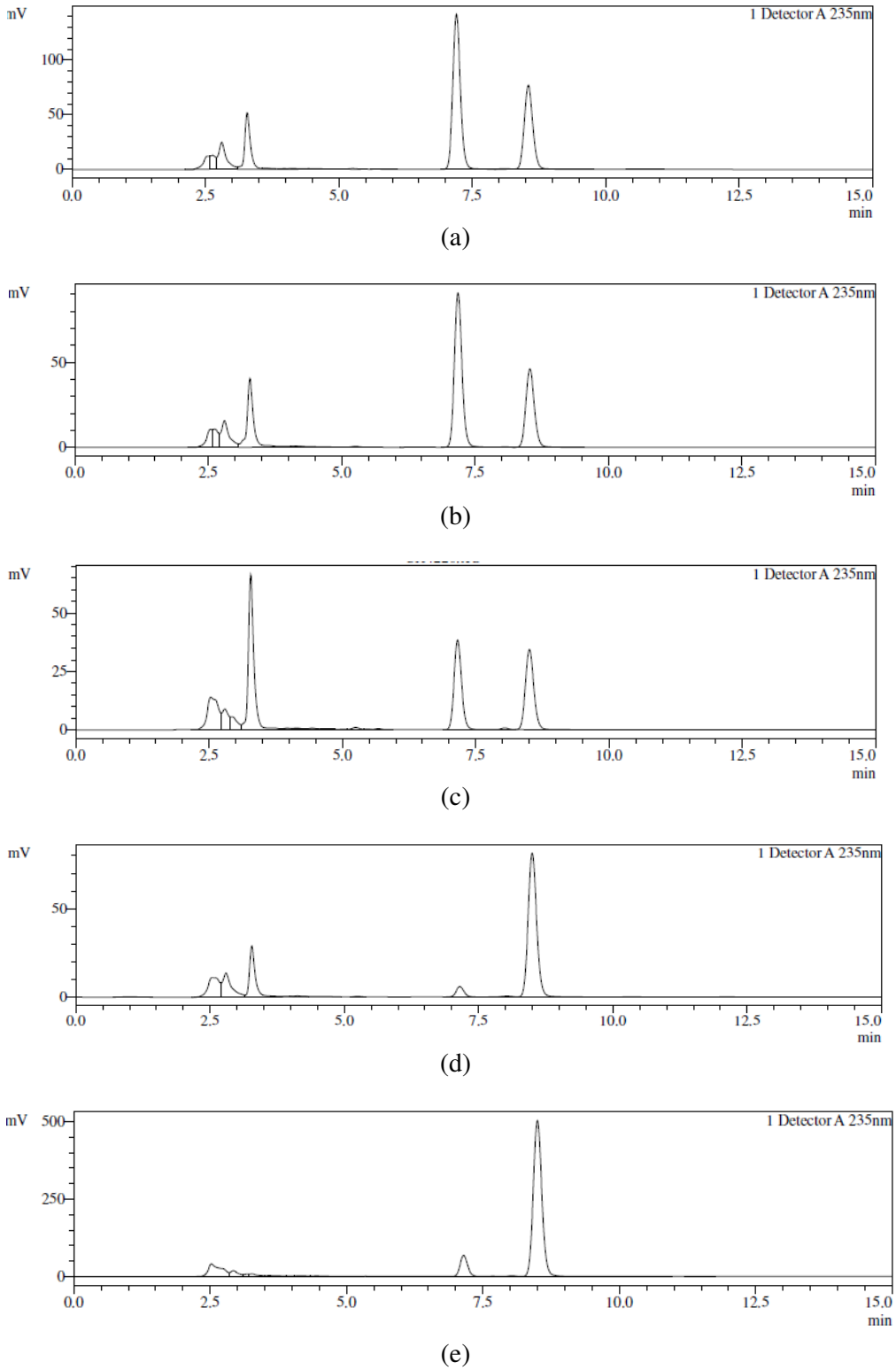

Figure-194: Chromatograms of fruit drink sample (a) FD 21, (b) FD 22, (c) FD 23, (d) FD 24 and (e) FD 25

