Review Article

VARIOUS METHODS INVOLVED IN STUDYING PERSISTENCE AND RESIDUES OF PESTICIDES IN SOIL

UNDER PEER REVIEW

Abstract

Pesticides protect plants or plant products against crop pests, plagues and reduce competition from weeds, in order to improve yields and protect the availability, quality, reliability and economical competitively of the production in benefit of farmers and consumers. The increase in yields in agricultural activities has been boosted by the introduction of new technologies from breeding to harvest. In every step of the production chain, these activities are supported using agrochemicals which in general jeopardise the environment sustainability, polluting soils, water and air. The deleterious effects of pesticides implicate a threat, not only to nearby natural habitats but also to cropland itself.

Keywords: pesticides, soil pollution, agricultural activities, Accelerated Solvent Extraction

Introduction

Soil is a key resource for agricultural activities whose integrity is basic for systems sustainability, so variations in soils ecosystems could have unpredictable consequences. It is the direct receptor of pesticides which are applied directly to it or by foliar application. The potential of pesticides to contaminate the soil is governed by many factors that include soil and pesticide physicochemical properties, crop management practices, among others. It was reported that the individual behavior of each pesticide in soil is different depending on soil texture. However, the general effect on the soil ecosystem of pesticides is due to the particular combinations of compounds that occur in it. To a better understanding of the whole phenomenon, it is necessary to determine the largest number of residues that may be present in this matrix(provide reference).

Accelerated Solvent Extraction (ASE), Pressurised Liquid Extraction (PLE), Soxhlet, QuEChERS or Solid-Liquid Extraction (SLE) with different types of agitation (manual shaking, ultrasonic, among others), in combination with different clean-ups, are typical examples of the methodologies reported for pesticide analysis in soil coupled to tandem mass spectrometry. Liquid

chromatography is being preferred for pesticides residues in soils over gas chromatography as the interest for the occurrence of new and more polar agrochemicals is gaining attention over the common, persistent and non-polar organochlorines(provide reference).. However, there are very little reports on analytical methods either for the multi-residues determination of polar compounds or mixed residues of compounds with a broad range of polarity in soils.

- Pesticides are the integral part of modern agriculture(provide reference)..
- The production of pesticides started in India in 1952 with the establishment of a plant for the protection of BHC near Calcutta(provide reference)..
- India is now the 2nd largest manufacturer of pesticides in Asia after China and ranks 12th globally. There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 102,240 metric tons in 2016(provide reference)..
- The per capita consumption of pesticides in India is 0.5 kg ha^{-1}which is lowest compared to other countries. The main reason for low per capita consumption of pesticides in India is low purchasing power and small land holdings(provide reference)..
- 800 pesticides molecules identified and 291 registered pesticides in India (pesticides Act - 1968)(provide reference).

Chromatography is a method in which the mixed components are separated on the adsorbent column in the flow system. For chromatographic separation, samples are included in the mobile phase flowing through the stationary phases. HPLC was used for the analysis of some thermolabile or highly polar compounds and also on compounds with high molecular weight. The success of an analysis is also strongly influenced by the accuracy of the chromatographers in selecting and using column, the stationary phase, and the mobile phase. The advantages of separation with HPLC compared to conventional methods include fast analysis times, low cost, and able to analyze compounds with low stability(provide reference)..

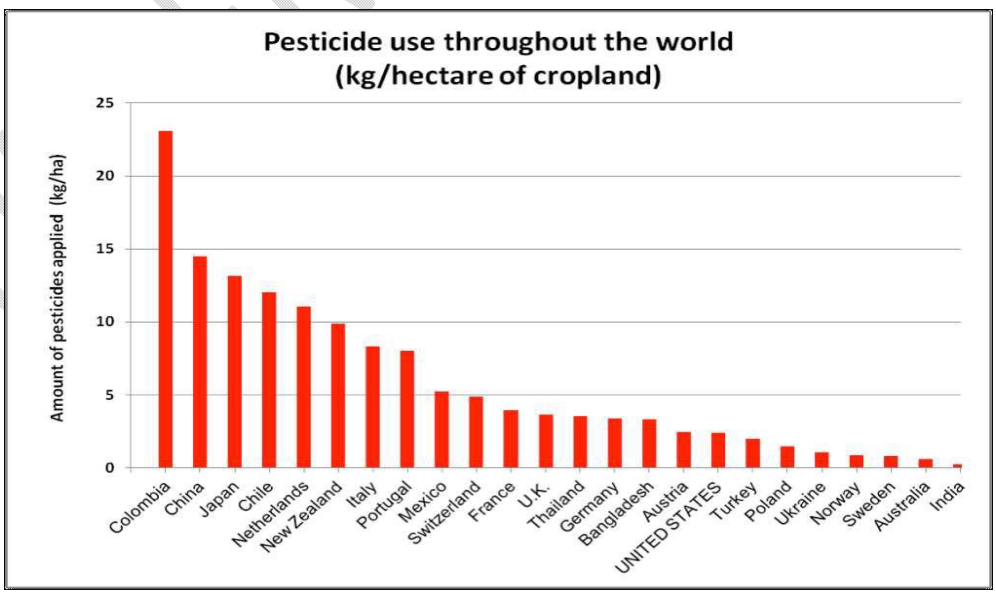


Image 1: Pesticide use throughout the world(provide reference).

UNDER PEER REVIEW

Colombia country use highest efficiency of pesticides kg ha^{-1} for agriculture practices followed by China, Japan and Chile etc. India country is the lowest consumption of pesticides kg ha^{-1}.(provide reference).

PESTICIDES

Any chemical, biological substance or mixture of substances intended for preventing, destroying, attracting, repelling or controlling any pest. Pesticides can target insects (insecticides), rats and mice (rodenticides), weeds (herbicides) and fungi (fungicides)(provide reference)..

Pesticides are known as synthetic active substances, which are widely sprayed in agricultural land, especially in grape-growing areas. Generally, pesticides have been sprayed on vegetables and fruits for fast and healthy growth. During the spray, pesticide-droplets may contaminate the soil. The soil was also contaminated by leaching of pesticides from plants and or decay of plants(provide reference)..

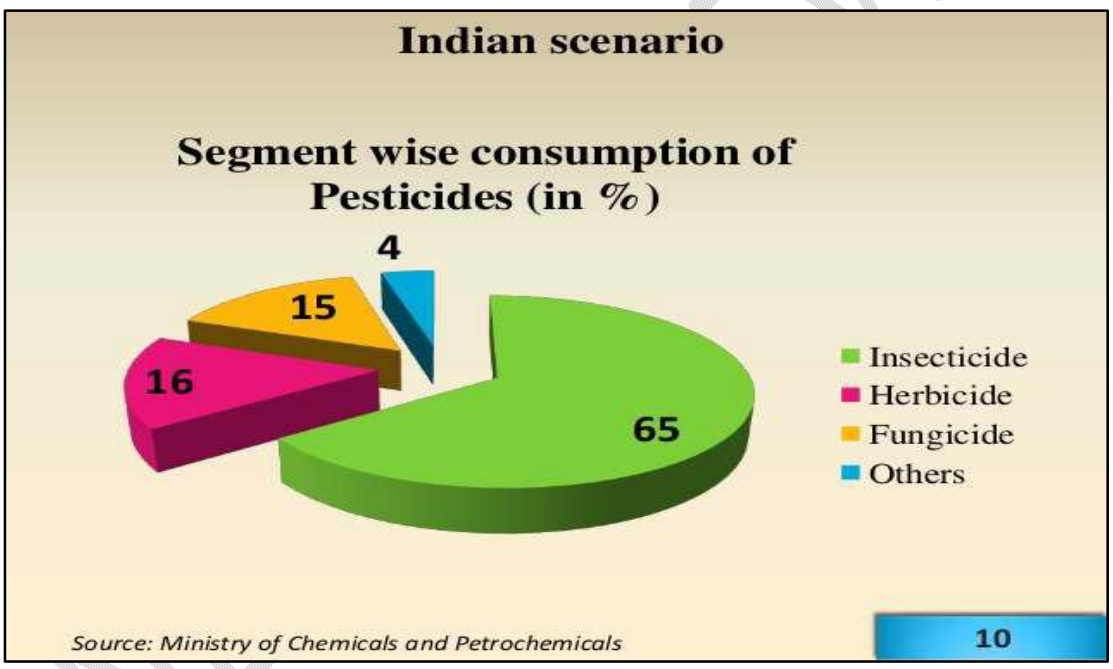


Image 2: Indian Scenario(provide reference).

