A DISSERTATION ON

"Quantitative Analysis of pesticides in Beverages by LCMS/MS and GCMS/MS"

SUBMITTED TO THE

DEPARTMENT OF BIOENGINEERING

FACULTY OF ENGINEERING
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Mohammad Almas Khan**, a student of **M.Tech Biotechnology** (II Year/IV semester), Integral University have completed my six months dissertation work entitled **"Quantitative Analysis of pesticides in Beverages by LCMS/MS and GCMS/MS"** successfully from the Fare labs under the Able guidance of Ms. Rita Singh, Technical Head, pesticides Department Fare labs Gurgaon.

I, hereby affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reputed in this study are genuine and authentic.

Name and Signature of Student with Date

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CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Mohammad Almas Khan,** a student of **M.Tech Biotechnology** (II year/IV semester); Integral University has completed his six months dissertation work entitled **"Quantitative Analysis of pesticides in Beverages by LCMS/MS and GCMS/MS" s**uccessfully. He has completed this work from under the guidance of Ms. Rita Singh, Technical Head, pesticides Department Fare labs Gurgaon. The dissertation was a compulsory part of his M.Tech Biotechnology degree.

I wish him good luck and a bright future.

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Assistant professor
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TO WHOM IT MAY CONCERN

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I wish him good luck and a bright future.

Dr. Alvina Farooqui
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DATE: NAME:

LIST OF ABBREVIATIONS

S. No	Name	ABBREVIATION
1	Food and Agriculture Organization	FAO
2	Integrated pest management	IPM
3	GAS chromatography	GC
4	Helium	He
5	Nitrogen	N2
6	Hydrogen	H2
7	Standard Atmosphere	atm
8	Micro Liter	µl
9	Meter	m
10	Millimeter	mm
11	Wall-Coated Open Tubular Columns	WCOT
12	Support-Coated Open Tubular Columns	SCOT
13	Thermal Conductivity Detectors	TCD
14	Retention Time	RT
15	Retention Value	RV
16	High-Performance Liquid Chromatography	HPLC
17	Normal Phase	NP
18	Reversed Phase	RP
19	Trinitrotoluene	TNT
20	Progressive Multifocal Leukoencephalopathy	PML
21	Joint Parliamentary Committee	JPC
22	Bureau of Indian Standards	BIS
23	Parts Per Million	PPM
24	Parts Per Billion	PPB
25	Food and Agriculture Division	FAD
26	Chromium	Cr
27	Copper	Cu
28	Cadmium	Cd
29	Potential of Hydrogen	pH
30	Dichlorodiphenyltrichloroethane	DDT

31	European Union	EU
32	Plant Growth Regulators	PGR
33	Persistent Organic Pollutants	POPs
34	World Health Organization	WHO
35	Granules	G
36	Emulsifiable Concentrate	EC
37	Wettable Powder	WP
38	Natural killer	NK
39	Lip polysaccharide	LPS
40	Adenosine Triphosphate	ATP
41	Dihydrorotenone	DHR
42	Pentachlorophenol	PCP
43	Tributyltin	TBT
44	Bifenthrin	BF
45	Organochlorines	OCP
46	European Commission	EC
47	Environmental Protection Agency	EPA
48	United States Department of Agriculture	USDA
49	Maximum Residue Limit	MRL
50	Good Agricultural Practices	GAP
51	Supervised Trials Mean Residue	STMR
52	Liquid Chromatography Tandem Mass Spectrometry	LC-MS/MS
53	Liquid Chromatography-Mass Spectrometry	LC-MS
54	Electrospray Ionization	ESI
55	Atmospheric pressure photo Ionization	APPI
56	Thermo spray and Plasma spray Ionization	TSPI
57	Standard Operating Procedure	SOP
58	Quick Easy Cheap Effective Rugged Safe	QuEChERS
59	Certified Reference Material	CRM
60	Acetonitrile	ACN
61	Limit of quantification,	LOQ
62	Below the Limit of Quantification	BLQ

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Chapter -1
Introduction

1. Introduction

Pesticides are substances or mixtures of substances used to prevent, destroy, repel, or mitigate pests Pesticides are defined by the Food and Agriculture Organization (FAO) as any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or substances which may cause harm during or otherwise interfering with the production, processing, storage.[1] The term encompasses substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing premature fruit fall. Also applied to crops before or after harvest to protect them from deterioration during storage and transportation.

Pesticides are classified into three categories Insecticides, Herbicides and Fungicides on basis of the types of pests they control.

Insecticides: - are pest-controlling agents of chemical or biological origin. Insecticides are chemicals that kill insects that feed on crops, leaves, roots, and other plant parts. Chloryiphos is one of the largest molecules available on a global scale among insecticides.[2]

Herbicide: - A weed killer, or herbicide, is a type of pesticide used to kill unwanted plants that act as parasites on agricultural crops.[3]

Fungicides: - These are used to keep crop diseases at bay. The widespread use of new hybrid seeds has resulted in an increase in diseases, resulting in higher sales over the last five years. Its use has increased from 8% to 16%. Mancozeb is the most popular product in this category.

Pesticides were widely used during the green revolution, and pesticide consumption has since increased. Pesticides have a variety of beneficial effects. Crop protection, food and material preservation, and vector-borne disease prevention are among them. Although pesticides have advantages, some have disadvantages, such as potential toxicity to humans and other animals. Short-term or long-term exposure, as well as low- or high-level exposure via skin contact, inhalation, or ingestion, can have negative consequences. Some pesticides are highly toxic, with a few drops causing extremely harmful effects; however, while other pesticides are less toxic, excessive exposure to them can also cause harm (http://www.gogov). So and there is growing concern about human pesticide exposure and its negative health effects.[4]

1.2 Pesticide consumption

With an annual production of 90,000 tones, India is currently the largest producer of pesticides in Asia and ranks twelfth in the world for pesticide use. When compared to the world average of 500 g, the consumption is 381 g. Pesticide use in India is restricted to approximately 25% of arable land (170 mha). The vast majority of India's population (56.7 percent) is engaged in agriculture and thus exposed to pesticides used in agriculture.[5] Pesticide production and use in the country differs from global trends. In the country, approximately 75% of insecticides are used, compared to 32% globally.

1.3 Pesticide exposure

Pesticide exposure occurs when pesticides come into contact with a surface or an organism. Pesticide exposure in humans refers to pesticides getting into or on the body. The toxic effect is determined by the length of exposure and the concentration of pesticides.[6] Pesticides can be ingested by humans in four ways: orally (by swallowing a pesticide), inhalationally (by breathing in a pesticide), ocularly (through the eyes), or dermally (through the skin).

1.4 Pesticide toxicity

Pesticide residues in food have been a source of concern for environmental and consumer groups due to their widespread use. Most pesticides, particularly organochlorines, are extremely resistant to microbial degradation.[7] As a result, they can accumulate in human body fats and the environment, posing health risks Pesticides and their residues may concentrate in the adipose tissues and blood serum of animals due to their persistence and lipophilicity, resulting in environmental persistence, bio-concentration, and bio-magnification throughout the food chain the risk of pesticide-related harm is referred to as a hazard. Hazard is determined by the pesticide's toxicity and the amount of pesticide exposure, and is frequently represented by the following equation:

Hazards = Toxicity * Exposure

Toxicity is a chemical's ability to harm human health. Pesticides can have three kinds of negative effects: acute, Delayed or chronic, and allergic.

1.5 Introduction to Chromatography

Chromatography is an analytical technique used to separate, purify, and identify constituents in a mixture.[8]They administer the mixture to be separated in a stationary phase (solid or liquid) and allow a pure solvent such as water or any mixed gas (mobile phase) to move slowly over the stationary phase, holding the components separately in the pure solvent according to their solubility

1.6 Type of Chromatography

Chromatography is classified into three types: liquid, gas, and supercritical fluid chromatography. Gas chromatography is further subdivided into gas-liquid and gas-solid chromatography. And there are four types of liquid chromatography: ion exchange, exclusion, partition (paper chromatography), and liquid-solid interaction (thin layer chromatography) **[9]**

1.7 GAS chromatography

G.C. was developed in the 1950s and first used in the late 1950s. The theory, like all chromatographic techniques, is based on the partitioning of the analyte between a stationary and mobile phase. Because the mobile phase in GC is a gas, the analyte must be volatile in order to move through the column, and the majority of analytes have little interaction with the mobile phase.[9]

1.8.1 It has two types:-

1. Gas-solid chromatography: - The stationary phase is in solid at this technique. This functions on the absorbent principle.

2. Gas- liquid chromatography: - The stationary phase is in liquid at this technique. This functions on the principle of partition and absorption.

- The number of peaks obtained determines the number of components in a sample
- The sum of a given component in a sample is determined by the region under the peaks.
- The retention time aids in the identity of components.

1.9 Liquid Chromatography

Liquid chromatography is a type of column chromatography in which liquid serves as the mobile phase and inert solid substances such as silica gel, alumina, or cellulose serve as the stationary phase. When constructing a liquid chromatography column, it is critical that the stationary phase be saturated with solvent because any air present will disrupt the smooth flow and result in inefficient or incomplete separation.[10]

Liquid chromatography is divided into different parts: -

- Ion exchange
- Exclusion
- Paper chromatography (partition)
- Thin Layer Chromatography (liquid-solid interaction) HPLC is the advance technology of liquid chromatography.

1.9.1 HPLC (high performance liquid chromatography)

One of the most widely used analytical techniques is high-performance liquid chromatography (HPLC). Due to the physical properties of the HPLC column, eluent flow through the column requires a high pressure. Because of this, the method was previously known as high pressure chromatography. [10]

1.9.2 Principle

The principle of separation is based on the relative solubility of the analyte's two liquid phases. HPLC employs various types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that pushes the mobile phase and analytes through the column, and a detector that provides the analyte with a characteristic retention time.[11] The retention time of analyte varies with column temperature, solvent ratio/composition, and mobile phase flow rate. A pump (rather than gravity) is used in HPLC to provide the higher pressure required to propel the mobile phase through the densely packed column and analyte.

1.9.3 Chromatographic analysis

The number of components in a sample is determined by the number of peaks recorded. The region under the peaks determines the amount of a given component in a sample. The retention time aids in the identification of components. HPLC is built on a stationary phase with small particle size that improves separation efficiency. This is due to the solute's ability to quickly balance between the two phases. Larger particle size stationary phase is the foundation of low-pressure liquid chromatography, in which the eluent is either gravity-fed or pumped by a low pressure pump through the column. It is less expensive to run but lacks high resolution.As the particle size of the stationary phase decreases, the surface area increases, this

indirectly increases the number of plates and thus the resolution. However, resistance to the mobile phase flow increases as capillary action increases with small particle sizes. Such resistance creates backpressure, which reduces the flow rate and makes gravity draining the column more difficult.[12]To overcome this high pressure, a high-pressure system must be used to keep the solvent flowing.

1.10 INTRODUCTION & ORIGIN OF THE STUDY IN BEVERAGES

Following the publication of PML's study on pesticide residues in soft drinks in August 2003, the Indian Parliament formed a Joint Parliamentary Committee (JPC) on "Pesticide Residues in and Safety Standards for Soft Drinks, Fruit Juices, and Other Beverages" to investigate the veracity of the PML's study and to establish soft drink safety standards.[13]JPC issued its report in February 2004, concluding that PML's study was correct and recommending that the government set pesticide standards for soft drinks.

1.11 Soft drink definition

Soft drinks are non-alcoholic, flavoured water drinks that can be sweetened, acidulated, or carbonated. Some carbonated soft drinks, particularly brown-colored cola drinks, contain caffeine.[14]

1.12 Standard Appiled

The Bureau of Indian Standards (BIS) Drinks and Carbonated Beverages Sectional Committee, FAD 14, has set a limit of 0.1 ppb for individual pesticides and 0.5 ppb for total pesticides. [13]The PML has used this standard, which has been finalised but has yet to be notified, in the current work. To compare the results of pesticide residue analysis in soft drinks, PML used the finalised BIS limits set for single and multiple residues.

1.13 Beverages Review

There is growing concern in the medical and scientific communities about the harmful effects of carbonated soft drink consumption, particularly among children, teenagers, and vulnerable populations such as pregnant women.[15]

The increased acid levels (citric, malic, and phosphoric acid) throughout the body cause gastronomic distress due to inflammation of the stomach and erosion of the stomach lining leading to painful stomach-ache as the stomach, which maintains a very delicate acid-alkaline balance, can be thrown out of balance by the consumption of a large amount of soft drinks, which can cause indigestion and gassiness by maintaining an acidic state.[16]

Numerous medical and scientific studies have clearly documented the harmful effects of some major soft drink ingredients, namely, carbon dioxide, artificial sweeteners such as aspartame, saccharin, acesulfame-K, and others, flavoring agents such as caffeine, acids such as phosphoric acid, some preservatives, and excessive sugar.

Objective:

- **Collection of Different beverages sample from Different Industries And its primary Analysis :-**
- **Colour**
- **pH**
- **Texture**
- **Preparation of Standard for pesticides Analysis in collecteds samples.**
- **Extraction of pesticides from different collected sample.**
- **Quantitative And Qualitative Analysis of Extracted pesticides by LCMS-MS And GCMS-MS**

Significance of the study:

The purpose of the study is to benefit the region in its efforts to contain and reduce pesticides in every drinks and food. The finding may also be applied to wider geographical areas. This research when finished with the proven result can have further commercial applications and can benefit the environment around all of us in the coming years.