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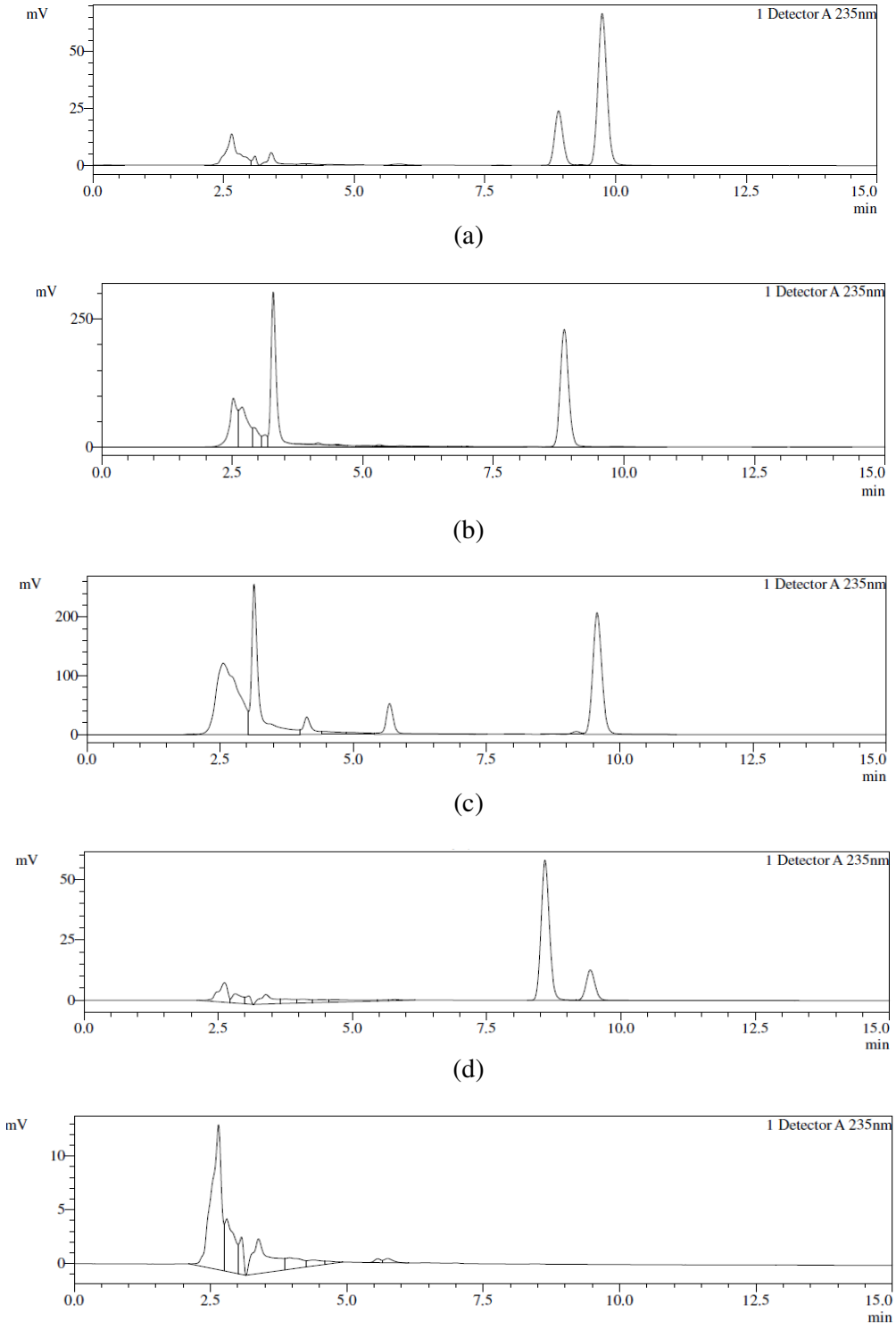
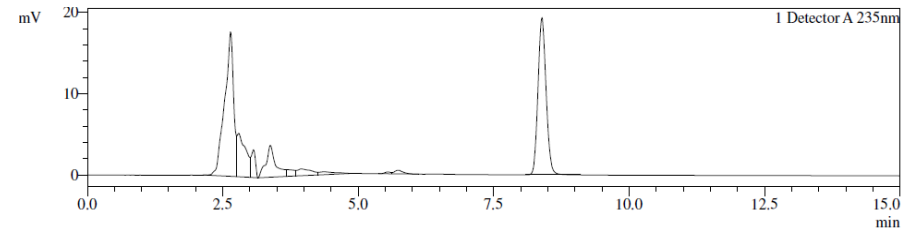
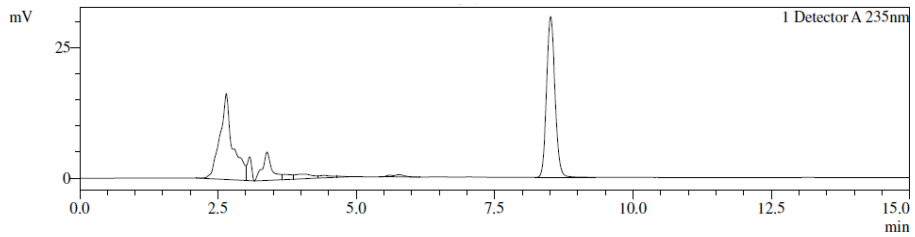