India use pesticides consumption in percentage as Insecticide 65percent Herbicide16 percent Funigicide 15 percent others 4 percent(provide reference)..

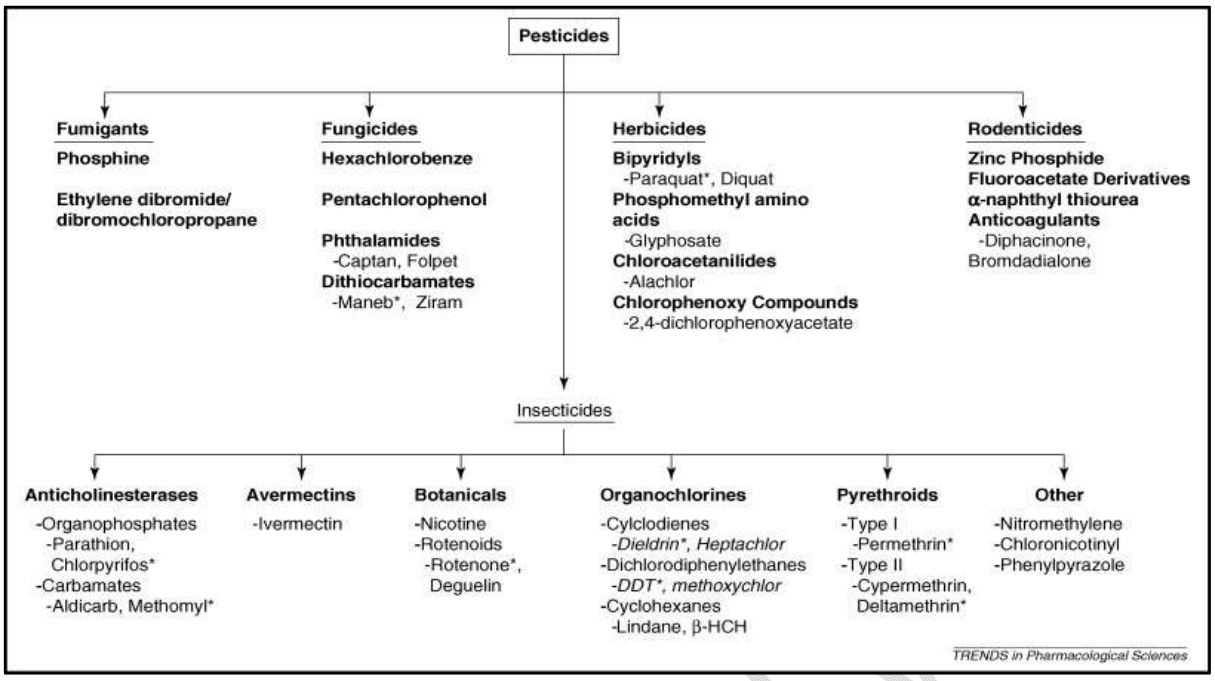


Image 3: Classification of pesticides

PESTICIDE RESIDUES

- Pesticide residue means any substances in food, agricultural commodities or animal feed resulting from the use of pesticide(provide reference)..
- The term includes any derivatives of the pesticide such as specified derivatives, degradation & conversion products, metabolites, impurities and reaction products etc. which are considered to be of toxicological significance

- Surface residues
- Terminal residues – presence of terminal residues in plants/crops produces at harvest is of great concern
- Penetrated residues
- Bound residues – residues of remaining in soil/plants after exhausting solvent extraction significance bound residues: bioavailability toxicity and accumulation nature
- Dislodgeable residues – amount of pesticide residues that can be dislodged from the two sided foliar surface of a plant during a well-defined producer which are deposited on and remain on surface afezter pesticide application(provide reference).

Residues

"The amount of insecticide left over after a lapse of time."

- Disappearance of pesticide residue takes place by two way i.e.
- Dissipation: In which the disappearance of the residue is fast

UNDER PEER REVIEW

- Persistence: In which there is a slow decrease in amount of residue

Ability of a pesticide to remain present and active for a long times. Provides for long-term pest control, but may harm sensitive plants and animals. May lead to illegal residues on rotational crops. Pesticide persistence often is expressed in terms of half-life. This is the length of time required for one-half of the original quantity to break down(provide reference).

Based on these 3 types:

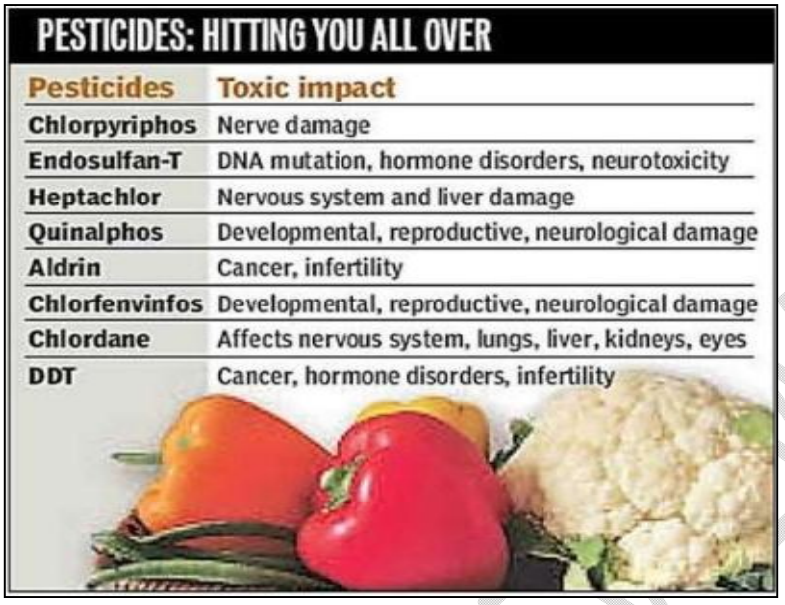
1. Non-persistent pesticides: <30 days
2. Moderately persistent pesticides: 30 to 100 days
3. Persistent pesticides: >100 days.