Chapter-2
Review of Literature

2.1 Pesticides

Pesticides are any substance or mixture of substances used to prevent, destroy, or control pests such as insects, fungi, rodents, or unwanted plant species that cause harm during crop production and storage Pesticides are a broad term that includes insecticides, herbicides, fungicides, and rodenticides, all of which can be used to kill specific pests. Pesticides are classified as chemical pesticides or biopesticides based on their source of origin. Host-specific biological pesticides they are highly specific in the sense that they only act on the target pest and closely related organisms, whereas chemical pesticides are nonspecific, acting on a wide range of nontarget organisms. Biopesticides are environmentally friendly because they are less toxic, easily decompose, and are only needed in small amounts Chemical pesticides pollute the environment because they are highly toxic and may not biodegrade. Furthermore, biopesticides have the advantage of being less susceptible to genetic modification in plant populations. This confirms the low likelihood of pesticide resistance in pests, which is uncommon with chemical pesticides. Pesticides classified as chemical are further classified as organochlorines, organophosphate, carbamate, and pyrethroids. Biopesticides are pesticides derived from natural sources such as animal, plant, and microorganism waste (bacteria, viruses, fungi, and nematodes). Microbial pesticides, plant-incorporated protectants, and biochemical pesticides are among them. Pesticides work in a variety of ways. Some are known as growth regulators because they either stimulate or retard pest growth, while repellents repel pests, attractants attract pests, and chemosterilants sterilize pests. Pesticides with a broad range of activities that are used to control more than one type of pest are difficult to categorise .Aldicarb, for example, is used in Florida citrus production and can be classified as an acaricide, insecticide, or nematicide depending on whether it controls mites, insects, or nematodes. Aside from established chemicals used as insecticides, other traditional methods are also used to reduce insect growth or limit their activity. Some of these compounds are intended for other applications but are being used as insecticides. Alcohols and oils are among them. Alcohols are used as surface compounds for mosquito larvae control. Agnique, a well-known larvicidal product, contains it as an active ingredient. The goal of using alcohol is to reduce the surface tension of water, which reduces the adherence of mosquito larvae or pupae, resulting in drowning and death. Similarly, oils are useful for surface applications. They do, however, prevent the larvae or pupae from getting enough oxygen to survive. This water surface control method has several advantages because its actions are more physical than conventional insecticides, which are more biochemical in nature. These prevent the organisms from developing pesticide resistance.[1]

2.2 History of pesticides

Human civilizations have attempted to apply the most effective and least time-consuming methods of cultivating and preserving their food resources since ancient times. An example of this is how they cultivated both venomous and nutritious vegetation in the same area due to the protective effect of toxic plants on insect elimination. Similarly, elemental sulphur was used throughout this time period. For millennia, these were the primary methods of pest control. Later came the Ebers papyrus, one of the oldest

still extant documents containing some of the techniques for removing insects from foods In parallel, traditional Chinese medicine also uses primitive sulfides. It is likewise interesting to note that Homer's epic work "Odysseus," written around the same time, describes the use of substances to remove insects. Around 1500's, the early stages of the use of the "Para-pesticides," namely mercury and arsenic, emerged. These substances were used until the start of synthetic pesticide era, initially for the destruction of food reserves during the World War II and later on as precious tools for cultivating processes of foods consumed daily. It is crucial to note that through this time, several scientists have highlighted the adverse effects of pesticides on human health when used for a long time. For instance, the drastic increase in the number of lymphoma patients is a topic that would be discussed until today.[2]

2.3 Present Era

The discovery of the first modern pesticide, dichlorodiphenyltrichloroethane (DDT) by Paul Muller in 1939, was a watershed moment in pesticide history. This discovery earned him the Nobel Prize in Medicine years later, owing to the reduction of pesticide damage in agriculture as well as health-related problems such as malaria and typhus. Despite its allure, DDT's use would be short-lived. Rachel Carson's book "Silent Spring," published in 1962, elaborated on the dangers of DDT. Following that, many states prohibited the use of DDT in favour of lower risk organophosphates and carbamates in the coming years. Currently, the public is concerned about the health effects of pesticide use (especially in old people and children). In contrast, as the world's population has grown exponentially over the last few decades, so has global pesticide production.Meanwhile, new EU regulations require producers to reduce pesticide production in order to reduce the number of serious illnesses in the population. However, much attention is now being paid to the arrival of pesticide-resistant herbs, which will undoubtedly have a significant impact in the future [3]

2.3 Pesticide Classification:

Pesticide classification is important because it provides useful information about pesticide chemistry, pesticide action, target pests, and so on. Some popular classification systems are mentioned.[4]

2.3.1 Classification Based on Chemical Nature

Pesticides can be classified as organic pesticides and inorganic pesticides. Organic pesticides are based on chemicals with carbon as the basis of their molecular structure; they may also contain oxygen, phosphorus, or sulphur in their molecules; they are complex compounds that do not dissolve easily in water. Organic pesticides are further classified into two categories: natural organics and synthetic organics. Natural organic pesticides are derived from naturally occurring sources such as plants, such as rotenone and pyrethrum, whereas synthetic organic pesticides or modern pesticides are created artificially through chemical synthesis and include DDT, permethrin, Malathion, lindane, and others. In comparison to organic pesticides, inorganic pesticides are simpler compounds that are crystalline, environmentally stable, and usually dissolve easily in water. The first chemical pesticides were inorganic and contained ingredients such as sulphur and lime.[5]

2.3.2 Classification Based on Target Pest Species

Pesticides are classified by target organism as insecticides, herbicides, defoliants, desiccants, fungicides, nematicides, avicides, rodenticides, plant growth regulators (PGRs), and others. Insecticides are pesticides that are used to target and kill insects and other arthropods. Herbicides are chemicals that are used to kill unwanted plants or weeds. Fungicides are chemicals that are used to kill or stop the growth of fungi and their spores. They could also be used to control mould, rust, and mildew. Algicides are chemicals that are used to control algae. Fumigants are any gas or volatile substance used to kill insects, nematodes, and other damaging animals or plants. To control disease-causing fungi, nematodes, and weeds, soil fumigants are sprayed into the soil. Miticides are chemicals that are used to kill mites, whereas molluscicides are chemicals that are used to kill or repel snails and slugs. Nematicides are a type of chemical pesticide that is used to kill nematodes, microscopic parasitic worms that can live in soil or water. Ovicides are designed to kill eggs of insects and mites similarly pesticides are poisonous to fish.[6]

2.4 Classification by Chemistry: Insecticides and Acaracides

2.4.1 Organochlorines (Chlorinated Hydrocarbons)

Organochlorines were the first significant synthetic organic pesticides to be classified as persistent organic pollutants (POPs). Organochlorines pesticides are highly persistent in the environment, have high toxicity, are bioaccumulative, and cause chronic toxicities through long-term exposure even at low doses .Its primary application is in the control of disease vectors such as malaria and dengue. They are also used in the preservation of grapes, lettuce, tomato, alfalfa, corn, rice, sorghum, cotton, and wood They kill insects through contact or ingestion. In humans, these substances or their metabolites primarily act at the central nervous system level, altering the electrophysiological properties and enzymatic neuronal membranes, causing changes in the kinetics of the flow of Na^+ and K^+ through the nerve cell membrane (resulting in the spread of multiple action potentials for each stimulus (causing symptoms such as seizures and acute poisoning death from respiratory arrest). The most commonly used pesticides in agricultural practise are organochlorines such as chlordane, dichlorodiphenyltrichloroethane (DDT), Dieldrin, and lindane.[7]

2.4.2 Organophosphates

Pesticides containing organophosphates are most commonly used in agriculture to control pests on crops such as tur, cotton, sugarcane, and many others. Some orgaophosphates used against pests include Malathion, parathion, profenofos chlorpyrifos, temephos, fenthione, and diazinon. Organophosphate is phosphorus-containing acid esters, thiol esters, or acid anhydride derivatives. In humans, these inhibit the acetylcholine esterase enzyme at nerve endings by phosphorylating the hydroxyl group in the enzyme's active site .Loss of reflexes, diarrhoea, vomiting, salivation, excessive sweating, agitation, other effects, respiratory failure, ataxia, hyperglycemia convulsion, acute pancreatitis, and even death are among the symptoms.[8]

2.4.3 Carbamates

Pesticides derived from carbamic acid are known as carbamate esters. They are widely used in homes, gardens, and agriculture as insecticides, herbicides, fungicides, and nematicides. Their mode of action is cholinesterase enzyme inhibition, which affects nerve impulse transmission in the same way that organophosphate insecticides do. Carbamate poisoning symptoms are similar to those caused by organophosphate pesticides; however, carbamates are less persistent than organochlorines and organophosphates. Carbamates that are commonly used include carbamyl, methomyl, and carbofuran.[9]

2.4.4 Pyrethrum

Pyrethrum is the powdered, dried flower head of Chrysanthemum cinerariaefolium, and pyrethrins are six insecticidal compounds found naturally in pyrethrum flowers. Pyrethrin I, Pyrethrin II, Cinerin I, Cinerin II, Jamolin I, and Jamolin II are the active ingredients. In most insects, the insecticidal action of pyrethrins is characterised by a rapid knockdown effect, hyperactivity, and convulsions. Pyrethrin-containing insecticides are neurotoxic to insects because they block voltage-gated sodium channels in nerve axons. Pyrethrins are moderately toxic to mammals, but commercial preparations are much less toxic. They are toxic to fish, are not persistent, and have low photostability.[10]

2.4.5 Pyrethroids

Pyrethroids are derived from pyrethrins, which are insecticidal substances found in natural pyrethrum extracted from chrysanthemum flowers, and are currently produced by nearly 100 different commercial products.They are neurotoxic or affect voltage-gated sodium channels, causing muscular paralysis and insect death. Pyrethroids have a higher activity and are more effective at lower doses. Pyrethroids are nonpersistent and photoinstable. Among the synthetic pyrethroids are bifenthrin, betacyfluthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, gamma cyhalothrin, Imiprothrin, permethrin, prallethrin, resmethrin, tefluthrin, tetramethrin, and tralomethrin.[11]

2.4.6 Biopesticides or Biorationals

Biorationals or biopesticides are not toxic to humans and are also non-hazardous to the environment. Biorationals are classified into two types: biochemical and microbial. Hormones, enzymes, chromosterilants, pheromones, natural insect and PGRs, insect growth regulators, and chitin inhibitors are examples of biochemicals. Biochemicals interfere with the target pest's natural growth process. They are extremely toxic to vertebrates. Viruses, bacteria, fungi, protozoa, and nematodes are examples of microbial pesticides. Microbial pesticides work by using toxins released by microbial organisms.[12]

2.5 Classification Based on How the Pesticides Target the Pest

2.5.1 Stomach Toxicants

The stomach poisons may be applied to the water where pests particularly mosquito consume the poison where it is absorbed in pests body. Ants, cockroaches, and other pest insects can also be controlled by using baits of various types. Rodents are controlled using ingested anticoagulants. Rodents die due to internal bleeding where blood does not clot.

2.5.2 Contact Toxicants

Contact toxicants are mostly applied to control mosquitoes. Chemicals enter the pest body through water treated leaves or through aerosol. These poisons act on the nerve and respiratory centers of pest.

2.5.3 Fumigants

Fumigants enter the insect's body in a gaseous phase.

2.5.4 Systemic Toxicants

The toxicant is ingested and absorbed when a pest organism feeds on the plant or animal. Some toxicants quickly kill the pest while others prevent the pest from maturing. Systemic toxicants are used for tick, flea control on pets, and for dog heartworm prevention.

2.6 Classification Based on Mode of Action

On the basis of Mode of action, pesticides are classified as Contact (non systemic), Systemic, Broad Spectrum,Disinfectant,(Eradicant),GerminationInhibitor,Nonselective, Nerve Poison, Protectants, Repellents and Stomach Poison. Another classification of pesticide is as per their acute toxicity, as classified by WHO (2009) and they are grouped into the following classes; Class Ia = extremely hazardous, Class Ib = highly hazardous, Class II = moderately hazardous, Class = III Slightly hazardous, and Class U = Unlikely to present acute hazard. Pesticides can also be grouped on the basis of their method of application and the timing of their application. Pesticides are also classified on how they are formulated as, liquid (emulsifiable concentrate EC, suspension concentrate, low concentrate liquid, flowable liquids etc.), dry [dust (D), granules (G), wettable powder WP, soluble powder, dry flowable, water-dispersible granule, baits, etc.], ULV, aerosols, etc.[6]

2.7 Health effects associated with pesticide exposure

Worldwide it is estimated that approximately 1.8 billion people engage in agriculture and most use pesticides to protect the food and commercial products that they produce. Others use pesticides occupationally for public health programs, and in commercial applications, while many others use pesticides for lawn and garden applications and in and around the home .Obviously, exposure to pesticides poses a continuous health hazard, especially in the agricultural working environment. By their very nature most pesticides show a high degree of toxicity because they are designed to kill certain organisms and thus create some risk of harm. Within this context, pesticide use has raised serious concerns not only of potential effects on human health, but also about impacts on wildlife and sensitive ecosystems.The World Health Organization has reported that roughly three million pesticide poisonings occur annually, resulting in 220.000 deaths worldwide.Agricultural pesticide poisoning is a major public health problem in the developing world, killing at least 250.000 – 370.000 people each year. The world-wide deaths and chronic diseases due to pesticide poisoning number about 1 million per year [13]

2.8 Toxicological impacts of pesticides on immune cells

The vertebrate immune system is a highly complex host defence system that protects the host from invading pathogens and environmental stimuli that can cause infectious diseases, cancer, and other.The immune

system is divided into two types: innate (antigen-nonspecific) and adaptive (antigen-specific), also known as humoral immunity and cell-mediated immunity. The immune cells that make up the immune system are also known as white blood cells or leukocytes. Neutrophils, eosinophils, basophils, lymphocytes, and monocytes are subsets of these. Each type of immune cell has unique functional characteristics, and they interact with one another in complex ways in the immune system. Lymphocytes are immune cells that include T cells, B cells, and natural killer (NK) cells. T cells, also known as T lymphocytes, are lymphocytes that develop in the thymus gland and play a role in cellular immune response [14]

2.8.1 Pesticides' Effects on T Cells

T cells are lymphocytes that are important in both adaptive and innate immunity. These cells are primarily involved in the activation of other immune cells and kill abnormal and cancerous cells directly. T cells are divided into several subtypes, including helper, effector, cytotoxic, memory, and regulatory T cells, and are involved in a variety of immune responses. Pesticides have been shown in previous studies to have negative effects on T cell viability and function by inducing apoptosis in various ways.

2.8.2 Pesticides' effects on B cells

B cells are lymphocytes that play a role in the adaptive immune system's humoral immunity. When the body is exposed to antigens, B cells mature into plasma cells capable of producing antigens via a T cell-dependent or -independent pathway. Memory B cells are a type of B cell that is activated by parental B cells and can initiate a stronger, faster antibody response. ATR, which has cytotoxic effects on T cells, has been found to affect the viability and function of B cells. In an in vitro experiment with mouse B cells treated with 0.001, 0.01, 0.1, 1, 10, and 100 M ATR, ATR inhibited LPS-stimulated B cell proliferation (lipopolysaccharide). ATR also reduced the number of developing B-blasts. B-blasts fusion with myeloma cells to form hybridomas, which produce monoclonal antibodies. As a result, ATR was discovered to reduce B cell proliferation as well as antibody production .Dihydrorotenone (DHR), a natural pesticide extracted from jicama vine seeds and stems, is produced by catalytic hydrogenation of retenone side chains. a natural pesticide derived from jicama vine seeds and stems. DHR is used to kill insects in plants by inhibiting mitochondrial function and ATP production .In a study of human plasma cells that had been differentiated from B cells and released various antibodies, DHR at concentrations of 5, 15, and 30 M induced apoptosis by decreasing mitochondria membrane potential and inducing ER stress and p38 signalling, interfering with the humoral immune response .

2.8.3 Pesticide effects on NK cells

NK cells are the most common type of innate lymphocyte that protects the body from tumours and infected cells. NK cells kill target cells by secreting cytokines, perforin, and granzyme, which cause apoptosis or necrosis in the target cells. The interaction between various receptors expressed on NK cells' surfaces and their ligands modulates their activities.Perforin and granzyme are primarily found in NK cells. Perforin creates pores in the cell membrane of target cells, while granzyme, also known as protease, enters the cytoplasm of target cells via the pores formed by perforin. These proteins cause apoptosis or osmotic lysis of

target cells in this manner.Previous in vitro studies on the immune toxicity of pesticides such as ziram, pentachlorophenol (PCP), atrazine, carbamate, and tributyltin (TBT) on human NK cells were conducted. Ziram is a dithiocarbamate fungicide used in agriculture and rubber products .PCP is a chemical that is used as an insecticide, fungicide, herbicide, molluscicide, and algaecide.As previously stated, carbamate, TBT, and atrazine are also widely used pesticides.