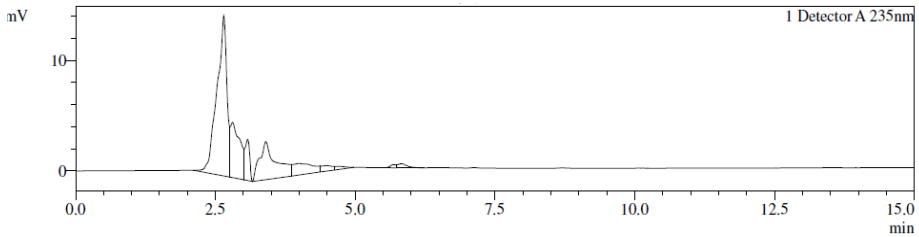
Figure-195: Chromatograms of tomato ketchup sample (a) TK 01, (b) TK 02, (c) TK 3, (d) TK 04 and (e)TK 05



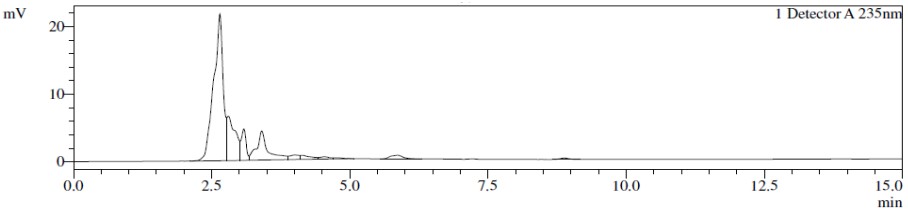
(a)



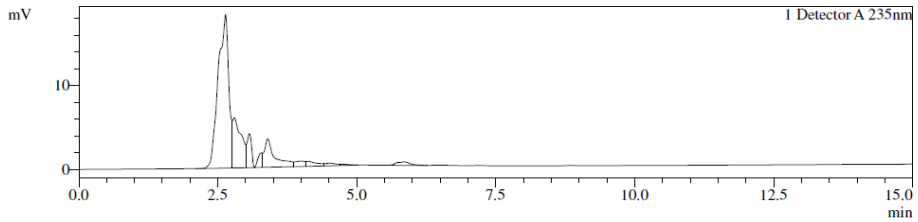
(b)



(c)



(d)



(e)

Figure-196: Chromatograms of tomato ketchup sample (a) TK 06, (b) TK 07, (c) TK 08, (d) TK 09 and (e) TK 10