What is the need to study pesticide residue

- persistence of the pesticide
- High quantity of pesticides (lack of awareness from farmers)
- Prolonged use of pesticides (Kerala incidence)
- Monitoring the residues in environment to fix the MRL, safe waiting peri

UNDER PEER REVIEW

Image 4: Effect of pesticides



PESTICIDES: HITTING YOU ALL OVER

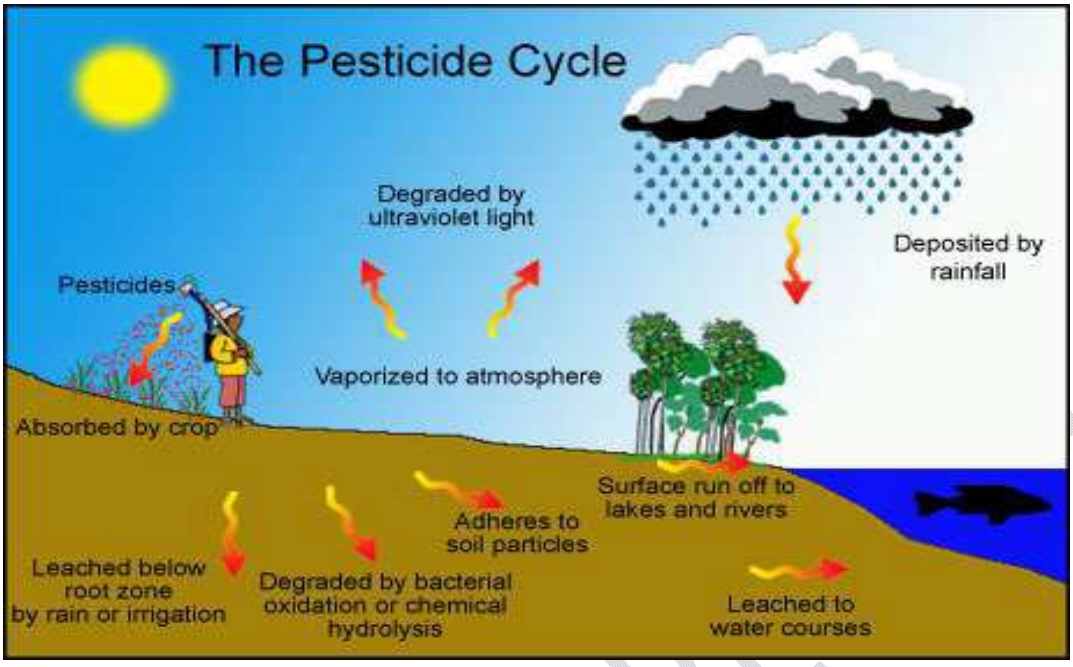
Pesticides	Toxic impact
Chlorpyriphos	Nerve damage
Endosulfan-T	DNA mutation, hormone disorders, neurotoxicity
Heptachlor	Nervous system and liver damage
Quinalphos	Developmental, reproductive, neurological damage
Aldrin	Cancer, infertility
Chlorfenvinfos	Developmental, reproductive, neurological damage
Chlordane	Affects nervous system, lungs, liver, kidneys, eyes
DDT	Cancer, hormone disorders, infertility

Image 5: Insecticide Exploration(provide reference).

Bhopal's pesticide plant was built in 1969 to manufacture Sevin- Asia to kill beetles, weevils and worms. The plant was operated by Union Carbide India, Limited, but an American company, Union Carbide Corporation, held > ½ of the stock. The leak began on December 2, 1984, when water entered a tank that was used to store methyl isocyanate, a toxic gas and a key ingredient in Sevin. The water reacted with the gas, causing extreme pressure and heat that possibly caused the tank to explode. The tank spewed 40 tons of poisonous gas into the air. The toxic cloud was mostly methyl isocyanate, a compound that can irritate the throat and eyes, cause chest pain and shortness of breath, and, in large doses trigger convulsions, lung failure and cardiac arrest.(provide reference).

The UNO classifies Endosulfan as highly dangerous insect killer and banned in 62 countries.Endosulfan, a highly toxic organochlorine pesticide was sprayed in the cashew plantations in Kasaragod District since 1976, till 2001 regularly three times every year(provide reference)..

Image 6: **Pesticide cycle:**



- **Adsorption**

Adsorption is the binding of pesticides to soil particles. The amount a pesticide is adsorbed to the soil varies with the type of pesticide, soil moisture, soil pH and soil texture. Pesticides are strongly adsorbed to soils that are high in clay or organic matter. They are not strongly adsorbed to sandy soils. Most soil-bound pesticides are less likely to give off vapours or leach through the soil(provide reference)..

- **Volatilization**

Volatilization is the process of converting solids or liquids into a gas, which can move away from the initial application site. Pesticides volatize most readily from sandy and wet soils. Hot, dry or windy weather and small spray drops increase volatilization. Incorporation of the pesticide into the soil can help reduce volatilization(provide reference).

- **Spray drift**

Spray drift is the airborne movement of spray droplets away from a treatment site during application

1. Droplet size – smaller- more likely they will drift.

2. Wind speed – stronger- more pesticide spray will drift.

3. Distance between nozzle and target plant or ground.

• Drift may also hazard to people, domestic animals, pollinating insects(provide reference)..

- **Runoff**

UNDER PEER REVIEW

Runoff is the movement of pesticides in water over a sloping surface. The pesticides are either mixed in the water or bound to eroding soil. Runoff can also occur when water is added to a field faster than it can be absorbed into the soil. Runoff from areas treated with pesticides can pollute streams, ponds, lakes and wells pesticide residues in surface water can harm animals and contaminate groundwater(provide reference).

- **Absorption**

Uptake of pesticides and other chemicals into plants or microorganisms. Pesticide residues may be broken down or remain inside the plant or animal, when the animal dies or as the plant decays released back. Some pesticides stay in the soil long enough to be absorbed by plants grown in a field years later. They may damage or leave residues in future crops(provide reference)..

- **Degradation (Breakdown Processes)**

Microbial breakdown is the breakdown of chemicals by microorganisms such as fungi and bacteria. Chemical breakdown is the breakdown of pesticides by chemical reactions in the soil. Photo degradation or Photolysis is the breakdown of pesticides by sunlight. All pesticides are susceptible to photo degradation to some extent. Hydrolysis: water also degrades pesticides by dividing large molecules intosmall(provide reference)..

METHODS FOR PESTICIDE RESIDUE ANALYSIS IN SOIL

STEPS INVOLVED

- Sampling
- Sample preparation
- Extraction
- Clean up
- Identification and Quantification

SAMPLING

- Previous history
- character of the sample
- Application of insecticide
- Formulation of insecticide
- Storage of samples

UNDER PEER REVIEW

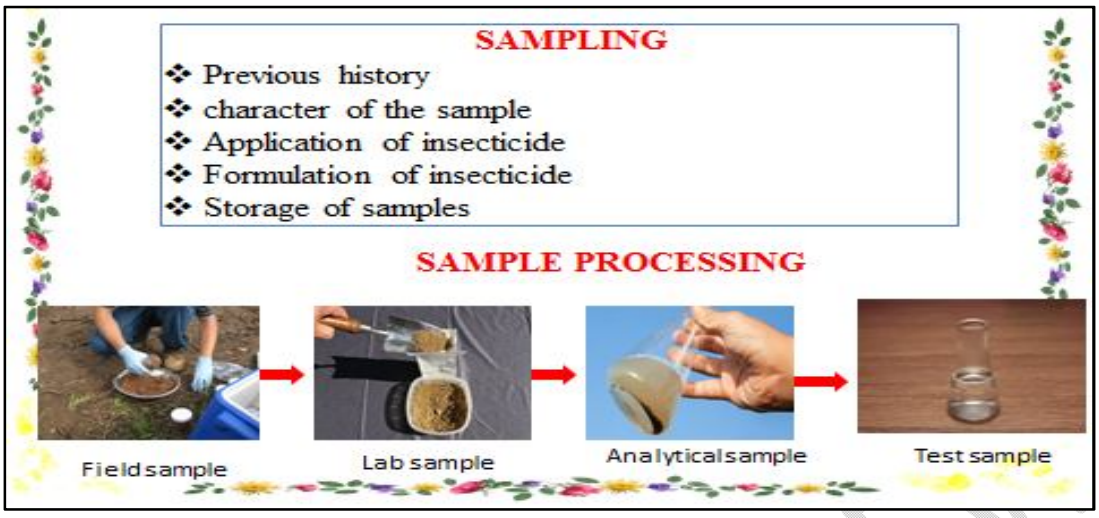


Image:7 Methods for pesticide residue analysis in soil

EXTRACTION

Extraction is the process by which toxicant is transferred from the treated bulky matrix material in to a solvent.