2.8.4 Pesticides effects on macrophages

Macrophages are phagocytic lymphocytes that regulate inflammation, pro-inflammation, and anti-inflammation in the innate immune system. Pathogen-recognition receptors on these cells cause phagocytosis and the production of various cytokines. Furthermore, macrophages aid in the initiation of adaptive immune responses by presenting antigen to T cells. Bifenthrin (BF) is a type of pyrethroid that is commonly used in pesticides and household insecticides.Because of its high photostability and low water solubility, BF has long residual times in soil .In mammals, BF is said to have neurotoxic, reproductive, hepatotoxic,nephrotoxic,and immune system interfering effects .BF at concentrations ranging from 0.5 to 20 g/mL decreased cell viability and induced apoptosis in Raw 264.7 cells, which are murine macrophages, by upregulating p53 and caspase 3 and downregulating Bcl-2. As a tumour suppressor, p53 has been shown to activate its downstream targets through an apoptotic pathway . Furthermore, BF inhibited the induction of IFN- mRNA expression in Raw 264.7 cells after infection with the Sendai virus, resulting in a loss of antiviral activity. BF also reduced the transcription of pro-inflammatory cytokines like IL-1, IL-6, and TNF-. These findings were linked to oxidative stress induced by BF in murine macrophages. Cypermethrin, another pyrethroid pesticide, induced apoptosis similarly to BF by increasing ROS and DNA damage. Cypermethrin at concentrations ranging from 0 to 200 m also caused macrophage death via cell cycle arrest and apoptosis mediated by the ROS-mediated MAPK pathway, as well as mitochondrial dysfunction with decreased mitochondrial membrane potential, ATP level, and mtDNA copy number. Furthermore, when treated with 25, 50, or 100 M cypermethrin metabolites such as 3-phenoxybenzoic acid (3-PBA), RAW 264.7 macrophages' phagocytotic activity was inhibited. Cypermethrin is an endocrine disrupting chemical that promotes cancer metastasis by inhibiting M1 macrophage development and increasing M2 macrophage polarisation.Silver nanoparticles (AgNPs) and organochlorine (OCP) pesticides were found to be immunotoxic, according to Glinski et al. AgNPs with antimicrobial and antifungal properties can enter organisms through the skin, lungs, and gastrointestinal tract and cause toxicity .Organic compounds containing covalently bonded chlorines are known as OCPs and have been used in a variety of applications, including pesticides. When primary mouse macrophages were treated with AgNPs at concentrations of 30, 300, or 3000 ng/mL and OPC at concentrations of 300 ng/mL, they reduced cell viability and phagocytosis activity via nitric oxide production, resulting in macrophage immunosuppressant.

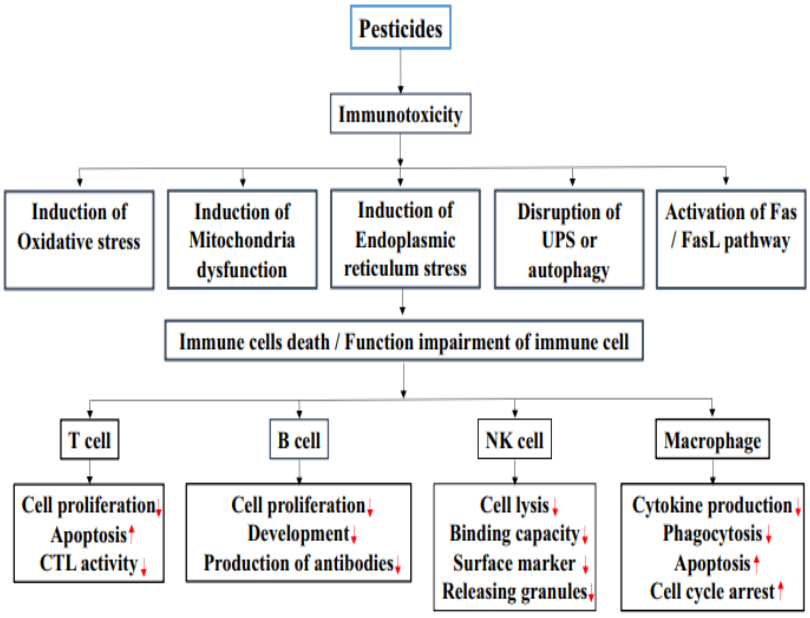


Figure 1: Pesticides affects on immune cells

2.9 Maximum residue level

Maximum residue levels are the highest levels of residues expected in food when pesticides are used in accordance with approved agricultural practises. MRLs are always set far below what is considered safe for humans. MRLs are not safety limits; a food residue can have a higher level than the MRL and still be safe for consumption. The safety limits are determined by comparing them to the acceptable daily intake (ADI) for short-term exposure or the acute reference dose (ARfD). Most countries have legal requirements regarding MRLs. In developed regions such as Europe, the European Commission (EC) is in charge of legislation, with input from member states, EFSA, and the Standing Committee on the Food Chain and Animal Health. The Environmental Protection Agency (EPA) is the leading agency in the United States, with input from the United States Department of Agriculture (USDA) and the Scientific Advisory Panel, while the New Zealand Food Safety Authority (NZFSA) is the leading agency in New Zealand, with input from the Environmental Risk Management Authority. MRL setting can be the responsibility of one or more authorities in a country and normally involves the health, agriculture and environmental agencies. MRL enforcement can be a responsibility of one or more agencies and may also depend on different food types. MRL setting is based on the national registered good agriculture practice (GAP) data combined with the estimated likely residue from the supervised trials mean residue (STMR) Children are exposed in different settings and by a variety of routes.

2.10 Introduction to beverages

Humans maintain their water balance by consuming an equal amount of water to that which is excreted. The body maintains water balance by controlling intake and excretion. The body, on the other hand, can suffer from either a negative or positive water balance. Negative water balance, also known as dehydration, is defined as a decrease in water and salt in varying proportions compared to the normal state, which can be

caused by a failure to replace obligatory water losses or a failure of the regulatory mechanism. Dehydration causes hypernatremia, which can be caused by one or more of the following factors: excessive sodium chloride loss, insufficient water intake, sodium chloride addition (salt poisoning), osmotic diuresis (with glucosuria), and diuretic therapy when free water intake is insufficient. Hyponatremic dehydration occurs when the body loses salt more than it loses water. Thirst motivates humans to consume fluids in order to maintain body fluid homeostasis and survive . To cover the water lost and maintain the water balance, a minimum of 1.44 L of water is required per day. Beverages may contribute to human water requirements. According to Troiano et al.beverages accounted for 20-24% of total energy intake. Drinks, in addition to meeting a basic need, are part of human society's culture. The types of beverages consumed have an impact on the composition of the modern diet. Despite the fact that all beverages contain water, water is not considered a beverage in and of itself. The term beverage has always been defined as referring to something other than water. Furthermore, these beverages have been reported to be among the top ten contributing foods for a variety of nutrients. Milk provides energy, protein, fat, calcium, and vitamin A, while fruit juices provide energy, calcium, iron, vitamin C, vitamin A, and fibre, and soft drinks provide energy and vitamin C (in fortified fruit drinks).

2.11 Types of beverages

According to Roethenbaugh, there are four primary sectors of the global commercial beverage market (Figure 1): hot drinks, milk drinks, soft drinks and alcoholic drinks. Hot drinks, include tea and coffee. Soft drinks have five main subcategories: bottled water; carbonated soft drinks; dilutables, (squash, powders, cordials and syrups); fruit juices (100% fruit juice and nectars (25–99% juice content); still drinks, including ready-to-drink (RTD) teas, sports drinks and other noncarbonated products with less than 25% fruit juice). Alcoholic drinks, including beer, wine, spirits, cider, sake and flavored alcoholic beverages. Amongst the different types of beverages, milk, soft drinks, and fruit juices are the most important and they are consumed in high amounts. Beverages could be also classified into alcoholic and non alcoholic drinks. An alcoholic beverage is a drink that contains ethanol. A non-alcoholic beverage is a drink that contains little or no alcohol. This category includes low-alcohol beer, non-alcoholic wine, and apple cider if they contain less than 0.5% alcohol by volume and they are called soft drinks.

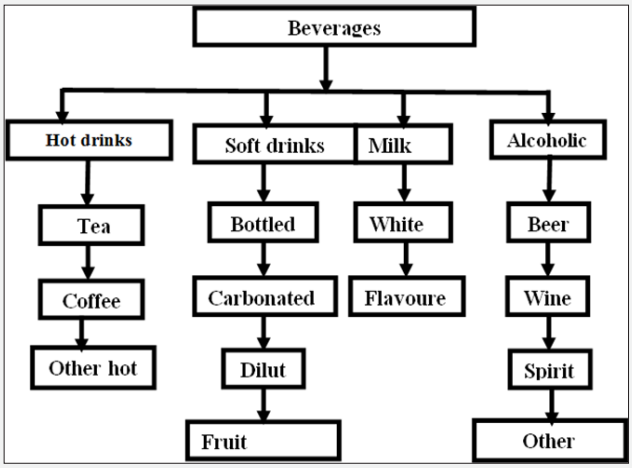


Figure 2: Beverage sectors and segments. Source: Soft drinks world, (2003).

Figure 2: Beverage sectors and segments

2.12 Soft drinks

There is no single definition available for soft drinks, but it is generally accepted that they are sweetened water-based beverages; usually with a balancing acidity. The term "soft drink" specifies the absence of alcohol in contrast to "hard drink". The term "drink" is neutral but often denotes alcoholic content. Small amounts of alcohol may be present in a soft drink, but the alcohol content must be less than 0.5% of the total volume if the drink is to be considered non-alcoholic. Soft drinks are refreshing beverages and they are typically formulated from 10 – 11% sugar content with about 0.3 – 0.5% of acid (usually citric) and flavoring, coloring and chemical preservatives, with the addition of carbon dioxide. A soft drink may also contain caffeine, fruit juice, or both. The widely sold soft drink flavors are cola, cherry, lemon-lime, root beer, orange, grape, vanilla, ginger, fruit punch, and sparkling lemonade. Various other additions can be made, including vitamins and minerals, clouding agents and foaming agents and plant extracts.

2.13 Types of soft drinks

Based on their ingredients, soft drinks are divided into carbonated and non-carbonated soft drinks. None carbonated soft drinks (NCSD): Non carbonated soft drinks are soft drinks without carbon dioxide and sparkling taste and they include fruit punch, fruit drinks, ice tea, coffee with sugar, and sport drinks. Non-carbonated soft drinks do not undergo carbonation process and do not have any sparkling flavor. The sugar used to sweeten the regular soft drinks is either sucrose or high fructose corn syrup. Regular soft drinks have approximately the same amount of sugar as a glass of pineapple or orange juice, 7-14g/100 mL. Diet soft drinks use aspartame, saccharine, acesulfam K, or sucralose as sweeteners A soft drink is slightly acidic in order to give pleasant tartness to the product and preserve it. The most common acidulants in soft drinks are citric acid and phosphoric acid.

2.14 Carbonated soft drinks (CSD)

Carbonated Soft Drinks are beverages with added carbon dioxide that gives an effervescent taste to the beverages. Carbonated soft drinks are further divided into colas and noncolas, as well as diet and regular soft

drinks. The cola-flavored carbonated beverages usually contain added phosphoric acid as acidulant because it can strengthen the acidity. Phosphoric acid has the same characteristics as the cola flavors, which are dry and sometimes balsamic. Cola soft drinks use cola nut from Cola nitida and Cola acuminata trees of Africa as their flavor agent. Non-cola soft drinks usually use citric acid as acidulant . Other categories of soft drinks are ready-to-drink soft drinks and dilute-to-taste soft drinks which are concentrated or in powder form.

2.15 Nutritional value of soft drinks

Water is an essential need for body. The need for water exists at the molecular, cellular, metabolic and functional levels. Water is a major solvent for the organic and inorganic chemicals involved in the biochemical reactions that are essential to life. Water is the principle medium that transports nutrients via body fluids to cell walls and act as carrier of nitrogenous waste products from the cells for ultimate elimination . Water also is a good regulator for body heat. About 60%, by weight, of a person body is water . A normal person experiences symptoms of dehydration when 5 – 10% of the body weight is lost as water and not soon replaced. Thirst drives human to drink fluid in order to preserve body fluid homeostasis and survive. Water may be obtained through several common beverages: plain water, bottled water, fruit and vegetable juices, fruit drinks, soft drinks, syrup, stimulant beverages, and milk. Several kinds of beverages are consumed not for food value but rather for thirst – quenching properties or for stimulating. Soft drinks are an essential vehicle for hydration. Soft drinks How to cite this article: Abdelazim Sayed and Abdelazim Abdellatif. The Beverages. Agri Res & Tech: Open Access J. 2018; 14(5): 555933. DOI: 10.19080/ARTOAJ.2018.14.555933 003 Agricultural Research & Technology: Open Access Journal are usually absorbed more readily than water (because of their osmolality), can replace lost salts and energy quickly and are rapidly thirst quenching. Their balance of sweetness and acidity, coupled with pleasant flavors, makes them attractive to all ages of consumers. Soft drinks and fruit juices are important beverages for adolescents because they are consumed at high amounts . Furthermore, Shachman reported that these beverages are in the top ten contributing foods for several nutrients, including carbohydrates, vitamins, minerals as well as energy. There are three main areas of particular nutritional significance for soft drinks. The first area is energy reported that 20 - 24% of energy intake came from beverages. Some soft drinks are formulated to deliver a rapidly assimilated energy boost to the consumer. Soft drinks generally contain soluble sugars, which are easy to administer. The second area of nutritional significance is that of the so-called isotonic drinks, which are of equivalent osmolality to body fluids. They promote extremely rapid uptake of body salts and water, and are very important products for sportspeople and others requiring almost instant hydration. Third, soft drinks have been widely formulated to low-calorie forms and these are now available for those who wish to enjoy such beverages and yet minimize their calorific intake. Other nutritional benefits that are claimed by some producers include the delivery of essential vitamins and minerals, especially to children.

2.16 Adverse effects of soft drinks

On the negative side, soft drinks have been claimed to have some adverse effects on human health. Wyshak [24] showed that all kinds of soft drink intake enhanced the bone fracture risk. Meanwhile, more recently, McGartland et al. [25] reported that the consumption of carbonated soft drinks did not affect bone health. The mechanism of this phenomenon is not known yet. Furthermore, cola beverage effects were more pronounced in more active girls and children. The phosphorus content in cola type carbonated beverages could have reduced levels of the active form of vitamin D and led to a decline in calcium absorption and to bone decalcification, increasing bone fracture risk [24,26,27]. The calcium: phosphorus ratio is a significant risk factor for bone fractures [27,28]. All cola beverages contain 40 to 70 mg phosphorus per 12 oz serving [29]. Studies indicated that caffeine could contribute to bone health. Heaney & Rafferty [30] detected higher calcium in urine after the subjects drank caffeinated cola beverages, indicating that the rise in calcium excretion was due to the caffeine. The authors proved that the mechanism of how phosphorus in soft drinks may impact on calcium metabolism is not through urinary calcium losses and they stated that drinking fruit juice can also lead to bone fracture. High potassium citrate in fruit juices was found to lower bone desorption Tylavsky et al. [31] and Marangella et al. [32]. The effect of sugars content of soft drinks was studied. Nguyen et al. [33] detected a negative impact of sugar on bone. Soft drinks contain a lot of sugar, especially fructose, but the diet soft drinks use primarily aspartame. Glucose intakes provoked a decrease in phosphatemia (high concentration of inorganic phosphates in blood) since phosphate followed glucose uptake into cells as required for glucose phosphorylation. The reduction of serum phosphate could stimulate mineral bone release, causing a rise in calcemia (excess calcium in blood).

