

A PROJECT REPORT ON
DETERMINATION OF PESTICIDE RESIDUE IN TEA SAMPLES BY USING
CHROMATOGRAPHY TECHNIQUES



Submitted by

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With regards,

Parismita Bordoloi

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ABSTRACT

The project work entitled "DETERMINATION OF PESTICIDE RESIDUE IN TEA SAMPLES BY USING CHROMATOGRAPHY TECHNIQUES" was carried out at Analytical service Department, Tocklai Tea Research Institute, Jorhat. The pesticide residue part describes the detection of the amount of pesticides residues in tea after application of pesticides formulation to control pest and diseases. The Biological formulation analysis work checks the presence of chemical pesticides in bio-products.

1. INTRODUCTION



Pests are organisms that invade an environment created by humans and destroy or damage crops or other resources. They include flies, caterpillars, fungi, weeds and microorganisms. Pesticides means any substance intended for preventing, destroying, repelling or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities or animal feeds or which may be administered to animals for the control of ectoparasites. Pesticides are vital for crop production and have been instrumental in continuous increase in food production.

If pesticides are used indiscriminately, it will lead to high residue in tea and invite other problems like pest-resistant and resurgence besides creating environmental pollution.

Today the global perception on quality of food has changed. The awareness on presence of undesirable substance like pesticide residues, heavy metals and micotoxins in food is on the increase. Presence of these substance residues the quality enhance the consumer acceptability of the food items.

Considering these facts and to keep the tea we drink safe, various national and international food safety agencies like 'Codex', 'European Union (EU)', 'Food Safety Standard Authority of India (fssai)' under the supervision of W.H.O. and F.A.O. has fixed an acceptable criteria called MRL (Maximum Residue Limit) for tea.

MRL is established taking into account the toxicological data of pesticides as well as that of residues of crops under good agricultural practices (GAP). It also reflects the residues that one would obtain using the minimum quantities of pesticides necessary to achieve adequate pests

control applied in a manner so that the amount of residue is the smallest practicable and is toxicological acceptable.

In recent times, a lot of emphasis is given towards switching over from chemical pesticide to biological pesticide to achieve pest/disease control as well as sustainability of the environment. Now a-days planters have been compelled to look for safer alternatives mainly in the form of bio-products. Bio-pesticides are mostly either microbial or plant origin products. There is a spurt in the production of plant based (botanical) pesticides and this is largely welcome, because these products are supposed to be free from any synthetic pesticides. Thus, application of these compounds should not result in any pesticide residues in tea. However, there are reports of detection of residue of chemical pesticides in tea produced organically or in conventional teas which was never been treated with the detected pesticide formulations but treated with market available bio pesticides. Doubts persists that the bio pesticides are mixed with chemical pesticides for better performance. Investigation of some major brands of commercial bio-pesticide formulation showed the presence of chemical pesticides. As a precautionary measure, a prior testing of bio-pesticide is advisable before use.

2. OBJECTIVE OF THE PROJECT WORK

DETERMINATION OF PESTICIDE RESIDUE IN TEA SAMPLES BY USING GAS CHROMATOGRAPHY AND SCREENING OF CERTAIN BIOLOGICAL FORMULATION BY USING GAS CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

3. METHODOLOGY

The project was carried out at the Analytical Services Dept., Tocklai Tea Research Institute, Jorhat, Assam.

DURATION OF PROJECT WORK: From 4.12.2017 to 29.12.2017

4. EQUIPMENT, APPARATUS & REAGENTS FOR PESTICIDE RESIDUE ANALYSIS IN TEA:

- **Glassware :**
 - (i) Stopped reagent bottle(500ml)
 - (ii) Measuring cylinder Class-A(50ml,250ml)
 - (iii) Separating funnel (500ml)
 - (iv) Stopped conical flask(100ml,250ml)
 - (v) Volumetric pipette- Class A(5ml)
- Analytical balance
- Homogenizer
- Rotary vacuum evaporator
- Gas liquid chromatograph
- Chemicals and reagent :
 - i) Acetone
 - ii) n -Hexane
 - iii) 5 % Sodium Chloride Solution
 - iv) Anhydrous Sodium Sulphate
 - v) Glass wool
 - vi) Alumina