TYPES:

- Liquid liquid extraction
- Soxhlet extraction
- Automated soxlet extraction
- Super-critical fluid extraction
- Solid phase extraction
- Solid phase micro extractions
- Accelerated solvent extraction
- Microwave assisted extraction
- QuEChERS technique

Solvents: Water, acetonitrile, ethyl acetate, methanol, acetone and n-hexane.

UNDER PEER REVIEW

EXTRACTION METHODS

1. Liquid-Liquid Extraction (LLE):

- ✓ Also known as solvent extraction and partitioning method
- ✓ Separates the compounds based on their relative solubility in two different immiscible liquids
- ✓ Usually the water and organic solvents

Ex: Hexane, Ether, Dichloromethane, Chloroform, Ethyl acetate etc…

- ✓ Performed using a separating funnel
- ✓ Most organic solvents float on the top of an aqueous phase,. Except the halogenated solvents (Dichlorometyhane)

2. Soxhlet Extraction (SE):

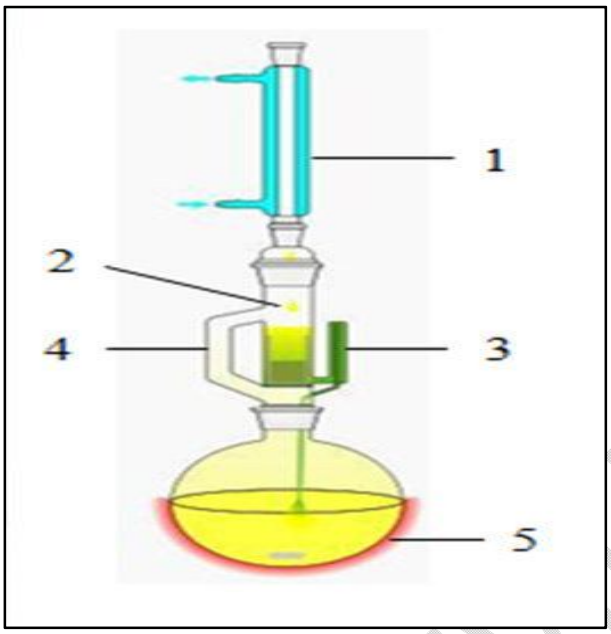
- ❖ Invented in 1879 by Franz Von Soxhlet
- ❖ Preferred where desired compound has limited solubility in a solvent and impurity is insoluble in that solvent

Operation procedure:

- ❖ Thimble
- ❖ soxhlet extractor
- ❖ Flask (extraction solvent)
- ❖ Condenser
- ❖ Sample housing chamber
- ❖
- ❖

Image 8: Soxhlet Extraction technique

UNDER PEER REVIEW



3. Automated Soxhlet Extraction (SSE) :

- ✓ Automated (or) semi-automated
- ✓ Allow fast and effective determination of organic compounds in soil, food etc.

Ex:

FOSS: Soxtec Systems(Boiling, rinsing and solvent recovery)

- ✓ Gerhardt GmbH: Soxtherm extractors (reduce extraction times)
- ✓ Except diethyl ether all solvent can used

Models available: Soxhlet standard

- Soxhlet warm
- Hot Extraction Soxhlet continuous extraction Soxhlet

UNDER PEER REVIEW

Image 9. •Hot Extraction Soxhlet

4. Super-critical fluid extraction (SFE):

- ✓ SFE uses supercritical fluid as an extraction solvent
- ✓ CO_2 is common solvent : safe nature, Unreactive, ready availability, inexpensiveness, low critical pressure and temperature point
- ✓ Fluid density solvation power changing extraction pressure & temperature
- ✓ Supercritical fluids have low viscosity even at high fluid densitygood penetration into matrix

<u>Advantage</u>: concentration of analytes after extraction is fast and convenientbecause supercritical CO_2 becomes gas after depressurization

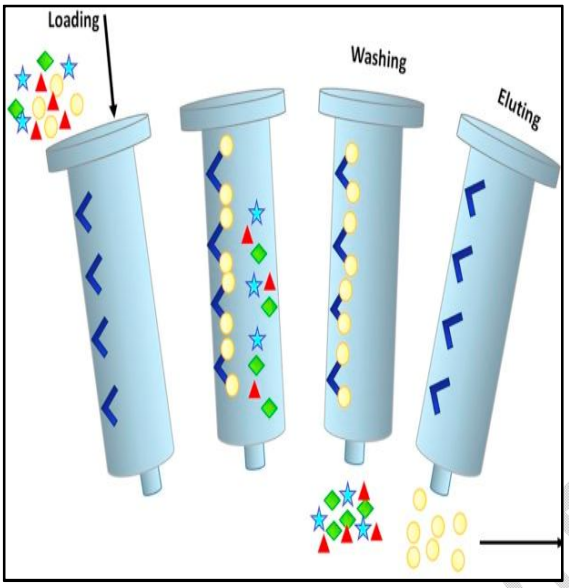
<u>Disadvantage</u>: large capital cost and method development is time consuming

5. Solid Phase Extraction (SPE):

- ✓ Based on chromatographic technique of preparation of samples by removing interfering substances that may be present
- ✓ Done by either retaining the substance of interest and washing off everything else or by retaining the interfering substances and eluting the product of interest
- ✓ Most widely used sample preparation method
- ✓ commonly used to clean up a sample before injection

Image 10: Solid Phase Extraction (SPE)

UNDER PEER REVIEW



6. Solid Phase Micro Extraction (SPME):

- ✓ Solid Phase Extraction only
- ✓ implemented by using micro-pipete tip, a 96 well plate , disk (or) coated fibers, later has its own name SOLID PHASE MICROEXTRACTION (or) SPME

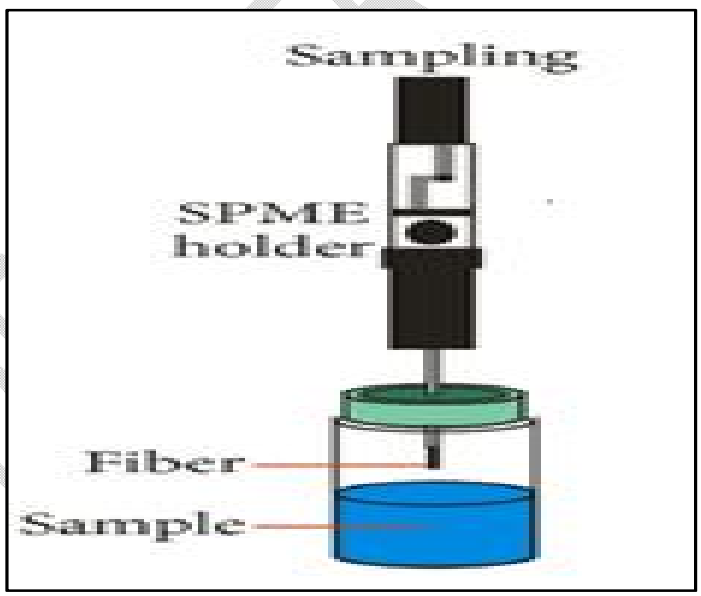


Image 11: Solid Phase Micro Extraction (SPME)

7. Accelerated Solvent Extraction (ASE):

- ❖ Also known as Pressurized Liquid Extraction (PLE)

UNDER REVIEW

- ❖ Similar in principle to Soxhlet extraction except that elevated temperature and pressure are used in enclosed vessels which allows the extraction by small amount of solvent (< 50 ml)
- ❖ Extraction will be completed in a very short time (< 20 min)
- ❖ Extraction of various organic compounds from different environmental samples few pesticides from soil including chlorinated and organophorous insecticides, the herbicides and fungicide hexaconazole.