2.17 Ingredients and formulation of soft drinks

The simplest form of soft drink contains a mix of the basic nutritional components; sugars, acids in water with flavoring, coloring and chemical preservatives, in addition to carbon dioxide to render the product ‘sparkling’, ‘effervescent’ or ‘fizzy’. Sadecka & Polonsky [39] reported a review on the determination of various compounds in beverage samples, including soft drinks, using electrophoretic methods. Taylor [53] summarized the components of soft drinks as shown in Table 1.

Component	Typical Use Level
Water	Up to 98%v/v
Sugars	7–12%m/v
Fruit juice	up to 10%, (widely variable usage)
High-intensity sweeteners	Use based upon sucrose equivalence (e.g. aspartame might be employed at 0.40–6%m/v as sole sweetener).
Carbon dioxide	0.30 –60%m/v
Acids (e.g. citric)	0.030–0.05%m/v
Flavors	Nature-identical and artificial: 0.10–28% m/m Natural: up to 0.5%m/m
Emulsion (flavor, color, cloud etc.)	0.1%m/v
Colors (natural or synthetic)	0-70ppm
Preservatives	Statutory limits apply (e.g. sorbic acid up to 250ppm in EU)
Antioxidants (e.g. BHA, ascorbic acid)	Less than 100ppm, subject to user-country legislation
Quillaia extract (saponins)	Up to 200mg/l (EU), up to 95mg/l (USA)
Hydrocolloids (mucilaginous gums)	0.1–0.2% per GMP, minimum amount required to create desired effect
Vitamins/Minerals	allowed daily intake applies

Table 1: Soft drink components

2.17.1 Water

Water is the main component of a soft drink, usually accounts between 85 and 95% of the product and acts as a carrier for the other ingredients. Water quality must conform to rigid requirements and not interfere with the taste, appearance, carbonation or other properties of the drink. It may be necessary to carry out treatment to improve the quality of the water used in the manufacture of soft drinks. Water should be free from: high levels of elements and mineral salts; objectionable tastes and odors; organic material. It is very important that water should also be clear; colorless and free from dissolved oxygen and microorganism [53].

2.17.2 Sweeteners

The profile of bulk and intense sweetener used in soft drinks has changed significantly over the last 10–15 years due to several reasons, including; reducing cost, developing new formulations and increasing consumer awareness of health leading to an increased number of low- and reduced-sugar formulations [54]. Sweeteners used in soft drink may be classified as carbohydrate sweeteners and artificial sweeteners. Carbohydrate-based sweeteners still represent the largest share of the global sweetener market and account for 81% of sweetener usage [55]. A number of carbohydrate sweeteners are used in soft drinks to provide different attributes, including sweetness, mouth feel, stability and, in some cases, color. Sucrose is regarded as the standard for a sweet taste. In soft drinks, glucose syrups are used to provide sweetness and mouth feel to products and occasionally specific physiological properties in sports and energy drinks.

2.17.3 Acidulants

A soft drink is slightly acidic in order to give pleasant tartness to the product and preserve it. The most common acidulants in soft drinks are citric acid and phosphoric acid [13]. The use of acidulants is an essential part of beverage formulation, with the acid component usually third in order of concentration. Acidulants performs a variety of functions in addition to their primary thirst-quenching properties, which are the result of stimulation of the flow of saliva in the mouth. Stampanoni [60] studied the influence of acid and sugar content on sweetness, sourness and the flavor profile of beverages and found that acid addition increased sourness and decreased sweetness, whereas sugar increased sweetness and depressed sourness. Melissa et al. [48]

2.17.4 Carbon dioxide and carbonation process

Beverages containing carbon dioxide are, nowadays, very popular products. The addition of CO_2 renders the product 'sparkling', 'effervescent' or 'fizzy', and the acid formed (carbonic acid) from carbon dioxide enhances the sharpness of taste [62]. Consumers enjoy their "pleasurable and sought after" sensation, despite the fact that they can be irritating, or even painful for some people. The sensation elicited by carbonated drinks are either of mechanical origin, due to the bursting CO_2 bubbles stimulating mechanoreceptors on tongue, or of chemogenic origin by formation of carbonic acid in a reaction catalysed by carbonic anhydrase, which stimulates polymodal nociceptors in the oral cavity [63]. It has been reported that bubbles appear when concentration levels of CO_2 are 3-5 times higher than the saturation equilibrium value and depend on the pre – existing gas liquid interfaces [64,65]. Numbers and sizes of these bubbles also have a sensory impact on the beverage, enhancing mass transport of CO_2 when the bubbles impinge upon the tongue and increase the "tingling" sensation [66,67]. The modification of the composition of soft drinks may dramatically affect the visual or taste perception of effervescence of the drink [66,67]. Also, the addition of CO_2 provides a very effective antimicrobial effect, especially against yeasts and moulds. Carbon dioxide is effective against yeasts because it tends to suppress the production of more CO_2 as a byproduct of the fermentation of sucrose to ethanol. It deprives moulds of the oxygen that most of them require for growth. Good hygiene standards are the norm in most soft drinks bottling operations today, and it is possible to produce carbonated drinks without chemical preservatives by flash-pasteurizing the syrup before it is mixed with carbonated water. The risk of microbiological spoilage is then low, but where multiserving containers are used the risk is increased because of the potential for subsequent contamination [54].

2.17.5 Flavors

A soft drink is slightly acidic in order to give pleasant tartness to the product and preserve it. The most common acidulants in soft drinks are citric acid and phosphoric acid [13]. Cola- flavored carbonated beverages use phosphoric acid as acidulant because it can strengthen the acidity and cola flavor at very low costs. Phosphoric acid has the same characteristics as the cola flavors, which are dry and sometimes balsamic [14,53]. Cola soft drinks use cola nut from *Cola nitida* and *Cola acuminata* trees of Africa as their flavor agent [15]. Non-cola soft drinks usually use citric acid. Flavors used in soft drinks can be divided into two main types based on solubility in water. Water-soluble flavors present no major problems when used in beverages, as there is ample water in a beverage formulation to dissolve and disperse the relatively small amounts of flavor required [12]. A flavor containing oil components that are not soluble in water cannot be used directly in the formulation. These components would separate out in the beverage and form a neck-ring in the beverage or, sometimes, in clear beverages, be seen as tiny suspended particles, giving the drink an unsightly appearance. To overcome this problem, an insoluble flavor is prepared by a flavor house in the form of an emulsion that can then be used in a soft drink formulation. These are the flavor emulsions that are used for flavoring the beverage as well as giving the beverage its cloudy appearance. It goes without saying that a flavor emulsion cannot be used in a clear beverage. Should a person want to use a particular water-insoluble flavor in a clear beverage, it is possible that the flavor house supplier may be able to modify the flavorant into a water-soluble form or supply a water-soluble variant of that particular flavor [52]. Most soft drink flavorants are used in liquid form, but powdered spray-dried flavors can also be used. Spray-dried flavors are mostly used in powdered instant beverage products. Again, the water-solubility factor must be taken into consideration. The flavor component of a typical carbonated soft drink (CSD) formulation may amount to anything from 10 to 50% of the total raw materials cost, including the sugar sweetener. Thus, the cost contribution level can sometimes be of great significance in the beverage formulation design. Therefore, it will depend on the sophistication involved in the product design, which, in turn, may depend on the target market as well as on the company's profit margin policies. All in all, flavorants present a costing factor that should not be ignored in beverage formulations, both in current existing products as well as in new product development. Flavorants are sometimes used in natural fruit juices as boosters of the natural fruit flavor.

Chapter-3

Instrumentation

3.1 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

3.1.1 Liquid Chromatography-Mass Spectrometry (LC-MS)

The High Performance Liquid chromatography (HPLC) is one of most common analytical technique used in pharmaceutical industry for determination and quantification of drug substances and its related substances. Due to high reproducibility and accuracy, HPLC is routinely used in pharmaceutical, chemical and pesticide industries. The Liquid Chromatography-Mass Spectrometry (LC-MS) is hyphenated analytical technique which is combination of Liquid Chromatography (LC) and Mass Spectrometry (MS). HPLC (LC) separates the components of mixtures by passing through chromatographic column. Generally, the separated components cannot be positively identified LC alone. Mass Spectrometry is also used for identification of unknown compounds, known compounds and to elucidate the structure. Mass spectrometry is alone not good for identifying mixtures because mass spectrum mixture is actually complex of overlapping spectra from separated individual components. It is difficult to connect Liquid chromatography (LC) with Mass spectrometry (MS). An interface is used to transfer the liquid eluents from LC to MS.LC-MS is more significantly used in invite dissolution, bioavailability, bioequivalence and pharmacy-dynamics studies [1]. Preparative LC-MS systems can be used for rapid mass-directed purification of specific substances from such mixtures that are important in basic research, pharmaceutical, agrochemical, food and other industries [2, 3].

3.1.2 Instrumentation - Liquid chromatography-mass spectrometry (LC-MS)

The Liquid Chromatography-Mass Spectrometry (LC-MS) is combination of Liquid Chromatography and Mass Spectrometry which is used with separation power of HPLC with detection power of Mass Spectrometry (MS). The schematic block diagram of LC-MS is shown in below figure 1. The different parts of LC-MS instrument are listed as below{Zhou, 2012 #21151}.

a. Liquid Chromatography (LC)

b. Mass Spectrometry (MS)

3.1.3 Liquid Chromatography (HPLC)

The Liquid Chromatography (LC) is a high performance liquid chromatography in which separation of components of mixture can be carried out by using liquid mobile and solid stationary phase. There are different types of chromatography like normal phase liquid chromatography, Reversed phase chromatography, Ion exchange liquid chromatography, chiral separation and affinity liquid chromatography [3]. By using different packing of columns with high efficiency small amount of complex mixture can be separated. The components of HPLC are listed below:-

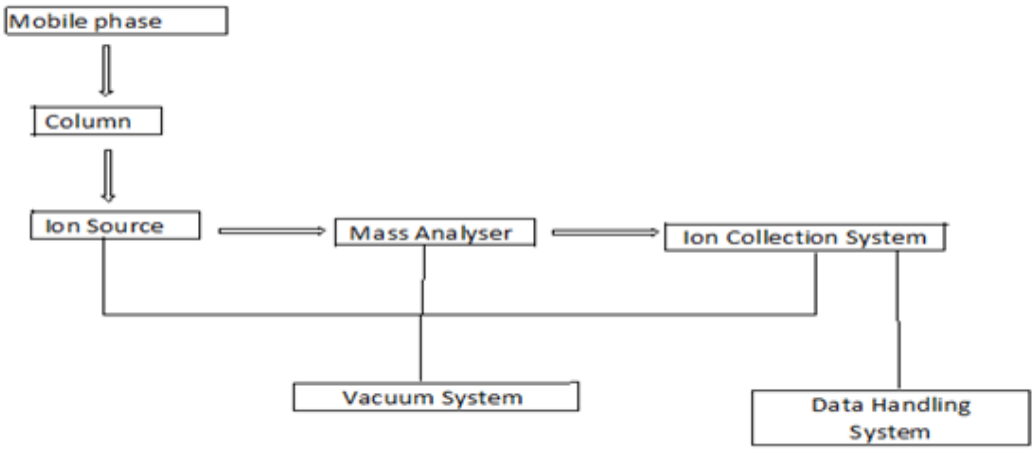


Figure 3: Schematic Block Diagram of LC-MS System.

3.1.4 Pump

It consists of material which is inert towards solvents or any mixed composition of aqueous buffer and organic solvents. It delivers high volume of mobile phase up to 10mL/min. There are three major types of pumps are used i.e. reciprocating pump, Syringe pumps and constant pressure pumps.

3.1.5 Sample injector

It is used to introduce sample volume into the chromatographic system. Generally sample volume from 1µL to 100µL can be injected. The injection volume can be increase by injector loop up to 2mL volume. There are two major types of injectors used i.e. Automatic injectors and Manual injectors. Automatic injectors are more comfortable and user friendly and are more accurate and precise as compare to manual injectors [3].

3.1.6 Columns

It is stationary phase which consists of silica material in combination with carbon chain. Generally the column length used is about 50mm to 300mm. The columns used in HPLC are consists of Octadecyl (C18), Octyl (C8), Cyano, Amino, Phenyl packing's. The columns are used on the basis of nature of compounds to be separated [4].

3.1.7 Detectors and recorder

The detectors is most important part of HPLC .There are different types of detectors used are UV-Visible detectors, PDA detectors, Refractive index (RI) detectors, Electrochemical detector, Fluorescence detectors and conductivity detectors. The signal received from detector can be recorded as peak and respective data can be stored in software.

3.1.8 Mass spectrometry

Mass Spectrometry is analytical technique based on the measurement of the mass to charge ratio of ionic species related to the analyte under the investigation.MS can be used to determine the molecular mass and elemental composition of an analyte as well as in depth structural elucidation of the analyte [5]. In LC-MS there are two key components, ionization source and Interfaces. Below listed are the different components of Mass spectrometers as below:-

A. Ionization Sources and Interfaces

B. Mass Analyzers

3.1.8.1 Ionization/Ion Source and Interfaces

The Liquid chromatography separates mixture of components which are in liquid form, usually contains methanol, acetonitrile and water. This liquid containing mixture of components is transferred into the ion source of mass spectrometer. As ion source is under high vacuum. Due to the difference in the pressure it is difficult to mass to vaporize the liquid drops without losing mixture of components. Hence interfaces are used to resolve this problem. The different types of interfaces commonly used in mass spectrometer are described as below.

3.1.8.1.1 Direct liquid Introduction (DLI)

The ionization in Direct Liquid Introduction (DLI) is generally accomplished by vaporizing solvent as a chemical ionization and reagent gas. Both the normal and reverse phase solvent system have been used. Reverse phase solvents used are methanol/water, acetonitrile/ water mixture up to 60% water. In general buffer with salts are not allowed as there is chance of capillaries to plug when heated.

The operation of Direct Liquid Introduction (DLI) is combination of thermal energy and liquid flow rate. The liquid enters the interface at limited flow rate only. The analyte ions produced with the help of thermal energy then transferred into ion source through capillary inlet or pinhole diaphragm [6,7].

3.1.8.1.2 Atmospheric-Pressure Ionization (API)

In Atmospheric-pressure ionization (API) contains three major steps i.e. Nebulization, Evaporation and Ionization. There are two main modes of API are Electrospray Ionization (ESI) and Atmospheric-pressure ionization (APCI). In Atmospheric-pressure ionization (API), when stream of liquid (solvent) containing a sample is passed through narrow capillary tube and nebulized at large chamber, mist of small droplets is produced .The ionization process takes place and the proportion of droplets carry an excess of positive or negative electric charge .In large heating chamber the evaporation of solvent takes place. The solvent evaporates from the droplets to form smaller and smaller. The collision takes place between the molecules and ions. The resulting ions then passed through capillary into mass analyser [2,8]. The Atmospheric-pressure ionization (API) is technique used for wide range of polar and non-polar analytes of moderate molecular weights.