Standard pesticide stock solution

EQUIPMENT, APPARATUS & REAGENTS FOR BIO-FORMULATION ANALYSIS:

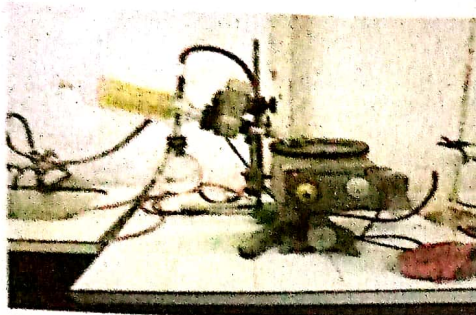


Fig: Rotary Evaporator

- **Glassware :**
 - (i) Test tube (25 ml.)
 - (ii) Pipette –Class A(2ml,5ml)
 - (iii) Measuring Cylinder (Class A-25ml.)
- **Analytical balance**
- **HPLC**
- **Standard pesticide stock solution**
- **Chemicals and reagent :**
 - i) Acetonitrile
 - ii) Methanol
 - iii) n-Hexane
 - iv) 10% Sodium Chloride solution
 - iv) N₂ gas

5. INSTRUMENTATION

Introduction to Gas liquid chromatography(GC) and High Performance Liquid chromatography(HPLC)

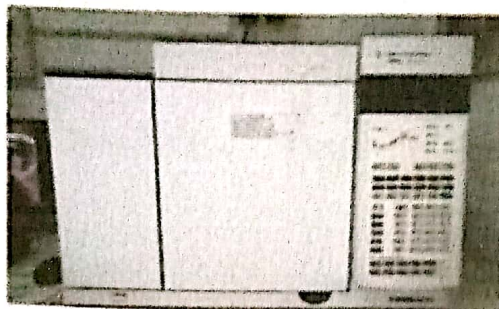


Fig:Gas Liquid Chromatography

- Gas Liquid chromatography,(GLC) is basically a separation technique in which the compounds of a vapourised sample are separated and fractionated as a consequence of partition between a mobile gaseous phase and a stationary phase held in column. The partition takes place between a gas and liquid or gas or solid. When the stationary phase is a liquid , the technique is known as gas liquid chromatography(GLC).Gas chromatography is also termed as a technique which is generally applied to separations of gas phase mixtures by interaction with a high boiling liquid or a solid . Gas chromatograph separates components of a gaseous mixture in a column and detects the components as they are eluted. The response of the detector is recorded as peaks.



a) COMPONENTS OF GC:

- A tank of carrier gas.
- An injection port of the sample.
- The column.
- Detector with appropriate readout.

Gas liquid chromatography(GLC) instrument set-up for analysis

- Model : Agilent 7890A
- Manufacturer : Agilent Technologies
- Carrier gas used : Nitrogen
- Column used : Agilent J& W CP-Sil 5 CB(30m x 0.25mm x 0.25 μ m)
- Detector used : ECD (Electron Capture Detector)