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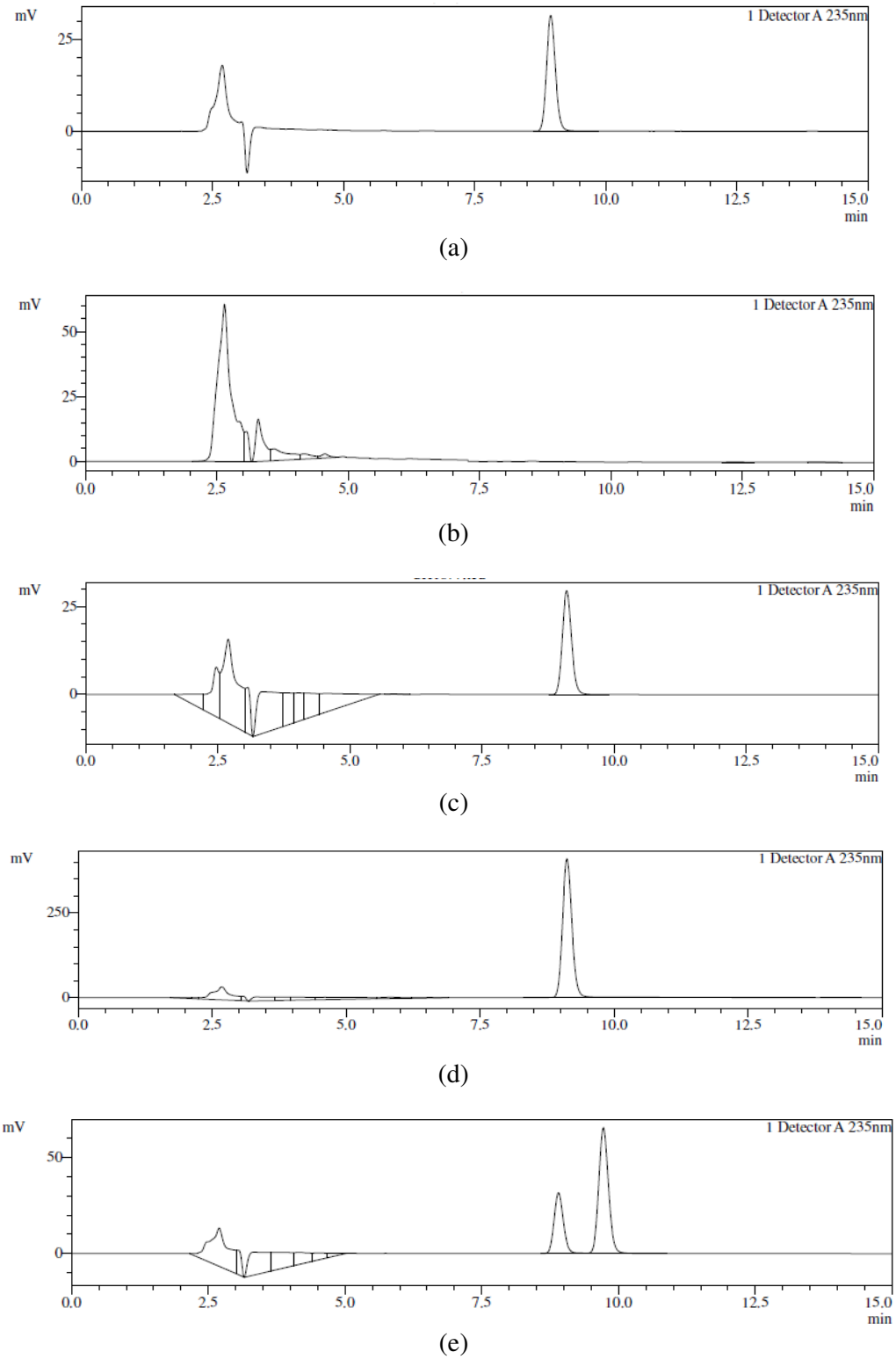

Figure-197: Chromatograms of tomato ketchup sample (a) TK 11, (b) TK 12, (c) TK 13, (d) TK 14 and (e) TK 15