3.1.8.1.3 Electrospray Ionization (ESI)

The Electrospray Ionization (ESI) is most useful ion source developed by Fenn and his colleague's. In Electrospray Ionization (ESI) the liquid sample passed through a stain steel capillary tube which is maintained at high positive or negative electric potential about 3-5kV [1]. Due to this the charged droplets are formed at the capillary tip which is then undergoes vaporization process. The solvent gets evaporated from droplets, and undergoes reduction in size and surface charge increases. The collision takes place until the highly charged droplets are converted into gas phase ions. These gas-phase ions pass through the capillary sampling orifice into the low pressure region of the ion source [9]. The major advantage of ESI is

that the ions are multiply charged, the number of charges increased by 1 to 3 for a molecule 1000Da or above 50000Da. This yields an m/z ratio that is always below 2000. LC-MS with an Electro spray ionization (ESI) is used to measure the molecular weight of peptides, Proteins, Biological samples, Polymers, nucleotides, sugars and organometallics. It is also used frequently in Biological research and medical analysis [10].

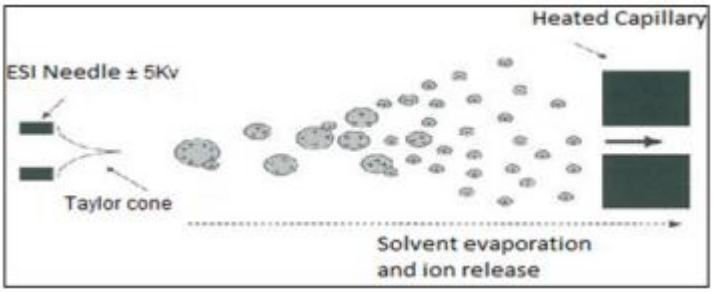


Figure 4: Electrospray ionization source.

3.1.8.1.4 Atmospheric Pressure Chemical Ionization (APCI)

The Atmospheric Pressure Chemical Ionization (APCI) include two major steps, evaporation /desolvations of analytes and charged transfer reaction in vapour phase to generate the vapour phase ions. In Atmospheric Pressure Chemical Ionization (APCI) liquid (solvent) containing sample is nebulised through narrow capillary tube and nebulized into large chamber. In large heating chamber the evaporation of solvent takes place at atmospheric pressure and small droplets are produced. The ionization takes place. Generally ionization takes place at 250 to 400 °C. The ions are then transfer the charges to molecules through chemical reactions. The resulting ions are pass through capillary orifice of mass analyser. It is widely used for less polar and non-polar analytes having moderate molecular weights [11].

3.1.8.1.5 Thermo spray and Plasma spray Ionization (TSPI)

The Thermo spray is used as both liquid inlet system as well as ionization source. Plasma spray is modification of thermo spray. In Thermo spray the liquid sample solution is passed through capillary tube which is heated and which causes the evaporation of solvent. The charged droplets are formed. Due to evaporation of solvent the droplets becomes smaller and smaller. The density of electric charge on the surface of droplets increases. The resulting ions are then passed into mass analyser with electrostatic voltage system [8]. The Plasma spray itself does not produce ions but the ions produce in thermo spray, with the help of corona discharge or plasma the number of ions can be increased. The electric discharge induces the more ionization in the neutral molecules. This enhancement increases the ionization of molecule. The plasma spray technique is more sensitive and it is widely used for analysis in clinical and medicine [12].

3.1.8.1.6 Atmospheric pressure photo Ionization (APPI)

In Atmospheric pressure photo Ionization (APPI), photons are used to excite and ionise the molecules. Atmospheric pressure photo Ionization (APPI) include mainly two steps i.e. excitation and ionisation of analyte from eluent. Like atmospheric pressure chemical ionization (APCI) in Atmospheric pressure photo

Ionization (APPI) the eluent from LC vaporize into gaseous phase. The APPI uses Kr lamp to produce photons. Kr lamp generates high energy photons which are used for excitation and ionization of molecules. The range of energy is selected to minimize the ionization of analytes. The ionized analytes are then transferred into capillary orifice into mass analyser (m/z). This technique is useful for non-polar analytes which very much difficult to ionize with Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) [13, 14].

3.1.8.1.7 Particle Beam Ionization

The Browner and his colleagues has developed particle beam interface to separate the solvent from solute with minimum loss of solutes. The nebulization and evaporation process are like Thermo spray (TSP), Atmospheric pressure chemical ionization (APCI), Electrospray ionization (ESI) [15]. In this liquid separated from HPLC or LC, eluent is passed through narrow tube. The liquid is injected with helium gas, due to this the spray of liquid droplets are formed with high velocity. The liquid drops from nebulizer passes through heating chamber, where the solvent begins to evaporate and liquid droplets becomes smaller and smaller. The spray of liquid droplets exits through heating chamber as a particle beam. Then this beam passes through ionization chamber similarly like Electro spray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) [2].

3.1.8.1.7 Continuous Flow Fast Atom Bombardment (FAB)

The FAB is simple, high sensitivity technique of interface. In FAB liquid target is bombarded by fast atoms such as Argon (Ar) or xenon. The sample is dissolved in glycerol and spread on thin layer metal plate /probe. Then this probe is inserted into mass spectrometer and a beam of fast moving atoms bombards on probe and ionise the samples which then pass into mass analyser (m/z). FAB is used for large and thermally unstable molecules. It used for surfactants and proteins [16,17].

3.1.8.2 Mass Analyser

After ionization the ions are transferred into mass Analyser where the separation of ions are done according to their mass to charge (m/z) ratio. Generally mass Analyser used is on its speed, time, rate and its reaction. Below are the mass Analyser:

- Quadrupole
- Time of flight
- Ion trap
- FTICR (Fourier transfer ion cyclotron resonance)

3.1.8.3 Quadrupole Mass Analyser

It is the most useful and commonly used mass Analyser. It consists of two plain of parallel rods which are located between an ion sources and a detector. The mass Analyser i.e. separation of ion according to their m/z in either time or space [6].The linear Quadrupole mass Analyser consists of four hyperbolic or cylindrical rods that are placed parallel in a radial array. Opposite rods are charged is a +ve or –ve direct current(DC) potential at which an oscillating radio frequency alternating current (RF) voltage is

superimposed [18]. The combination of DC and RF applied to the rods, trajectories of the ions of one particular m/z are stable these ions are transmitted towards detector. On the other hand ions of unstable m/z are discharged on the rods. The ions introduced into Quadrupole by mean of low accelerating potential. The ions are oscillating in plane perpendicular to the rod length as they trends through Quadruple filter. Ions of carrying m/z consequently be travelled towards detector by applying DC and RF voltage at constant ratio. The resolution depends on ratio of DC and RF potentials. Generally the Quadrupole is operated at < 4000 m/z and scan speed up to 1000m/z passes. The unit mass resolution means that mass accurately is seldom better than 0.1 m/z. [19].

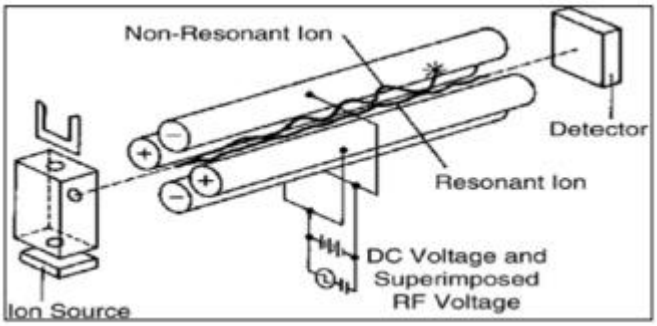


Figure 5: Schematic Diagram of Quadrupole Mass analyser.

3.1.8.4 Time of Flight Analyser (TOF)

The time Flight is most robust used for wide variety of ions sources and inlet systems. In this is no any magnetic field, maintenance and calibration it is just simple electrostatic and straight forward. The ions are extracted from source and subjected to an accelerating voltage. The time taken to travel the length of the drift or flight to be depends upon the mass of ion and its charge [20]. For single charged ions (z=1, m/z =w) the time taken to reach the detector is proportional to mass of the ions.When the ions trends towards the detector the lighter ions will strikes the detector first[18]. Scanning of all the ions are detected simultaneously. The scanning the mass range is very rapid and can be used for very large m/z values. The schematic diagram of Time Flight mass analyser is shown below figure 4.

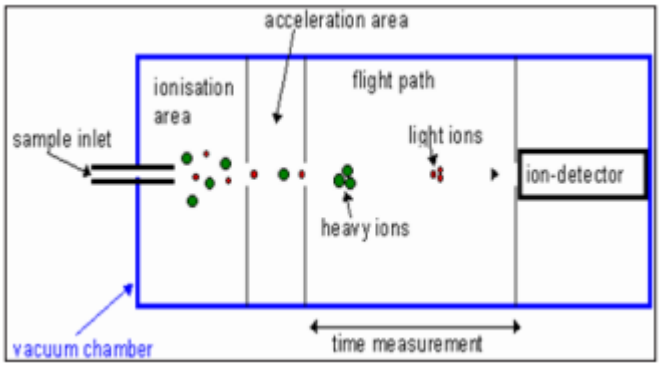


Figure 6: Time of flight mass Analyser

3.1.8.5 Ions trap mass Analyser

Ion trap mass Analyser is high resolution; high sensitivity and multiple product ion scan capability. A Quadrupole ion trap is a three dimensional ion trap. It consists of cylindrical ring electrode to which Quadrupole field is applied. Another two are end capped electrodes [21]. One end cap electrode has single small central aperture through which electrons or ions are introduced into the trap while other one has several apertures or holes through which ions are passed to a detector. A Helium bath gas is present in the trap to stabilize the ion trajectories. The collision takes place between helium bath gas and ions. Due to this the motion of ions increases the trapping efficiency of analyser. The ions are ejected from the trap on the basis of mass to charge (m/z) values to create the mass spectrum [22]. The schematic diagram shown below figure

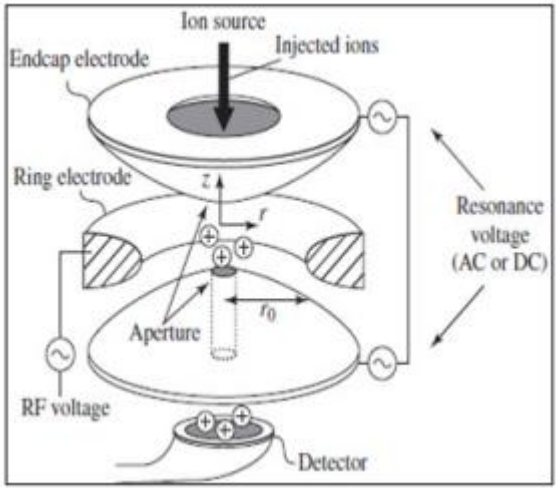


Figure 7: Ion Trap mass Analyser.

3.1.8.6 Fourier Transfer Ion Cyclotron Resonance (FT-ICR)

The Fourier transfer ion cyclotron resonance (FT-ICR) is most important mass analyser. The ions arrived from ionization source are passed into mass analyser where they are separated according to their m/z ratio. The ions entered in chamber are trapped in circular orbits. The ions are accelerated by both electric field and magnetic field. Due to this the ions get excited and generate time dependent current. The ions trapped separated according to mass to charge (m/z) ratios. Detectors the detector is an important tool of mass spectrometer that produces the current that is proportional to the number of ions strike it. Once the ions are formed passed from analyser they have to be detected and transformed into signal. Below listed are the types of detectors commonly used.

3.1.8.7 Point Ion Collectors Detector

In this the ions collectors are placed at fixed point in mass spectrometer. All the ions are focused upon the detector situated at single point. The arrivals of ions can be recorded by the flow of electric current and the data can be recorded. The electric current flow is proportional to the ions arriving at point ion detector.

Array Detector: An Array detector is collection of point collectors placed in plane. The ions are arrived at a point or across the plane in array detector. The ions with mass to charge (m/z) values are separated and are recorded along plane using point ion collector. Spatially differentiated ions with the mass range are detected simultaneously at the same time in array detector [23,24]

3.2 GAS CHROMATOGRAPHY

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture. In some situations, GC may help in identifying a compound. Only volatile compounds are tested in GC. GC is the most sensitive.

There are three components of GC, these are:

- Injector
- Oven
- Detector

Also there are two phases in GC:

Stationary phase - Column (different types of column according to the compounds).

Mobile phase – Gas

The gases used in GC are Nitrogen, Hydrogen and Zero Air. Nitrogen goes in column and is used as carrier gas. Hydrogen is the fuel gas while zero air is used for ignition and contains 20% O2.

In gas chromatography, the mobile phase is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. Helium remains the most commonly used carrier gas in about 90% of instruments although nitrogen is preferred for improved separations. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chromatograph.

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

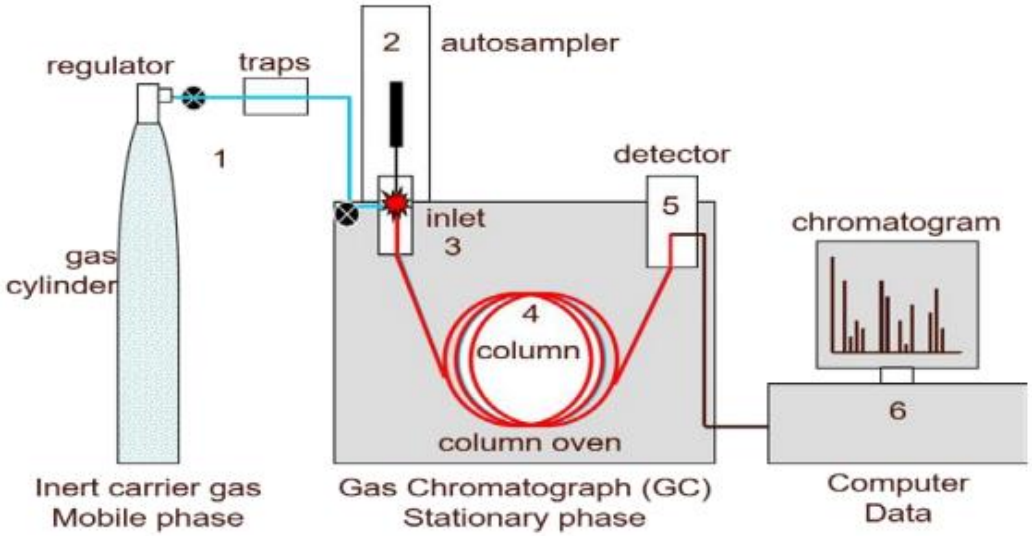


Figure 8: Diagram of gas chromatography instrument

The process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled. Finally, the concentration of a compound in the gas phase is solely a function of the vapour pressure of the gas. Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point differences.

3.2.1 MASS SPECTROMETRY

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. In a typical MS procedure, a sample is loaded onto the MS instrument and undergoes vaporization. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions). The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields. The ions are detected, usually by a quantitative method. The ion signal is processed into mass spectra. Additionally, MS instruments consist of three modules:

The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

3.2.2 COMPONENTS

The instrument consists of three major components

- Ion Source: For producing gaseous ions from the substance being studied.
- Analyzer: For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.
- Detector System: For detecting the ions and recording the relative abundance of each of the resolved ionic species.