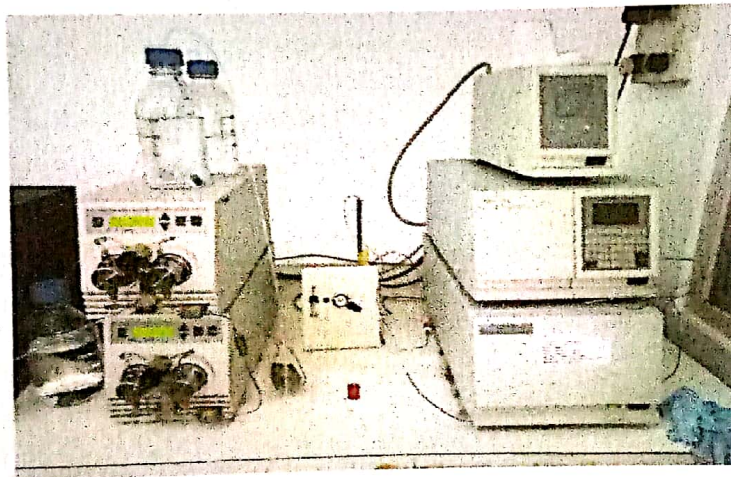


Fig: High Performance Liquid Chromatography

- The High Performance Liquid Chromatography ,(HPLC) is a method of separation in which the stationary phase is contained in a column, one end of which is attached to a source of pressurized liquid eluent (mobile phase).A mixture of solutes injected to the column is separated into components on moving down the column and the individual solutes are scanned by the detector and recorded as peaks. HPLC columns can be run isocratically (i.e. with a constant composition eluent) or they may run in the gradient elution mode in which the composition of mobile phase varies during the run.

b) COMPONENTS OF HPLC:

- A high pressure pump
- A column
- A detector
- Injector system.

High performance liquid chromatography instrument set-up

- Model : Waters
- Manufacturer : Waters
- Column used : Sunfire C18 (5 μ m x 4.6 μ m x 250mm)
- Detector used : PDA (Photo Diode Array)
- Pump used : Waters HPLC M515
- Mobile phase : Methanol

6. LIST OF PESTICIDE RESIDUE ANALYSIS FACILITIES IN TEA AT ANALYTICAL SERVICES DEPARTMENT, TOCKLAI

Dicofol
Chlorpyrifos
α - endosulfan
β -endosulfan
Deltamethrin
Fenprothion
Dimethoate
Hexaconazole
Ethion
Quinalphos
Lambda cyhalothrin
Bifenthrin

Profenofos
Flufenoxuron
Oxyflurfen
Spiromesifen
β Cyfluthrin
Propiconazole
Cypermethrin
Propargit
H.C.B
Trifloxystrobin
Alpha B.H.C
β B.H.C
Gamma B.H.C
Delta B.H.C
Heptachlor
Aldrin
Heptachlorepoxyisomer B
Gamma -chlordane
Alpha chloedane
Endosulfan I(alpha)
4,4- DDE
Dieldrin
Endosulfan II
4,4- DDD
Endrin aldehyde
Endosulfan sulphate
4,4-DDT
Endrin ketone
Methoxychlor

7. MATERIALS AND METHODS

a) MATERIAL AND METHOD FOR PESTICIDE RESIDUE ANALYSIS IN TEA:

SAMPLE DESCRIPTION:

Two commercial tea samples purchased from market to be analyzed for pesticide contamination.



Fig; Tea Samples

The sample is extracted by blending with hexane/acetone (4:1). An aliquot of the organic layer is washed with 5% aqueous sodium chloride solution. An aliquot of the washed organic phase is concentrated and added to the top of a column of acidic Activity 5 alumina. Pesticides are eluted from the column with hexane and two portions of 2% acetone in hexane. Eluent fraction are concentrated and examined by gas chromatography using ECD.



Fig: Samples after blending

b) MATERIAL AND METHOD FOR BIO-PESTICIDE ANALYSIS:

SAMPLE DESCRIPTION:

Two commercial samples sent by clients to Analytical Services Dept. were screened for their pesticide contamination.



The samples were extracted by mixing at first with acetonitrile and then n-Hexane. The fats present in the samples dissolves in n-Hexane and this layer is rejected. These were then shacked with 10% Sodium Chloride solution and n-Hexane. From the upper n-Hexane layer a portion was pipetted , dried in N₂ gas, dissolved in Methanol for analysis in HPLC. Gas liquid chromatography analysis was carried out from the n-Hexane layer directly.

8. RESULTS AND DISCUSSIONS

[1] Pesticide residue analysis in tea

Two made tea samples (R-74 & R-75) were analyzed between 4th Dec to 29th Dec using the standard operation procedure of the laboratory for detection of pesticide residues in them using GLC. No pesticides were detected ,

[2] Bio-pesticide formulation analysis:

Two bio-pesticide samples (F-65 & F-66) were analyzed between 4th Dec to 29th Dec using the standard operation procedure of the laboratory for detection of pesticide residues in them using HPLC. No pesticides were detected.