8. Microwave assisted Extraction (MAE):

❖ Applied to soil, food, vegetables, oils, dairy products, sediments and other samples for extraction of polycyclic aromatic hydrocarbons, pesticide residues, trace elements etc.

❖ Applied generally when residues level in sample is very less and uses less extraction solvent (1/10)

❖ Requires different solvent from conventional method: iso-octane, n-hexane / acetone, benzene/ acetone, methanol/ acetic acid, methanol/ n-hexane, iso-octane / acetonitrile etc.

9. QuEChERS technique

- ❖ 10 g soil sample in 50 ml centrifuge tube
- ❖ Add 20 ml of acetonitrite (methyl cyanide)
- ❖ Add 1 g of NaCl and 4 g of MgSO4 mix well by shaking gently
- ❖ Centrifuge for 3 minutes at 3300 RPM
- ❖ Transfer 10 ml of supernant solution into 15 ml centrifuge tube
- ❖ Add 1.5 g $MgSO_4$ and 0.25 g PSA
- ❖ Mix well and centrifuged for 10 minutes at 3300 RPM
- ❖ 1 ml of extract was was transferred into vials for LC- MS analysis

CLEAN -UP

Different Clean – up methods are:-(provide reference).(provide reference).

- ✓ Liquid - Liquid Partitioning: Dimethylformamide, hexane (lipids)
- ✓ Column Chromatography: florisil, alumina and silica (carotenoids, ES)
- ✓ Solid Phase Extraction: carbon C-8, C-18 (organic acids and colloids)
- ✓ Chemical clean up: H_2SO_4 and chromic acid (lipids, ES, H_2SO_4–robust organochlorines)
- ✓ Gel permeation / Size Exclusion Chromatography: (lipids, proteins)

UNDER PEER REVIEW

✓ Sweep co-distillation: hexane, diethyl either (groups of OCs)

Identification and Quantification

1. Gas – Liquid Chromatography

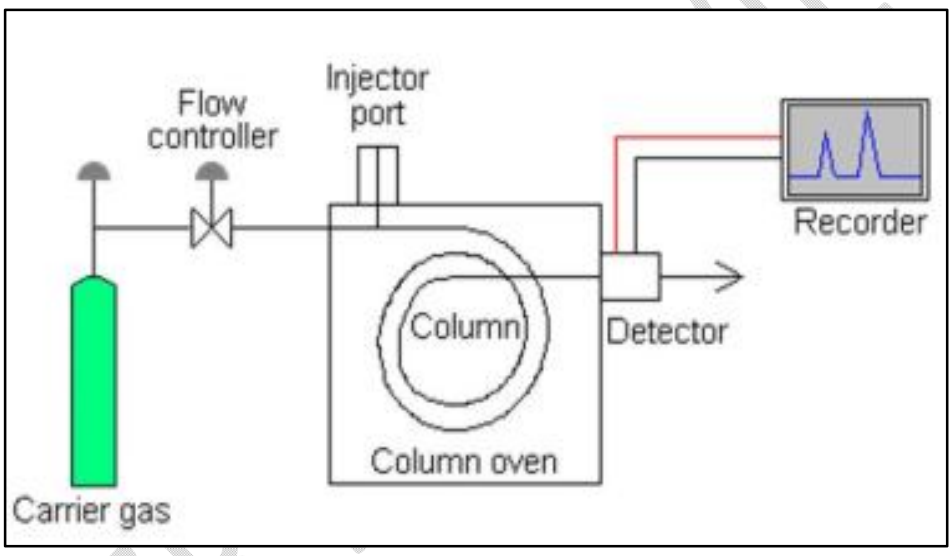
2. High – performance Liquid Chromatography

3. GLC coupled with Mass – Detector (GC–MS or GC-MS^n)

4. HPLC coupled with Mass Detector (LC- MS or LC-MS^n)

1. Gas – Liquid Chromatography (GLC / GC):-

Image 12:Principles of Gas chromatograph



A Gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample

- Gas – Liquid chromatography (GLC) is one of the most useful techniques in analytical chemistry. Claessonpublished one of the first important accounts of gas liquid chromatography in 1946(provide reference)..

- Gas – liquid chromatography is a form of partition chromatography in which the stationary phase is a film coated on a solid support and the mobile phase is an inert gas like Nitrogen (N_2) called as carrier gas flowing over the surface of a liquid film in a controlled fashion(provide reference)..

- The sample under analysis is vaporized under conditions of high temperature programming. The components of the vaporized sample are fractionated as a result of partitioning between a mobile gaseous phase and a liquid stationary phase held in a column(provide reference)..

- In gas-liquid chromatography the **mobile phase is an unreactive gas**such as nitrogen (the carrier gas) and the stationary phase comprises of a small amount of **nonvolatile liquid** heldon a finely-divided inert solid support(provide reference)..

➢ Gas liquid chromatography runs on the **principle of partition**

➢ In GLC the components of vaporize samples are fractionated due to partition between a gaseous mobile phase and a liquid stationary phase held in column(provide reference)..

➢ When the vapours of sample mixture move between the stationary phase (liquid) and mobile phase (gas) the different components of a sample mixture will separate according to their partition coefficient between the gas and liquid stationary phase(provide reference)..

Concnof solute in liquid (w/cc)

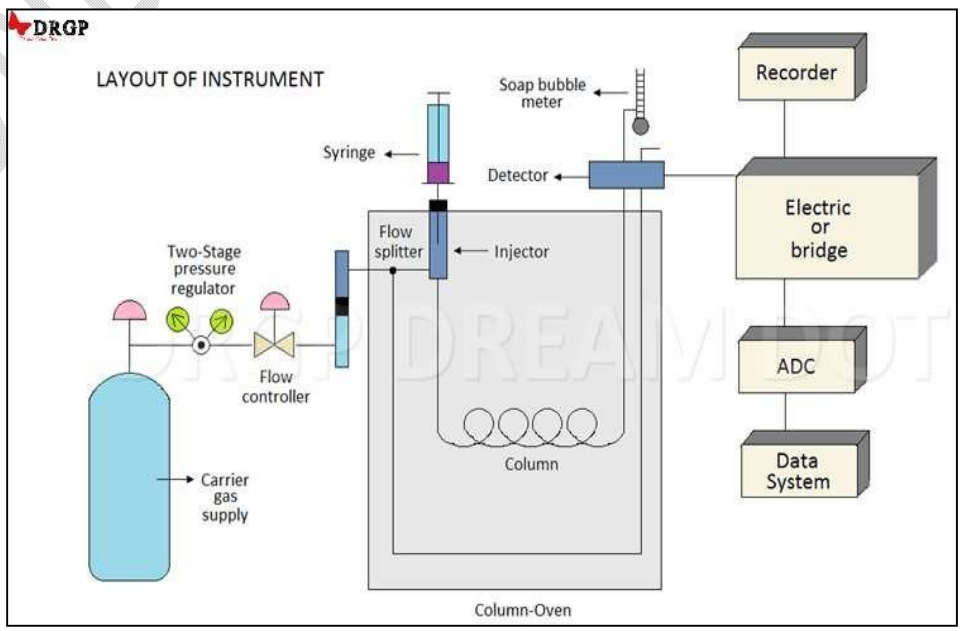
➢ Partition coeff.(Kg) = ----------

Concn of solute in gas (w/cc)

Instrumentation

- Tank of carrier gas
- Flow regulator and flow meter
- Injection port
- Column
- Temperature controlled device
- Detector
- Microprocessor/recorder

Image 13: Instrument layout(provide reference).



UNDER PEER REVIEW

The Mobile Phase (Carrier Gas)

- An inert gas such as He or N_2
- ❖ Function is to transport sample vapors through column
- ❖ No chemical interaction with sample
- Typical parameters
- ❖ Column inlet pressure:10-50 psi (above ambient)
- ❖ Flow rate:25-50 mL/min (packed column)
- Precise control of carrier gas flow rate is critical to obtaining reproducible retention times(provide reference)..