In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10-6 to 10-8 mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries

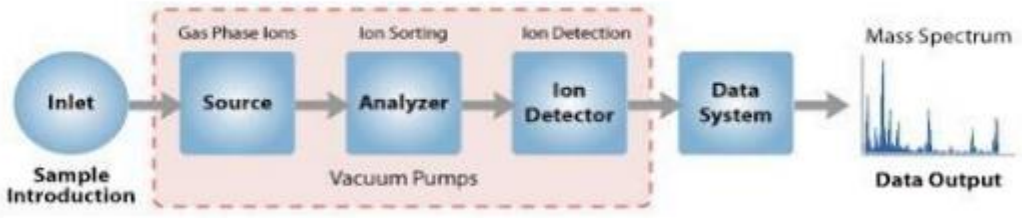


Figure 9: Components of a Mass Spectrometer

With all the above components, a mass spectrometer should always perform the following processes:

- Produce ions from the sample in the ionization source.
- Separate these ions according to their mass-to-charge ratio in the mass analyzer.
- Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
- Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
- Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

3.3 GC-MS Gas chromatography–mass spectrometry (GC-MS)

Is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also

be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. Like liquid chromatography–mass spectrometry, it allows analysis and detection even of tiny amounts of a substance. GC-MS has been regarded as a "gold standard" for forensic substance identification because it is used to perform a 100% specific test, which positively identifies the presence of a particular substance. A nonspecific test merely indicates that any of several in a category of substances is present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification. However, the high temperatures (300°C) used in the GC-MS injection port (and oven) can result in thermal degradation of injected molecules, thus resulting in the measurement of degradation products instead of the actual molecule of interest.

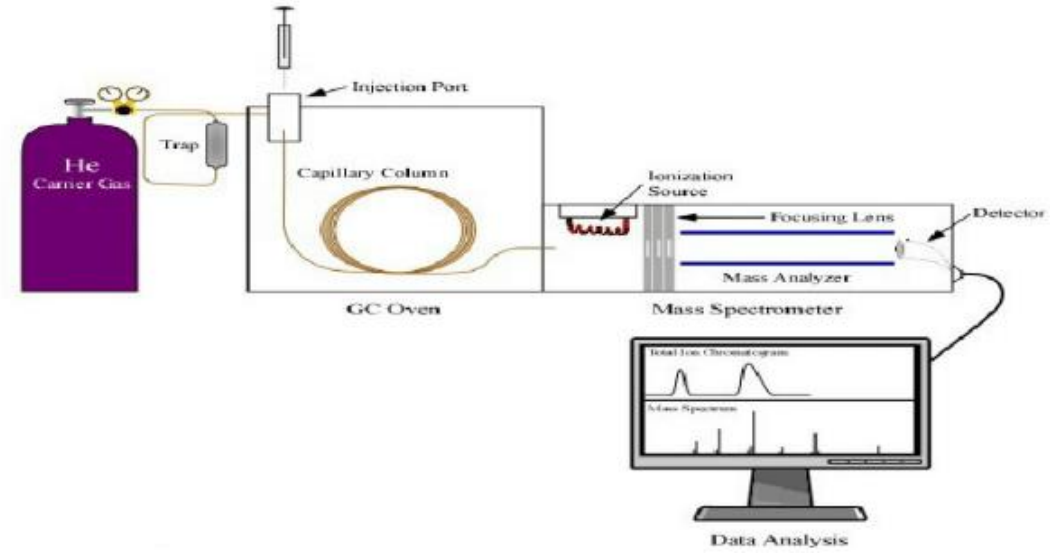


Figure 10: Schematic working of GC-MS.

3.4 GC-TANDEM MS (GC-MS/MS)

When a second phase of mass fragmentation is added, for example using a second quadrupole in a quadrupole instrument, it is called tandem MS (MS/MS). MS/MS can sometimes be used to quantitate low levels of target compounds in the presence of a high sample matrix background.

The first quadrupole (Q1) is connected with a collision cell (Q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed. Types of analysis include product ion scan, precursor ion scan, selected reaction monitoring (SRM) (sometimes referred to as multiple reaction monitoring (MRM)) and neutral loss scan. SRM is highly specific and virtually eliminates matrix background.

Samples being analyzed by GC-MS/MS are separated in a gaseous state based on the various physical and chemical properties of analytes of interest and their interaction with the analytical column's stationary phase. Upon exiting the analytical column, the analytes enter the tandem mass spectrometer (MS/MS) which consists of two scanning mass analyzers separated by a collision cell. Fragments selected in the first analyzer are reacted with an inert gas in the collision cell, resulting in further fragmentation. These daughter product ions are then resolved in the third quadrupole for analysis.

GC-MS/MS analysis can be performed on liquids, gases or solids. For liquids, the sample is directly injected into the GC. For gases, gastight syringes are used to transfer the gaseous components directly into the GC. For solids, the analysis is carried out either by solvent extraction, outgassing or pyrolysis analysis. The analytes of interest are then quantified through comparison to external or internal standards. In addition to the quantification, GC-MS/MS is well-suited for the identification of unknown volatile components using the mass fragmentation patterns and mass transitions associated with the unknown analyte.

Chapter-4

Materials and Methods

4.1 QUECHERS Method

In recent years, QuEChERS has been recognized as the most modern procedure for extraction. In recent years, the trends have been directed towards the decrease in the sample amount for analyses with the approach which is safer and less damaging to the environment, such as QuEChERS, and which, at the same time, implies a quicker and simpler way of sample preparation, ensuring high yields and good precision.

Anastaideset al. (2003) developed quick, easy, cheap, efficient, rugged and safe method (QuEChERS) in order to overcome the limitations of the existing preparation methods, which was successfully used in the analyses of pesticides from various matrices. With certain modifications, QuEChERS has become a method of interest for the extraction and purification of samples and for the Mycotoxins determination.

The QuEChERS procedure is based on an initial single-phase extraction with Acetonitrile. A liquid- liquid partition is created by adding excess salts and buffers to the extract. After centrifugation, the acetonitrile layer containing the analytes of interest is collected. The sample matrix can be further cleaned and excess water removed with a single d-SPE step by mixing acetonitrile extract with anhydrous MgSO4 and primary secondary amine (PSA) sorbents. Additional sorbents such as C18 or graphitized carbon black (GCB) could be added as needed for specific matrix cleanup. The final extract can be analyzed directly by either GCMS-MS or LS-MS/MS with a simple dilution.

A modification of the well-known and accepted QuEChERS sample preparation procedure AOAC Official 2007.01 Method was used for analysis of samples. In the method, MgSO4 was used to remove residual water, PSA for the removal of polar interferences, including sugars and organic acids, Graphitized carbon black (GCB) was used to effectively remose pigments such as chlorophyll and carotenoids.

4.2 SOP FOR ESTIMATION OF PESTICIDE RESIDUES IN Alcoholic and Non-Alcoholic Beverages

4.2.1 SCOPE

This SOP is applicable for estimation of Pesticide Residues in Alcoholic and Non- alcoholic Beverages by GCMS/MS.

4.2.2 PRINCIPLE

Pesticide Residues is extracted from fruits and vegetables with Acetonitrile by QuEChERS method for GC/MS-MS.

4.2.3 EQUIPMENTS AND CONSUMABLES

4.2.4 EQUIPMENTS

- Gas Chromatography -Tandem Mass Spectrometer - Agilent 7000C Series , 7890B GC-MS/MS (or) equivalent
- Ultra-Homogenizer with 15000 rpm
- Nitrogen Concentrator Low Volume , temperature controlled

- Centrifuge to hold 100 ml centrifuge tubes to operate @ 4°C
- Micropipettes 10-100 µL capacity and 100-1000 µL capacity, calibrated
- Refrigerated Centrifuge capacity at minimum 10000 rpm , -10 °C to ambient
- Ultrasonic Bath, temperature controlled
- Mechanical Shaker , Horizontal
- Vortex Mixer

4.2.5 CHEMICAL AND GLASSWARE

- Volumetric Flasks - 10ml and 100 ml
- Polypropylene Centrifuge Tubes 50ml and 15 ml
- Auto sampler vials
- Glass beaker 500 mL
- Sodium Chloride, AR Grade
- Sodium Sulphate, AR Grade
- Ethyl acetate, HPLC Grade
- Cyclohexane
- MTBE (Methyl Tert-butyl ether)
- Anhydrous Sodium Sulphate, AR Grade
- Acetonitrile MS / HPLC Grade
- Certified Reference Materials

4.3 PROCEDURE

4.3.1 Preparation of Standards

4.3.1.1 Preparation of Pesticide Standard Stock Solution (1000 mg/L)

Weigh equivalent to 10 mg of standard into a 10ml volumetric flask and dissolve in HPLC grade Ethyl acetate for GC-MS/MS. Make up the volume with the same. Beverages mix (10 ppm) stock solution Date of preparation (10/04/2022) And Date of expiry (10/4/2023), solution in a refrigerator @ 4-8 °C and store for 6 months. Validity is applicable for the reconstituted standards as per Certificate of Analysis if the 10 mg is fully consumed for stock preparation. Prepare the stock standards individually:-

Working Std. Conc.(mg/L)	Volume Taken from Stock (mL)	Volume of Diluent (mL)	Final Volume (mL)	Final Conc. (mg/L)	Validity Store @	Solvent for dilution
100	1	9	10	100	4-8 °C ± 2°C	GC = Ethyl Acetate
1	0.1	9.9	10	1	4-8 °C ± 2°C	

Preparation Working Standard: Table- 2

Working Std. Conc.(mg/L)	Volume taken from Working Std (µL)	Volume of Diluent (µL)	Final Volume (mL)	Final Conc. (ppb)	Solvent for dilution
1	5	995	1	5	GC = Ethyl Acetate
1	10	990	1	10	
1	20	980	1	20	
1	50	950	1	50	
1	100	900	1	100	
1	150	850	1	150	

Calibration Standards – GCMS-MS: Table-3

4.4 EXTRACTION PROCEDURE

4.4.1 For NON- Alcoholic Beverages

- Take 15 ml sample into a 50 ml centrifuge tube.
- Add 15 ml Acetonitrile Containing 1% acetic acid and vortex for 1 min.
- Add 6 g $MgSO_4$ and 1.5g Sodium Acetate shake vigorously and Vortex for 1 minute.
- Centrifuge @ 6000rpm for 5 minute.
- Transfer 8.0ml of Supernatant into 15 ml centrifuge tube containing 150 mg $MgSO_4$ and 150 mg PSA and Vortex for 30 Sec.
- Centrifuge @ 10000rpm for 5 min.
- Transfer 2 mL of supernatant and evaporate upto dryness under nitrogen.
- Reconstitute the residue in 2 mL ethyl acetate, vortex for 30 sec and transfer the residues in GC-MS auto-sampler vials.

4.5 EQUIPMENT METHODS

Agilent 7000C & 7890B GCMS-MS Conditions for Multi Residues Method:

Equipment	Gas Chromatograph- Mass Spectrometer (GCMS-MS)		
Make & Model	AGILENT- 7000C; 7890B		
Column	HP5-MS (30 m X 250 mm x 0.25 µm)		
Gas Chromatography Conditions			
Injection Volume	1 µl		
Oven Program			
	Temp (°C / min)	Temp (°C)	Hold Time (Min.)
		70	2
	25	150	0
	3	200	0
	8	280	10
Injector temperature program	Temp (°C / min)	Temp((°C)	Hold Time (Min.)
Total Flow	25 ml/min -	280	-
Purge Flow to split vent	30 ml/min		
Carrier Gas	Helium		
Carrier gas flow rate	2.66 ml/min		
Maximum Temperature	325 °C		
Run time	41.86 min		
MSMS Conditions			
Acquisition Mode		MRM	
Collision Gas		Nitrogen	
Ion Source Temperature			

Agilent 7000C & 7890B GCMS-MS Conditions for Multi Residues Method-Table 4

4.6 SOP FOR ESTIMATION OF PESTICIDE RESIDUES IN Alcoholic & Non-Alcoholic Beverages

4.6.1 SCOPE

This SOP is applicable for estimation of Pesticide Residues in Alcoholic & Non-Alcoholic Beverages by LC-MS/MS.

4.6.2 PRINCIPLE

Pesticide Residues is extracted by liquid-liquid extraction and after extraction method for LC-MS/MS.

4.6.3 EQUIPMENTS AND CONSUMABLES

4.6.3.1 EQUIPMENTS

- Liquid Chromatography -Tandem Mass - Agilent 6460 with 1260 Series LC-MS/MS (or) equivalent
- Ultra-Homogenizer with 15000 rpm
- Nitrogen Concentrator Low Volume , temperature controlled
- Centrifuge to hold 50 ml and 15 ml centrifuge tubes to operate @ 4°C

- Micropipettes 10-100 µL capacity and 100-1000 µL capacity, calibrated
- Refrigerated Centrifuge capacity at minimum 10000 rpm , -10 °C to ambient
- Ultrasonic Bath, temperature controlled
- Mechanical Shaker , Horizontal
- Vortex Mixer

4.6.4 CHEMICAL AND GLASSWARE

- Volumetric Flasks - 10ml and 100 ml
- Polypropylene Centrifuge Tubes 15ml and 50 ml
- Auto sampler vials
- Primary Secondary Amine (PSA),Agilent(Part No: 5982-5753)
- C18, Agilent (Part No. 1221-3024)
- Sodium Chloride, AR Grade
- Magnesium sulphate anhydrous (≥99.5%)
- Tri sodium Citrate dehydrate
- Disodium hydrogen citrate sesquihydrate
- Anhydrous Sodium Sulfate, AR Grade
- Ammonium formate, MS Grade
- Acetonitrile MS / HPLC Grade
- Methanol, MS Grade
- Milli-Q-water
- Formic Acid 99% MS Grade
- Certified Reference Materials

4.7 PROCEDURE

4.7.1 Preparation of Standards

4.7.1.1 Preparation of Pesticide Standard Stock Solution (1000 mg/L)

Weigh equivalent to 10 mg of standard into a 10ml volumetric flask and dissolve in HPLC grade Acetonitrile for LC-MS/MS. Make up the volume with the same. Beverages mix (10 ppm) stock solution Date of preparation (10/04/2022) And Date of expiry (10/4/2023), and store the solution in a refrigerator @ 4-8 °C and store for 6 months. Validity is applicable for the reconstituted standard as per Certificate of Analysis if the 10 mg is fully consumed for stock preparation. Prepare the stock standards individually.

Working Std. Conc.(mg/L)	Volume Taken from Stock (mL)	Volume of Diluent (mL)	Final Volume (mL)	Final Conc. (mg/L)	Validity Store @	Solvent for dilution
100	1	9	10	100	4-8 °C ± 2°C	LC = Methanol
1	0.1	9.9	10	1	4-8 °C ± 2°C	

Preparation Working Standard: Table-5

Working Std. Conc.(mg/L)	Volume taken from Working Std (µL)	Volume of Diluent (µL)	Final Volume (mL)	Final Conc. (ppb)	Solvent for dilution
1	5	995	1	5	LC = Methanol
1	10	990	1	10	
1	20	980	1	20	
1	50	950	1	50	
1	100	900	1	100	
1	150	850	1	150	

Calibration Standards – LCMS MS: Table-6

4.8 EXTRACTION PROCEDURE

4.8.1For NON-Alcoholic Beverages

- Take15 ml sample into a 50 ml centrifuge tube.
- Add 15 ml Acetonitrile Containing 1% acetic acid and vortex for 1 min.
- Add 6 g $MgSO_4$ and 1.5g Sodium Acetate shake vigorously and Vortex for 1 minute.
- Centrifuge @ 6000rpm for 5 minute.
- Transfer 8.0ml of Supernatant into 15 ml centrifuge tube containing 150 mg $MgSO_4$ and 150 mg PSA and Vortex for 30 Sec.
- Centrifuge @ 10000rpm for 5 min.
- Transfer 2 mL of supernatant and evaporate upto dryness under nitrogen concentrator at 40 degree temperature.
- Reconstitute the residue in 2 mL (50:50) ACN: WATER, vortex for 30 sec and transfer the residues in LC/MS/MS auto-sampler vials.