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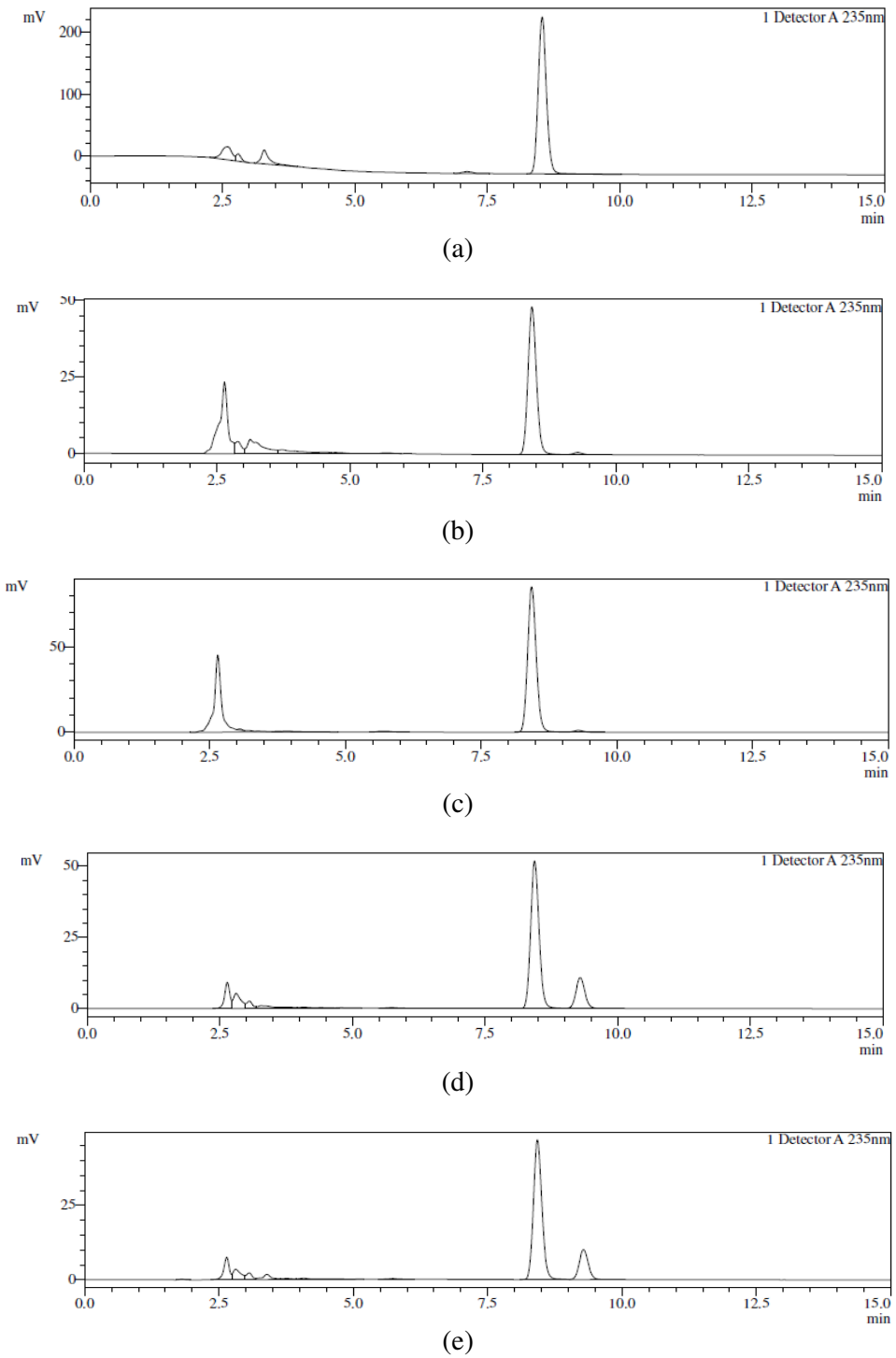

Figure-198: Chromatograms of tomato ketchup sample (a) TK 16, (b) TK 17, (c) TK 18, (d) TK 19 and (e) TK 20