Sample Injection

- ▪ Sample is injected using a syringe into a flowing stream of hot mobile phase
- ▫ High temperature (at least 50°C above boiling point of sample) causes vaporization of sample
- ▫ Introduces a narrow plug of sample vapor onto the column
- ▪ Various designs
- ▫ For packed columns, inject 1 to 5 mL of sample
- ▫ For capillary columns, a split valve is used to introduce a small fraction of sample onto column

Columns

- Column is heart of GC, which decides the separation efficiency
- It is made up of glass or copper
- Columns are two types based on it's use:

Analytical column:Length 1-2 mts, outer diameter 3-6 mm

Preparative column:Length 3-6 mts, outer diameter 6-9 mm

UNDER PEER REVIEW

Column Oven

- Precise control of column temperature
- Column temperature should be slightly below the boiling points of the solutes (but above the dew point; i.e., no condensation)
- For complex mixtures with a broad range of boiling points, use programmed temperature
- Precise control of oven temperature is critical to obtaining reproducible retention times.

Detectors

Generate an electrical signal proportional to solute concentration or mass flow rate

A detector provides specific response for the separated components. Majority of the organic compound applications require flame ionization detector.

Ideal characteristics

- High sensitivity
- Rapid response time
- Non-destructive technique
- Applicable to wide range of samples
- Easy to use
- Stable, predictable response

Types of Detectors in Gas Chromatography

- 1. Flame ionisation detector
- 2. Thermal conductivity detector
- 3. Electron capture detector
- 4. Flame photometric detector
- 5. Photo ionisation detector
- 6. Nitrogen phosphorus detector

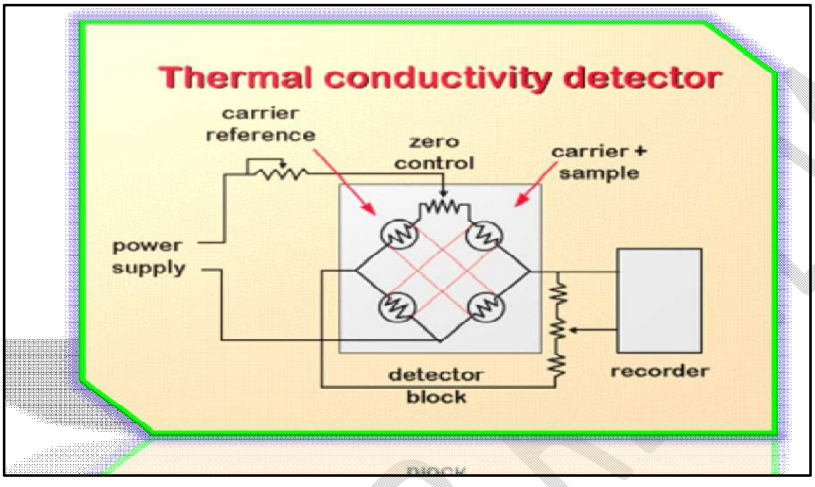
Thermal conductivity detector

- Element is electrically heated at constant power.
- Temperature depends on thermal conductivity of surrounding gas.
- Measure conductivity with respect to a reference.

UNDER PEER REVIEW

- When analyte comes off, filament temperature goes up, resistance goes down.
- **Mechanism**: A detector cell contains a heated filament with an applied current. As carrier gas containing solutes passes through the cell, change in the filament current occurs. The current change is compared against current in reference cell. The difference is measured and a signal is generated

Image 14: Thermal conductivity detector



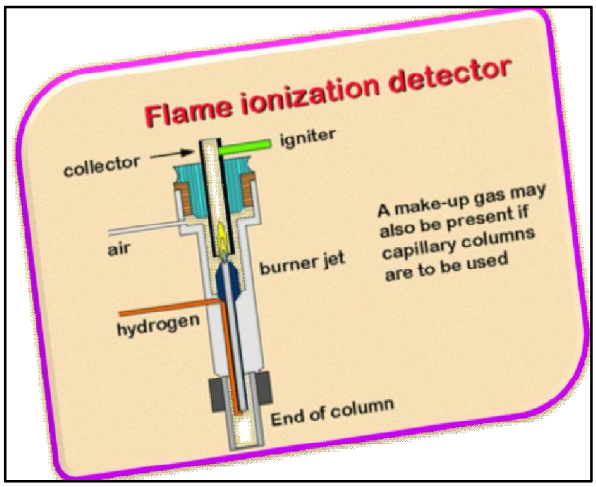
- **Sensitivity**: 5-20 ng
- Selectivity: All compounds
- **Gases**: Hydrogen, Helium
- **Temperature**: 150-250^{0}C

Flame Ionization Detector

- Column effluent is passed through a H_2-airflame produces ions and electrons.
- Charged particles are accelerated by voltage applied between jet and collector - results in current

Image 15: Flame Ionization Detector

UNDER PEER REVIEW



- Less sensitive to non-hydrocarbon groups
- **Mechanism:** Compounds are burned in a hydrogen-air flame. Carbon containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated
- **Sensitivity:** 0.1-10 ng
- **Selectivity:** compounds with C-H bonds
- **Gases:** combustion– hydrogen and air, makeup-He, N_2
- **Temperature:** 250-300^{0}C

Electron capture detector

- Carrier gas (and analyte) passes over **β-emitter,** resulting in ionization and electron production
- Produce current between electrodes
- Most commonly used for **halogenated organics**
- **Mechanism:** Electrons are supplied from a 63Ni foil lining the detector cell. A current is generated in the cell. Electronegative compounds capture electrons resulting in a reduction in the current. The amount of current loss is indirectly measured and a signal is generated
- **Sensitivity:** 0.1-10 ng
- **Selectivity:** Halogens, nitrates
- **Gases:** Nitrogen or argon
- **Temperature:** 300-400^{0}C(provide reference).

Recorder

- Recorder is a device that draws the chromatogram that results from a chromatographic process onto chart paper

- The device can have a full scale deflection (FSD) voltage that commonly ranges from 1 mv to 10v

- The time scale of the chart movement normally ranges from about 1 cm per second to 1 cm per hour

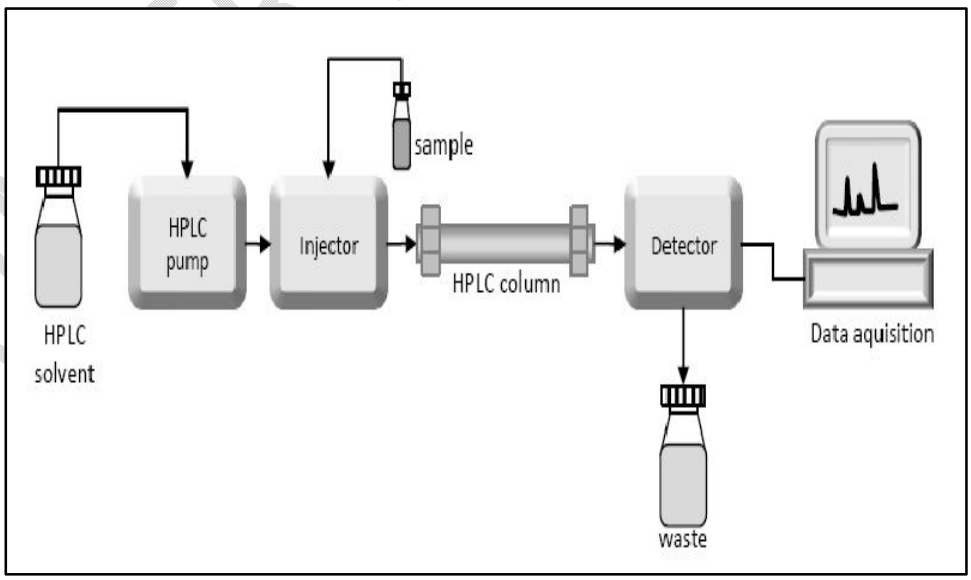
UNDER PEER REVIEW

Advantages of GLC

- Both qualitative and quantitative analysis are possible
- Instrument is simple, time of analysis is short
- High sensitivity
- The method is applicable to about 60% of organic compounds
- Very small samples sizes can be used
- Analysis can be highly accurate and precise.
- Analysis of pesticides
- Quantitative information based on peak areas
- Isolation and identification of drugs or metabolism in urine plasma, serum.
- Determining various cosmetics and perfumes
- Analysis of petroleum products, gasoline, waxes etc.
- Determination of water in creams, ointments, pastes.