4.9EQUIPMENT METHODS

Agilent 6460 & 1260 Series LC-MSMS Conditions -Multi Residue Method

Equipment	Liquid Chromatography-Tandem Mass Spectrometer (LCMSMS)		
Make & Model	AGILENT 6460 –MSMS, 1260 Infinity –LC		
Column	Zorbax RRHD-C18,1.8 µm, 3.0 X 100 mm, Pressure-600 bar		
Liquid Chromatography Conditions			
Mobile Phase A	5mM Ammonium formate and 0.01% Formic acid in water		
Mobile Phase B	Acetonitrile (0.1%)		
Flow Rate	0.5ml/minute		
Injection Volume	2.0 µl/min		
Column Temperature	40°C		
Mode	Gradient		
	Time (Min.)	**A %**	**B %**
	0	90	10
	1	90	10
	3	10	90
	8	10	90
	9	90	10
	15	90	10
MSMS Conditions			
Mode	ESI Positive, Negative		
Gas temperature (°C)	300		
Gas flow (l/min)	5		
Nebulizer (psi)	45		
Sheath Gas Temp.	250		
Sheath Gas Flow	5		
Capillary voltage	3000		
Delta EMV	500		

Agilent 6460 & 1260 Series LC-MSMS Conditions -Multi Residue Method Table-7

4.10 BATCH SEQUENCE AND QUALITY CONTROL ACCEPTANCE CRITERIA

S. No.	QC Point	Criteria	Run
1	Solvent Blank	Analyte Free	
2	Calibration Standard- 5 Levels	$R^2 \geq 0.9500$	each 1
3	Matrix Blank	Analyte Free (or) <30% LOD	1
4	QC Recovery @ LOQ	70% to 120% Refer Validation data (or) as prescribed in the Standard	1
5	Reagent Blank	Analyte Free	1

Batch Sequence and Quality control Acceptance criteria- Table 8

4.11 FORMULAE

Reference Material – Salt & Purity Correction Formula:

$$X = \frac{(Molecular\ weight\ of\ Compound\ with\ salt - \text{Salt Mass})}{Molecular\ weight\ of\ Compound\ with\ salt} * Reference\ Material\ Weight * Purity$$

Purity = x/100 where x = percentage of compound

Final concentration in solution Y (mg/L) = X *1000/V where V is the makeup volume.

4.12 CALCULATION

$$Conc, mg/Kg = \frac{(Concentration\ From\ calibration\ X\ Volume\ made\ up\ )}{Wt.of\ the\ sample}$$

$$Conc, ppm = \frac{(Sample\ Area\ XStandard\ Conc.in\ ppmX\ Volume\ make\ up\ )}{Standard\ Area\ X\ Volume\ \ of\ the\ sample\ Taken}$$

4.13 Results with Recovery Correction

Calculated concentration X Recovery factor

Chapter-5

Results

5.1 ANALYTICAL VALIDATION

5.1.1 LINEARITY

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration (amount) of analyte. Linearity should be evaluated visually to plot signals as a function of analyte concentration.

For the establishment of linearity a minimum of 5 different concentrations are recommended. Linearity is determined by analyzing reference material of at least 5 different concentrations within the linear range and calculates the regression coefficient, y-intercept, slope of the regression line.

Acceptance Criteria for linear quantification, regression coefficient (R2) for analytical standard solution should be ≥ 0.99

5.2 RECOVERY

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated oils samples were spiked with known concentration of the pure Aldrin and Dieldrin standard solutions. The concentration of each pesticide in the final extracts was calculated by:

$$\textit{Recovery}\ \% = \frac{\textit{Spiked Value}}{\textit{Spiked Concentration}} \times\ 100$$

According to sante guidelines, recovery should be in range between 70% - 120%. And from recovery quantitative analysis of both Aldrin and Dieldrin is done by:

$$\textbf{Result (ppb)} = \frac{\textbf{Sample Response}}{\textbf{Recovery}} \times 100$$

Table:-9 Results of quantitative analysis of Beverages:-

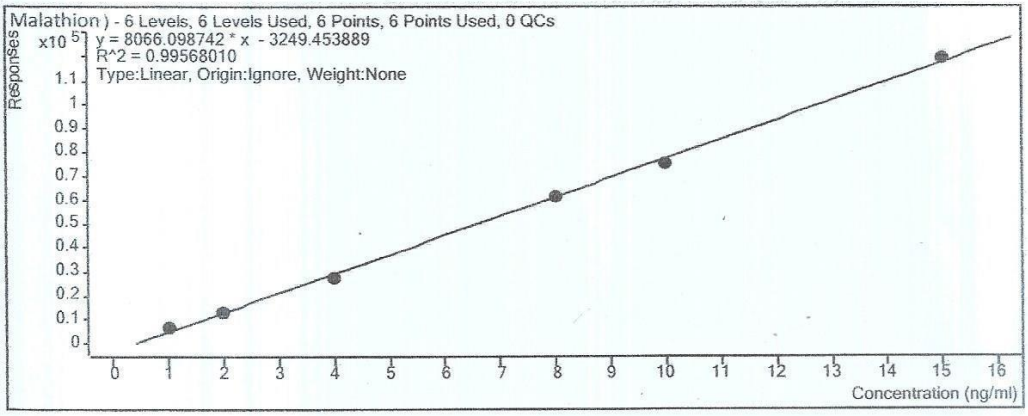
These results are calculated from below graph by using above formula:-

S.NO	Name of Parameter	MRL (µg/Kg)	Coca-Cola (µg/Kg)	Maaza (µg/Kg)	String (µg/Kg)	7 UP (µg/Kg)
1	Malathion	10	1	2.318	2.0814	0.999
2	Lindane	Not Specified	0.822	0.922	0.9488	0.9036
3	Chloryiphos	Not Specified	0.521	0.1569	0.0056	0.1267
4	Atrazine	Not Specified	0.625	0.121	0.00314	0.1805

GRAPH

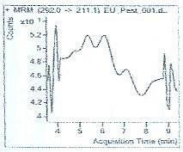
Quantitative Analysis Compound Report

Malathion



Data File	Type	Name	Compound	Final Conc	Units
Beverage_project_01.D	Blank	Blank	Malathion	0.0021	ng/ml
Beverage_project_02.D	Blank	Reagent Blank	Malathion	0.0043	ng/ml
Beverage_project_03.D	Blank	Matrix Blank	Malathion	0.0022	ng/ml
Beverage_project_04.D	Calibration	MB_0.01 ppb	Malathion	10.1125	ng/ml
Beverage_project_05.D	Calibration	MB_0.025 ppb	Malathion	25.4465	ng/ml
Beverage_project_06.D	Calibration	MB_0.05 ppb	Malathion	49.9865	ng/ml
Beverage_project_07.D	Calibration	MB_0.075 ppb	Malathion	76.2352	ng/ml
Beverage_project_08.D	Calibration	MB_0.1 ppb	Malathion	98.7786	ng/ml
Beverage_project_09.D	Calibration	MB_0.150ppb	Malathion	150.2125	ng/ml
Beverage_project_10.D	Blank	Blank	Malathion	0.0036	ng/ml
Beverage_project_11.D	Sample	Cocacola_sample	Malathion	0.0019	ng/ml
Beverage_project_12.D	Sample	Cocacola_spike_0.01 ppb	Malathion	9.4859	ng/ml
Beverage_project_13.D	Sample	7UP_sample	Malathion	0.0028	ng/ml
Beverage_project_14.D	Sample	7UP_spike_0.01 ppb	Malathion	9.4786	ng/ml
Beverage_project_15.D	Sample	String_sample	Malathion	0.0049	ng/ml
Beverage_project_16.D	Sample	String_spike_0.01 ppb	Malathion	9.3549	ng/ml
Beverage_project_17.D	Sample	Maaza_sample	Malathion	0.0035	ng/ml
Beverage_project_18.D	Sample	Maaza_spike_0.01 ppb	Malathion	9.2846	ng/ml
Beverage_project_19.D	Blank	Blank	Malathion	0.0047	ng/ml

Blank



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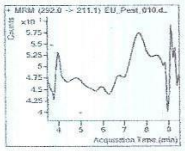

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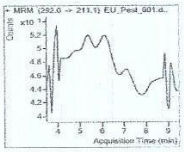

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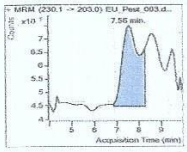
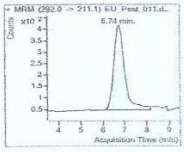


MB_0.01 ppb



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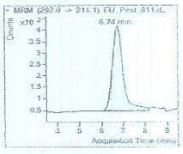

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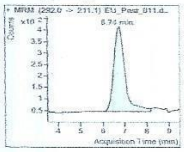
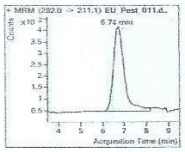
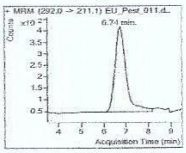


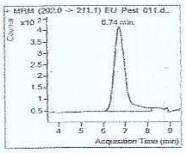
MB_0.075 ppb



MB_0.1 ppb



MB_0.150 ppb



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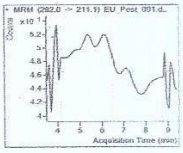
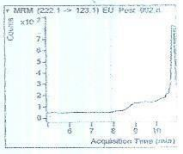
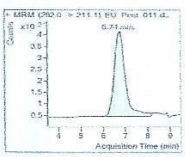


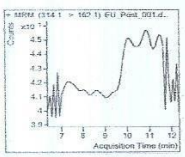
Cocacola_sample



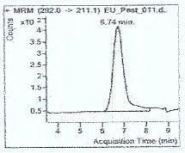
Cocacola_spike_0.01 ppb



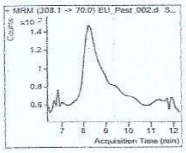
7UP_sample



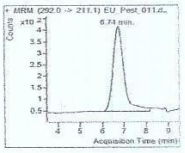
7UP_spike_0.01 ppb



String_sample

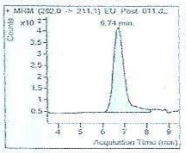


String_spike_0.01 ppb



Maaza_sample

Maaza_spike_0.01 ppb



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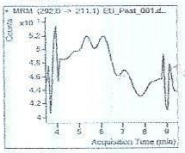
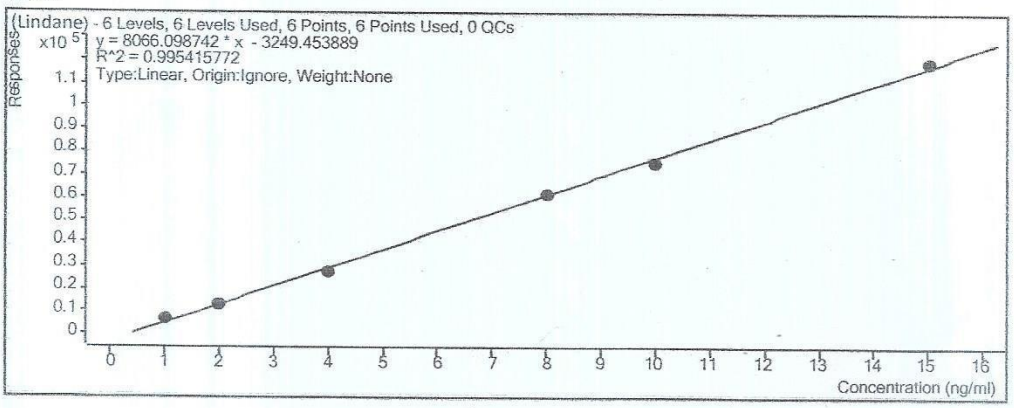
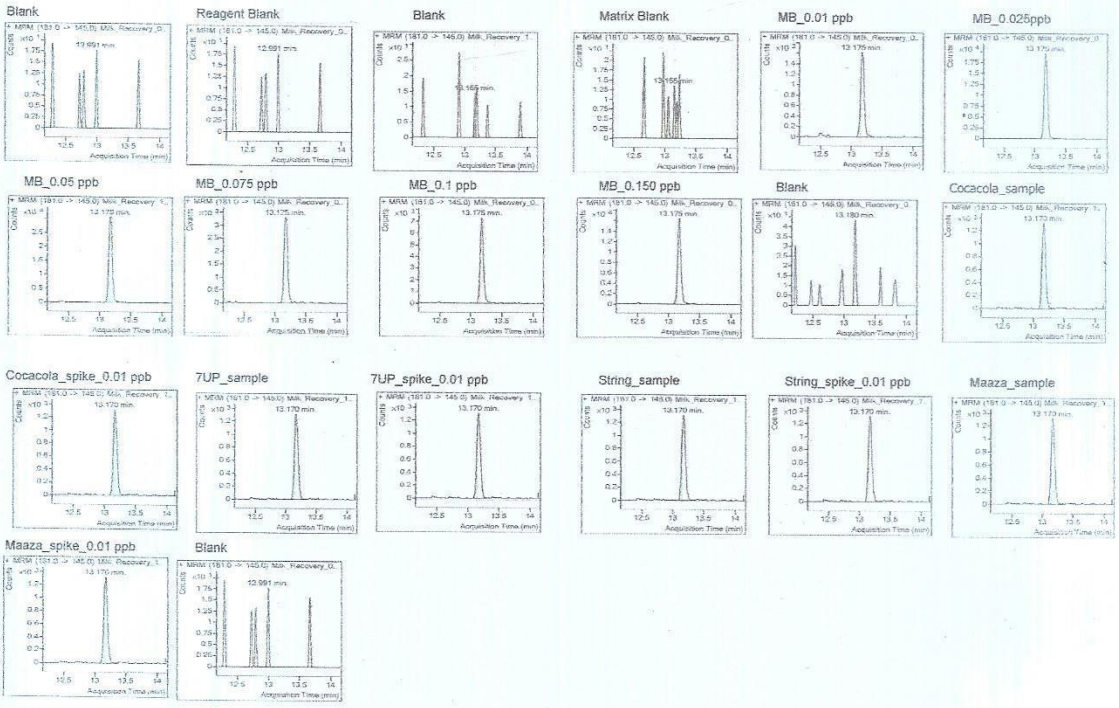