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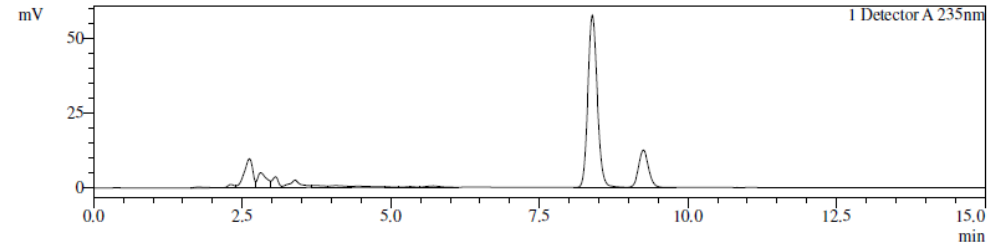
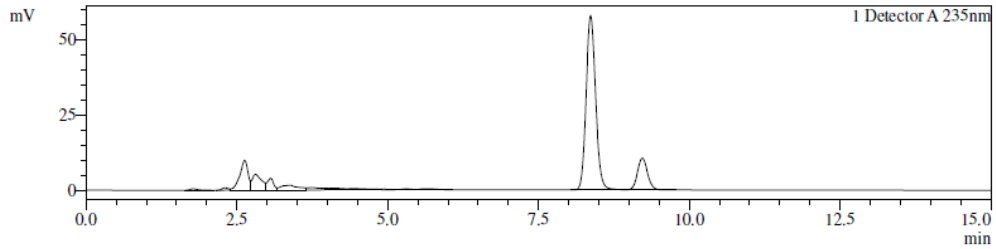
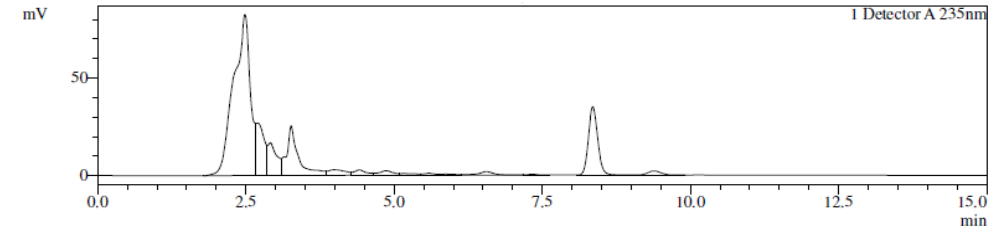


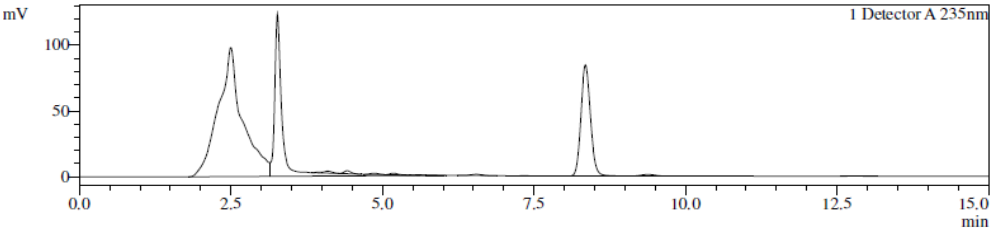
(a)



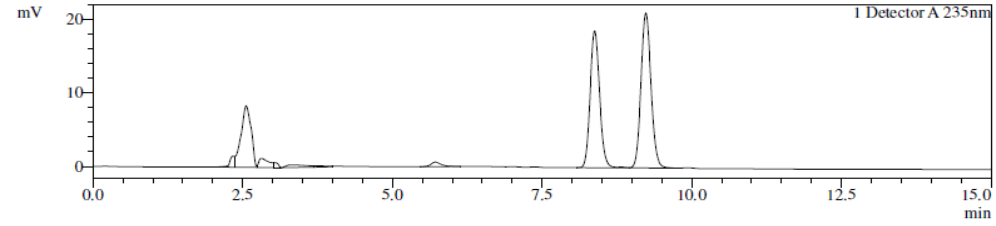
(b)



(c)

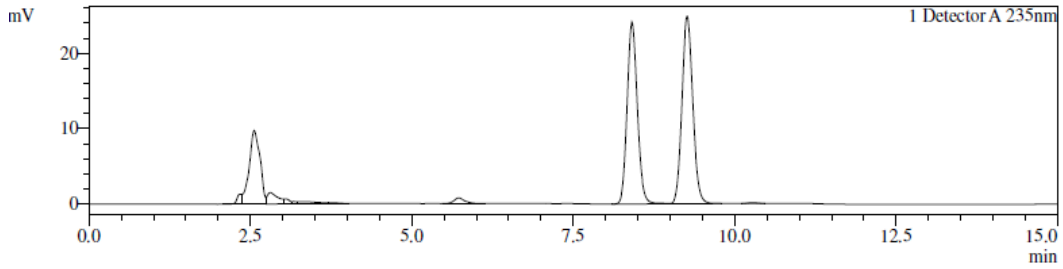


(d)