2. High – performance Liquid Chromatography (HPLC)

Image 16: High – performance Liquid Chromatography (HPLC)



With change in particles size (3 – 10 µ) & efficient pumps

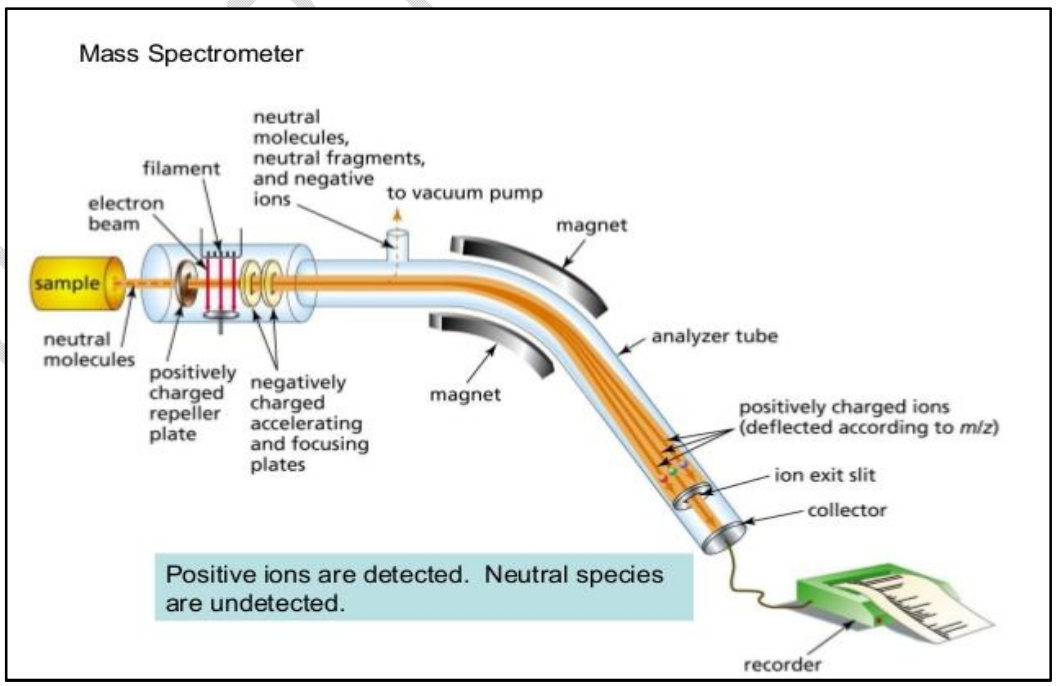
UNDER PEER REVIEW

High performance liquid chromatography is basically a highlyimproved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

High-performance liquid chromatography (HPLC- formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

3. GLC coupled with Mass – Detector (GC–MS)

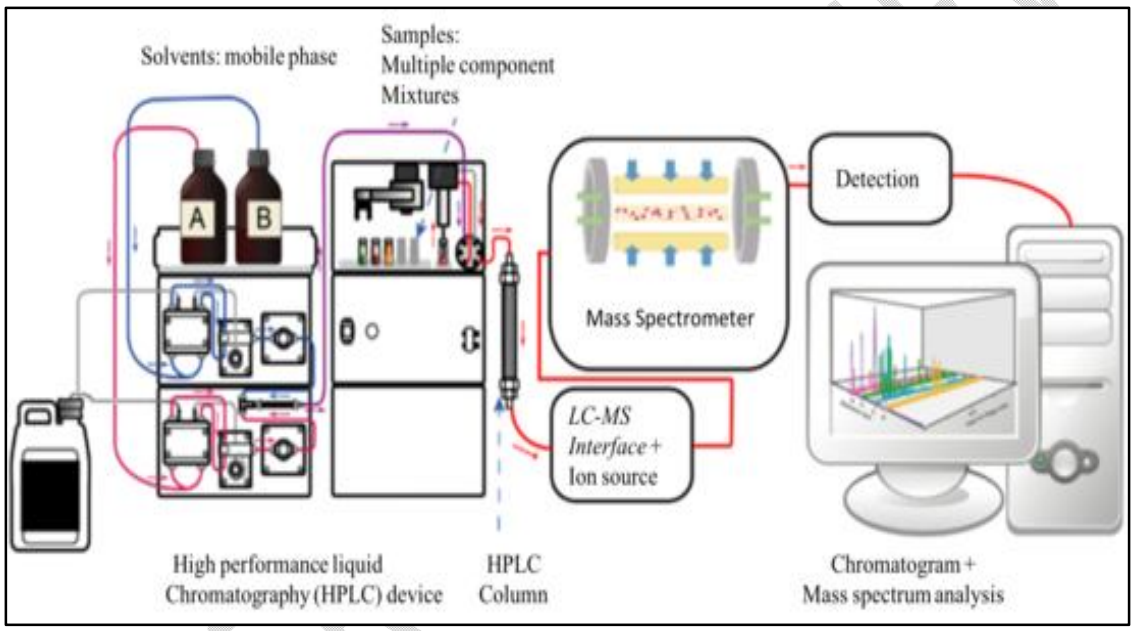
Image 17: GLC coupled with Mass – Detector (GC–MS)



Separation based on mass to charge ratio of the particles/substances

4. **HPLC coupled with Mass – Detector (GC–MS)**

Image 18: HPLC coupled with Mass – Detector (GC–MS)



Separation by liquid chromatography and Quantification based on mass to charge ratio of the particles / substances

RESULT AND DISCUSSION

Determination of Organophosphorus Pesticides in Soil by Dispersive Liquid–LiquidMicroextraction and Gas Chromatography

Zhonghua*et al.* (2012) study experiment at Bejing, China on Rapid and sensitive sample pretreatment technique for the determination of organophosphorus pesticides (OPPs) in soil samples is developed by using dispersive liquid–liquid microextraction(DLLME) combined with gas chromatography–flame photometric detection. Experimental conditions, including the kind of extraction and disperser solvent and their volumes, the extraction time, and the salt addition, are investigated, and the following experiment factors are used: 20 mL chlorobenzene as the extraction solvent; 1.0 mL

acetonitrileas the disperser solvent; no addition of salt; and an extraction time of1 min. Under the optimum conditions, the linearities for the three target OPPs (ethoprophos, chlorpyriphos, and profenofos) are obtained by five points in the concentration range of 2.5–1500 mg/kg, and three replicates are used for each point.