Quantitative Analysis Compound Report

Lindane

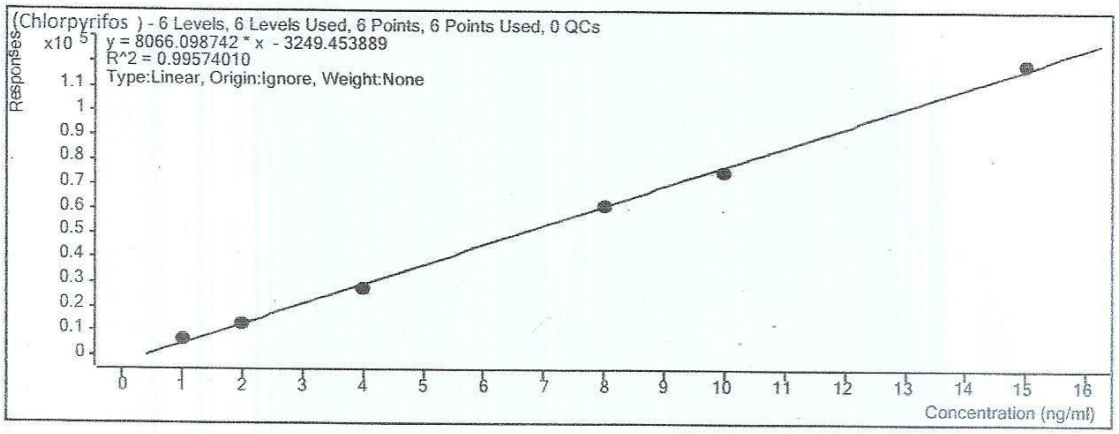


Data File	Type	Name	Compound	Final Conc	units
Beverage_project_01.D	Blank	Blank	Lindane	0.0023	ng/ml
Beverage_project_02.D	Blank	Reagent Blank	Lindane	0.0048	ng/ml
Beverage_project_03.D	Blank	Matrix Blank	Lindane	0.0015	ng/ml
Beverage_project_04.D	Calibration	MB_0.01 ppb	Lindane	10.2515	ng/ml
Beverage_project_05.D	Calibration	MB_0.025 ppb	Lindane	25.0215	ng/ml
Beverage_project_06.D	Calibration	MB_0.05 ppb	Lindane	50.8459	ng/ml
Beverage_project_07.D	Calibration	MB_0.075 ppb	Lindane	74.9585	ng/ml
Beverage_project_08.D	Calibration	MB_0.1 ppb	Lindane	100.5483	ng/ml
Beverage_project_09.D	Calibration	MB_0.150ppb	Lindane	15.4850	ng/ml
Beverage_project_10.D	Blank	Blank	Lindane	0.0021	ng/ml
Beverage_project_11.D	Sample	Cocacola_sample	Lindane	0.0024	ng/ml
Beverage_project_12.D	Sample	Cocacola_spike_0.01 ppb	Lindane	9.2548	ng/ml
Beverage_project_13.D	Sample	7UP_sample	Lindane	0.0025	ng/ml
Beverage_project_14.D	Sample	7UP_spike_0.01 ppb	Lindane	9.2890	ng/ml
Beverage_project_15.D	Sample	String_sample	Lindane	0.0032	ng/ml
Beverage_project_16.D	Sample	String_spike_0.01 ppb	Lindane	9.4255	ng/ml
Beverage_project_17.D	Sample	Maaza_sample	Lindane	0.0022	ng/ml
Beverage_project_18.D	Sample	Maaza_spike_0.01 ppb	Lindane	9.2488	ng/ml
Beverage_project_19.D	Blank	Blank	Lindane	0.0020	ng/ml

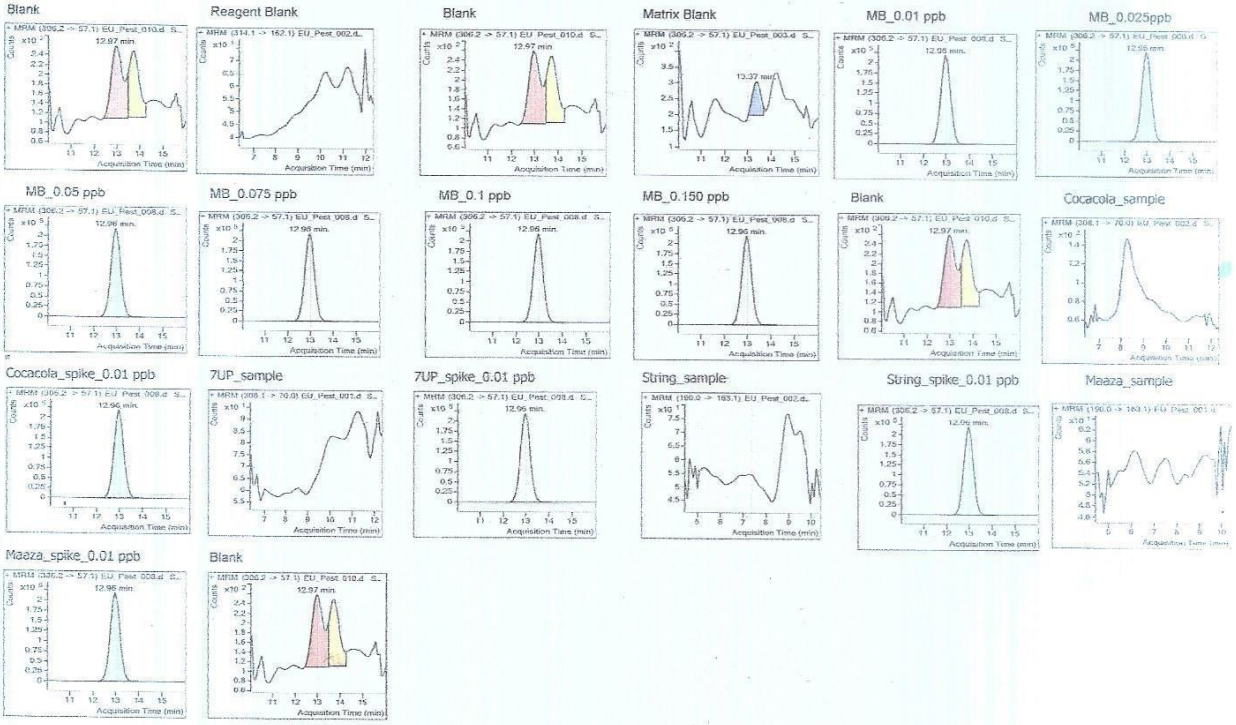


Quantitative Analysis Compound Report

Chlorpyrifos

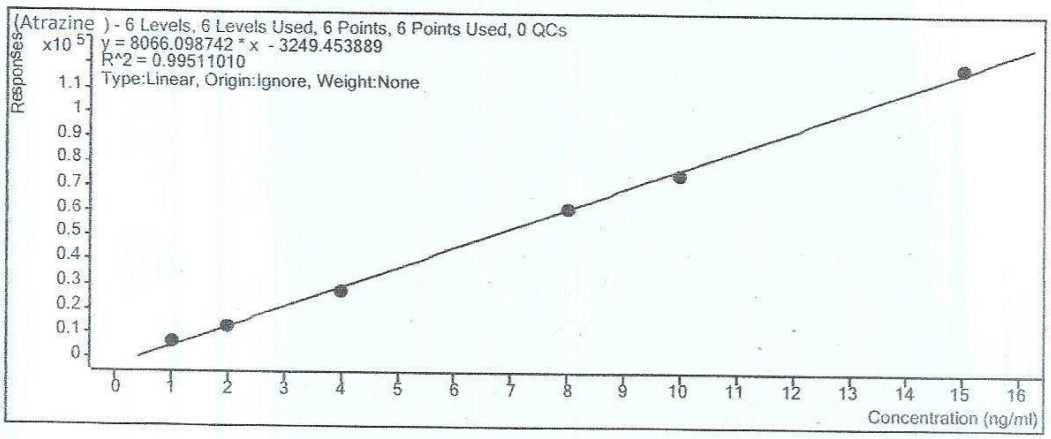


Data File	Type	Name	Compound	Final Conc	units
Beverage_project_01.D	Blank	Blank	Chlorpyrifos	0.0017	ng/ml
Beverage_project_02.D	Blank	Reagent Blank	Chlorpyrifos	0.0026	ng/ml
Beverage_project_03.D	Blank	Matrix Blank	Chlorpyrifos	0.0045	ng/ml
Beverage_project_04.D	Calibration	MB_0.01 ppb	Chlorpyrifos	10.1525	ng/ml
Beverage_project_05.D	Calibration	MB_0.025 ppb	Chlorpyrifos	25.0145	ng/ml
Beverage_project_06.D	Calibration	MB_0.05 ppb	Chlorpyrifos	50.1248	ng/ml
Beverage_project_07.D	Calibration	MB_0.075 ppb	Chlorpyrifos	75.0143	ng/ml
Beverage_project_08.D	Calibration	MB_0.1 ppb	Chlorpyrifos	100.6260	ng/ml
Beverage_project_09.D	Calibration	MB_0.150ppb	Chlorpyrifos	150.2459	ng/ml
Beverage_project_10.D	Blank	Blank	Chlorpyrifos	0.0021	ng/ml
Beverage_project_11.D	Sample	Cocacola_sample	Chlorpyrifos	0.0078	ng/ml
Beverage_project_12.D	Sample	Cocacola_spike_0.01 ppb	Chlorpyrifos	9.4264	ng/ml
Beverage_project_13.D	Sample	7UP_sample	Chlorpyrifos	0.0045	ng/ml
Beverage_project_14.D	Sample	7UP_spike_0.01 ppb	Chlorpyrifos	9.1254	ng/ml
Beverage_project_15.D	Sample	String_sample	Chlorpyrifos	0.0062	ng/ml
Beverage_project_16.D	Sample	String_spike_0.01 ppb	Chlorpyrifos	9.3482	ng/ml
Beverage_project_17.D	Sample	Maaza_sample	Chlorpyrifos	0.0037	ng/ml
Beverage_project_18.D	Sample	Maaza_spike_0.01 ppb	Chlorpyrifos	9.3585	ng/ml
Beverage_project_19.D	Blank	Blank	Chlorpyrifos	0.0047	ng/ml



Quantitative Analysis Compound Report

Atrazine



Data File	Type	Name	Compound	Final Conc	units
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Beverage_project_02.D	Blank	Reagent Blank	Atrazine	0.0046	ng/ml
Beverage_project_03.D	Blank	Matrix Blank	Atrazine	0.0032	ng/ml
Beverage_project_04.D	Calibration	MB_0.01 ppb	Atrazine	10.3252	ng/ml
Beverage_project_05.D	Calibration	MB_0.025 ppb	Atrazine	25.8476	ng/ml
Beverage_project_06.D	Calibration	MB_0.05 ppb	Atrazine	49.8956	ng/ml
Beverage_project_07.D	Calibration	MB_0.075 ppb	Atrazine	75.4859	ng/ml
Beverage_project_08.D	Calibration	MB_0.1 ppb	Atrazine	101.6326	ng/ml
Beverage_project_09.D	Calibration	MB_0.150ppb	Atrazine	150.4860	ng/ml
Beverage_project_10.D	Blank	Blank	Atrazine	0.0023	ng/ml
Beverage_project_11.D	Sample	Cocacola_sample	Atrazine	0.0025	ng/ml
Beverage_project_12.D	Sample	Cocacola_spike_0.01 ppb	Atrazine	9.3585	ng/ml
Beverage_project_13.D	Sample	7UP_sample	Atrazine	0.0034	ng/ml
Beverage_project_14.D	Sample	7UP_spike_0.01 ppb	Atrazine	9.4252	ng/ml
Beverage_project_15.D	Sample	String_sample	Atrazine	0.0015	ng/ml
Beverage_project_16.D	Sample	String_spike_0.01 ppb	Atrazine	9.3486	ng/ml
Beverage_project_17.D	Sample	Maaza_sample	Atrazine	0.0031	ng/ml
Beverage_project_18.D	Sample	Maaza_spike_0.01 ppb	Atrazine	9.4152	ng/ml
Beverage_project_19.D	Blank	Blank	Atrazine	0.0019	ng/ml

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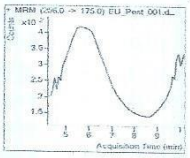

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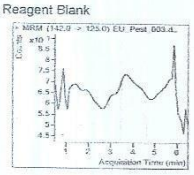

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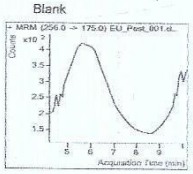

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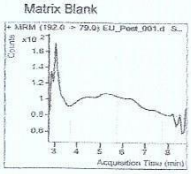

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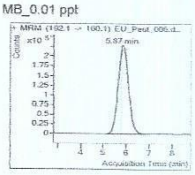

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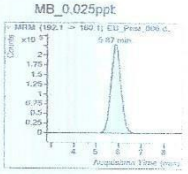

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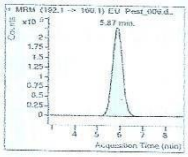

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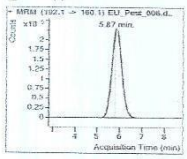

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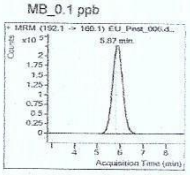

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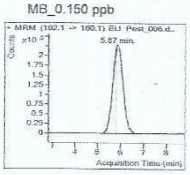

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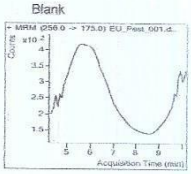
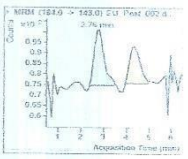


Cocacola_sample



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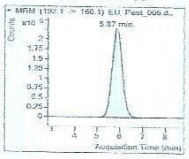
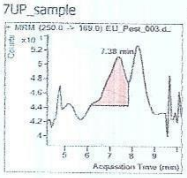


7UP_sample



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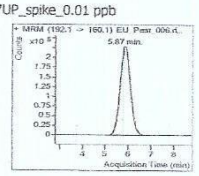

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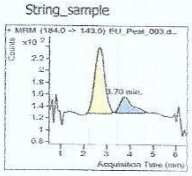

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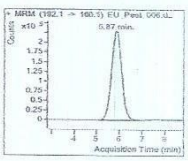
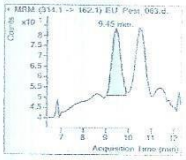
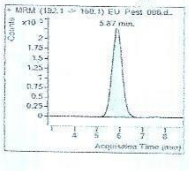


Maaza_sample



Maaza_spike_0.01 ppb



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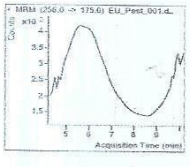

CONCLUSIONS

Pesticide prevalence is becoming increasingly inevitable due to the inability to absolutely control insects and pest. Pesticide contamination in Beverages cannot be overlooked as far as since these are the staples food of major population in India. However, with appropriate measures such as awareness creation and proper management practices, the prevalence of the toxin can be minimized, if not eradicated.

In the experimental part of this work, we have done quantitative analysis of Pesticide in Beverages Plant by liquid chromatography tandem to mass spectrometry & amp; Gas Chromatography mass spectrometry, which is distinguished by its simplicity, speed and sufficient precision to separate the contaminated samples and this, concluded that:

The Pesticide residue was found to be very less than the MRL (Maximum Residue Limit) which is the amount or limit of residue that should be present in a food product given by legal authority.

In the tested Beverages the result was BLQ (Below Limit of Quantification) which means our Beverages are safe to drink and are not harmful.

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