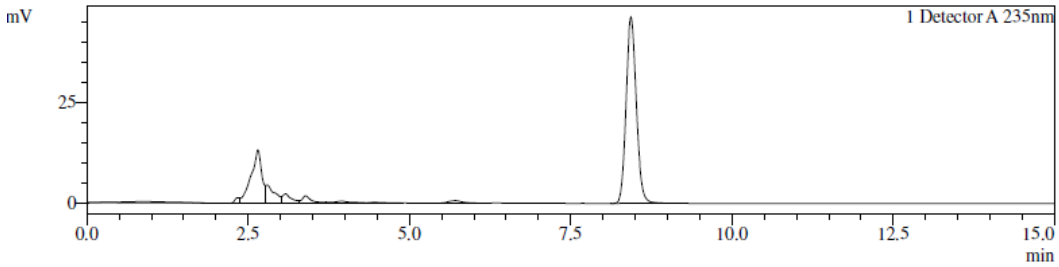


(e)

Figure-199: Chromatograms of tomato ketchup sample (a) TK 21, (b) TK 22, (c) TK 23, (d) TK 24 and (e) TK 25



(a)



(b)

Figure-200: Chromatograms of tomato ketchup sample (a) TK 26 and (b) TK 27

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Investigation on Chemical Contaminants in Selected Food Stuffs

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Abstract

This study describes determination of pesticide residues and heavy metals in fruits and vegetables, heavy metals in turmeric powder, aflatoxins in wheat and maize, benzoic acid and sorbic acid in fruit drinks and tomato ketchup, sudan red in chili powder and antibiotic residues in pasteurized milk. Quick, easy, cheap, effective, and rugged method was used for pesticide and antibiotic residues. Gas chromatograph equipped with electron capture detector (GC-ECD), gas chromatograph-mass spectrometer (GC-MS) and liquid chromatograph-mass spectrometer (LC-MS/MS) were the major equipment for analysis of pesticide residues in fruit and vegetable samples. Microwave digester was used for sample preparation of fruits, vegetables and turmeric powder for analysis of heavy metals by atomic absorption spectrophotometer (AAS). Aflatoxins B_1, B_2, $G_1$and G_2 were determined in maize and wheat by high performance liquid chromatograph (HPLC) equipped with fluorescence detector and coring cell as post column derivatization system. Phosphate buffered saline was used for extraction of sample and clean-up by immunoafinity column for analysis of aflatoxins. Benzoic acid and sorbic acid in fruit drinks and tomato ketchup was determined by HPLC. Extraction of benzoic acid and sorbic acid from sample was performed using a mixture of ammonium acetate buffer solution and methanol, under pH 4.5. Sudan dyes I, II, III, IV have been determined in chili powder by HPLC. Sudan dyes I, II, III, IV were extracted by ethanol. Antibiotic residues were extracted by methanol water mixture and were clean-up by $MgSO_4$, PSA and C18. Antibiotic residues in pasteurized milk were analyzed by LC-MS/MS. Tomato and cabbage were used as representative matrix for method validation of pesticide residues. The LOD for pesticide residue was in the range of 0.02-0.81 µg/kg and LOQ was in the range of 0.08-2.71 µg/kg. Linear correlation coefficient (R^2) value ranged from 0.996 to 0.999. Recoveries were in the range of 81-97%. Potato was used as representative matrix for method validation of heavy metals. For arsenic, lead and cadmium LOD were 2.49, 2.39, 0.09 µg/kg and LOQ were 8.30, 7.96, and 0.29 µg/kg, respectively. Linear correlation coefficient (R^2) value for As, Pb and Cd were 0.998, 0.996 and 0.998, respectively. Recoveries for As, Pb and Cd were 98%, 95%, 96%, respectively. Turmeric powder was used as a representative matrix for determination of lead and chromium. For Pb and Cr LOD were 1.71, 2.17 µg/kg and LOQ were 5.69, 7.22 µg/kg, respectively. Linear correlation coefficient (R^2) value for Pb and Cr were 0.996 and 0.995. Recoveries for Pb and C were 98% and 96%, respectively. Wheat was used as representative matrix for method validation of aflatoxin. LOD of aflatoxin G_2, G_1, B_2 and B_1 were 0.006, 0.021, 0.020, 0.046 µg/kg and LOQ were 0.020, 0.069, 0.066, 0.153 µg/kg, respectively. Linear correlation coefficient (R^2) value were in the range of 0.998-0.999. Recoveries (%) were in the range of 85-96%. Apple fruit drink was used as representative matrix for method validation of benzoic acid and sorbic acid. LOD of benzoic acid and sorbic acid were 0.15 and 0.09 mg/kg and LOQ were 0.49 and 0.30

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mg/kg, respectively. Linear correlation coefficient (R^2) value for benzoic acid and sorbic acid was 1. Recovery (%) of benzoic acid and sorbic acid with apple fruit drink was 99%. Chili powder was used as representative matrix for method validation of sudan red. LOD of sudan red-I, II, III and IV were 0.22, 0.50, 0.38 and 1.49 mg/kg and LOQ were 0.72, 1.66, 1.25 and 4.96 mg/kg, respectively. Linear correlation coefficient (R^2) value for sudan red-I, II, III and IV was 0.999. Recoveries (%) of sudan red-I, II, III and IV with chili powder were in the range 93-99%. Pasteurized milk was used as representative matrix for method validation of antibiotic residues. LOD of six antibiotics in pasteurized milk were in the range of 1.53-4.87 µg/kg and LOQ was in the range of 5.09-16.25 µg/kg. Linear correlation coefficient (R^2) value ranged from 0.995 to 0.999. Recoveries (%) of antibiotic were in the range of 84-101%. Fruits (n= 280) and vegetables (n= 455) samples were analyzed for pesticide residues. Chlorpyrifos was detected in 2 samples of cabbage which were within maximum residue limit (MRL) of 1.0 mg/kg set by Bangladesh Food Safety Authority (BFSA). Dimethoate was detected in 4 samples of green chili which were within MRL of 0.5 mg/kg set by BFSA. Carbofuran was detected in 2 sample of tomato and in 2 sample of eggplant. All these four samples were within MRL of 0.01 mg/kg set by European Commission (EC). Arsenic, lead and cadmium were analyzed for fruits (n= 280) and vegetables (n= 455) samples. Arsenic was detected in 13 potato samples, in 01 tomato samples, in 11 eggplant samples and in 1 carrot samples. Cadmium was detected in 6 potato samples. All these samples were within maximum limit of 0.1 mg/kg set by BFSA. Lead and chromium were analyzed in 17 turmeric powder samples. High amount of Pb and Cr were found in 8 turmeric powder samples. Eight samples exceeded maximum limit of Pb of 2.5 mg/kg set by Bangladesh Standards and Testing Institution (BSTI). Aflatoxin B_1, B_2, G_1 and G_2 were analyzed in 25 wheat and 25 maize samples. No targeted aflatoxin was detected in any sample of wheat and maize. Benzoic acid and sorbic acid were analyzed in 25 fruit drink and 27 tomato ketchup samples. Benzoic acid was detected in 17 fruit drink samples and in 21 tomato ketchup samples. Eleven fruit drink sample exceeded maximum limit of 120 mg/kg set by BSTI and 1 tomato ketchup sample exceeded maximum limit of 750 mg/kg set by BSTI. Sudan I, II, III and IV were analyzed in 20 chili powder samples. Sudan III was detected in 1 sample out of 20 samples. Six antibiotic residues were analyzed in 42 samples of pasteurized milk. No targeted antibiotic was detected in any sample. In this study analysis result showed that pesticide residues detected in 1.36 % sample of fruits and vegetables. Arsenic was detected in 3.54 % sample and cadmium was detected in 0.82% sample of fruits and vegetables. Lead and chromium was detected in 47.06% of the turmeric powder sample. Aflatoxin B_1, B_2, G_1 and G_2 was not detected in any wheat and maize sample. Benzoic acid was detected in 68% fruit drink sample and in 77.78% tomato ketchup sample. 64.71% of benzoic acid detected fruit drink sample exceeded maximum limit and 6.76% of benzoic acid detected tomato ketchup sample exceeded maximum limit. Sudan III was is detected in 5% chili powder sample. No targeted ciprofloxacin, levofloxacin, enrofloxacin, tetracycline, oxytetracycline and chlortetracycline were detected in any sample of pasteurized milk.