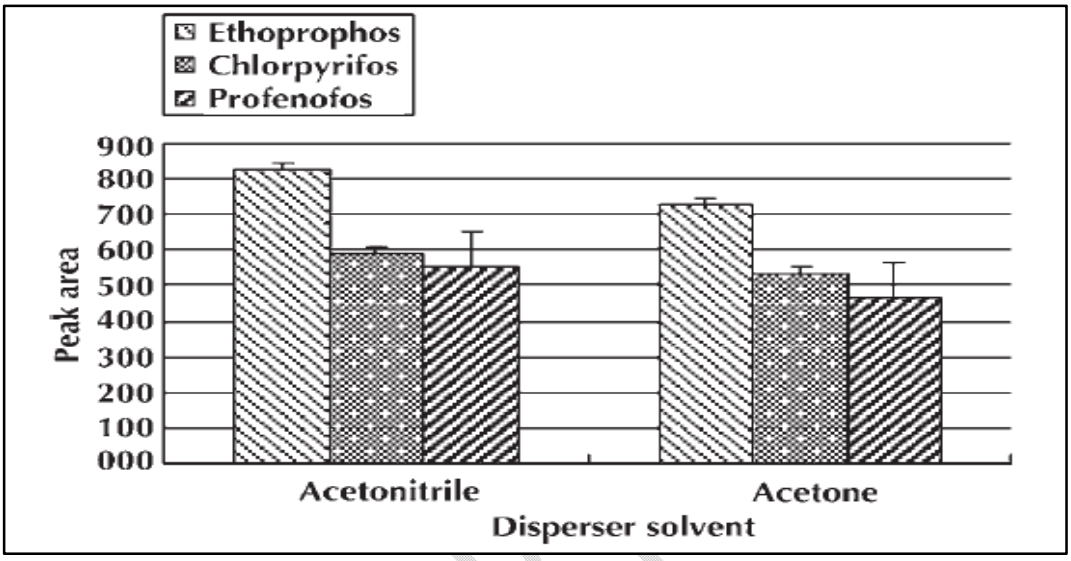


Fig 1. The effect of disperser solvent on DLLME

For a DLLME method, the disperser solvent must play tworoles. Firstly, it must efficiently extract analytes from soil samples. Secondly, it must be used as a disperser solvent in the DLLME step. The selection of the disperser solvent is based on its miscibility with both the organic and the aqueous phase. MeCN and acetone were the most used solvents for the extraction of OPPs from the samples; all of them have demonstrated acceptable recoveries(Anastassiades*et al.* 2003). In view of these considerations, MeCN and acetone were evaluated for this study. A series of sample solutions were investigated by using 1.0 mL each of the disperser solvents containing 20.0 mL chlorobenzene. As shown in Figure 1, the best extraction efficiencies were obtained when MeCN was used as a disperser solvent. Hence, MeCNwas selected.(provide reference).

UNDER PEER

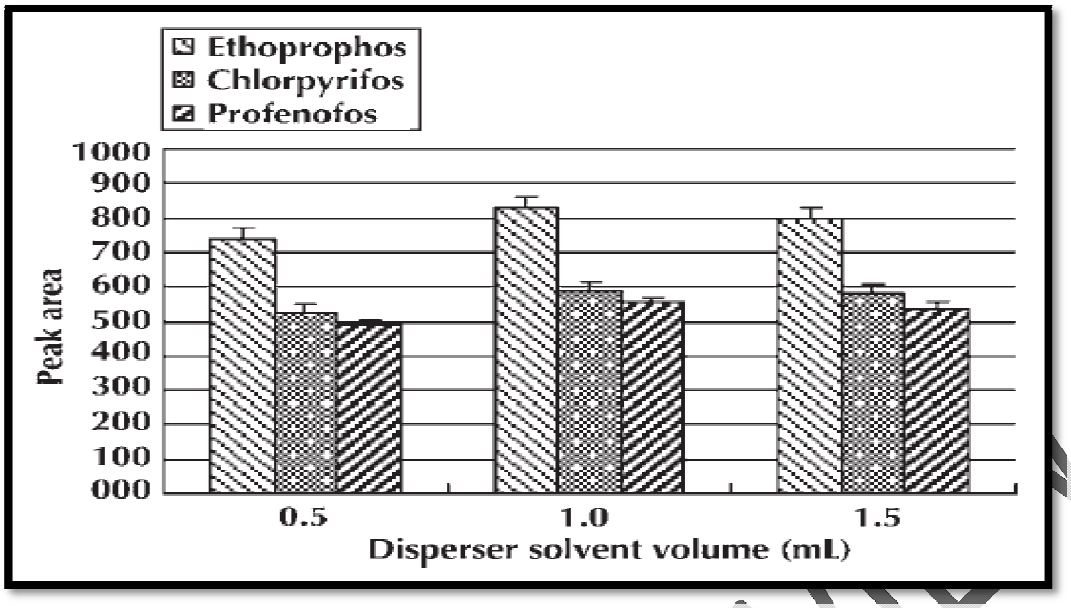


Fig 2. Effect of the disperser solvent volume on DLLME

The effect of the disperser solvent volume was investigated byusing different volumes of HPLC-grade acetonitrile (0.50, 1.0, and 1.5 mL) containing 20 mL chlorobenzene. The results shown in (Fig.2) demonstrate that the extraction efficiencies increased by increasing the volume of acetonitrile at first andthen decreased with further increase of the acetonitrile volume; this phenomenon may be attributed to the fact that at a low volume of acetonitrile, a cloudy state is not well formed. Based on the experimental results, 1.0 mL acetonitrile was chosen for the subsequent study(provide reference)..

Table 1. Analytical results in soil samples

UNDER PEER REVIEW

PESTICIDES	ADDED	FOUND	RECOVERY %
Ethoprophos	0	ND	-
	10	10.4	104.0
	20	19.8	98.8
	50	44.0	87.9
Chlorpyrifos	0	ND	-
	10	10.8	108.0
	20	18.1	90.5
	50	44.1	88.2
Profenofos	0	ND	-
	10	10.4	104.3
	20	18.1	90.5
	50	46.3	92.6

In order to investigate the developed method, the proposed method was applied to the analysis of OPPs in real soil samples (S1, S2, and S3) As a result, the three types of the target analytes were not found in the real samples. In order to assess the matrix effect on the developed method, a 20.0-g soil sample, which was free of OPPs, was accurately weighed and put into a 100 mL centrifuge tube. The individual stock standard solutions (see the Reagents and materials section) were added to the tube, and the soil sample was spiked with each OPP at three levels of 10.0, 20.0, and 50.0 mg/kg. Then, the mixture was air-dried at room temperature to obtain the spiked soil sample and was analyzed using the proposed method. The results are summarized in (Table 1). The recoveries ranged from 87.9% to 108.0%, 87.4% to 108.0%, and 86.7% to 107.2%, with RSDs varying from 2.8% to 7.1%, 2.1% to 5.6%, and 3.2% to 7.6%, respectively. These results demonstrated that the soil matrix in the present context had little effect on DLLME.

UNDER PEER REVIEW

Method development for pesticide residue analysis in farmland soil using High Perfomance Liquid Chromatography

Marselina*et al.* (2017), conducted experiment atBumiaji Sub-district, Batu City,Malang.method for the determination of diazinon and chlorantraniliprole in soil samples has been developed. The analyte was extracted with acetonitrile from farmland soil sample.Determination and quantification of diazinon and chlorantraniliprole were perfomed by high perfomance liquid chromatography (HPLC) with an UV detector. Several parameters of HPLC method were optimized with respect to sensitivity, high resolution of separation, and accurate determination of diazinon and chlorantraniliprole. Optimum conditions for the separation of two pesticides were eluent composition of acetonitrile:water ratio of 60:40, 0.4 mL/min of flow rate, and 220 nm of wavelength.

The chromatogram of diazinon and chlorantraniliprole separation resulted in four variations ofacetonitrile:water composition can be seen in (Fig. 3) that the more acetonitrile solvent used, the faster the retention time of the compound. This was caused by nonpolar columns and polar solvents so that the more non-polar compounds would be retained longer in the column. At 60:40 of mobile phase compositions, both compounds separated well at the retention time of each compound, chlorantraniliprole 9.8 and diazinon 18.64 and a short retention time of fewer than 20 minutes.

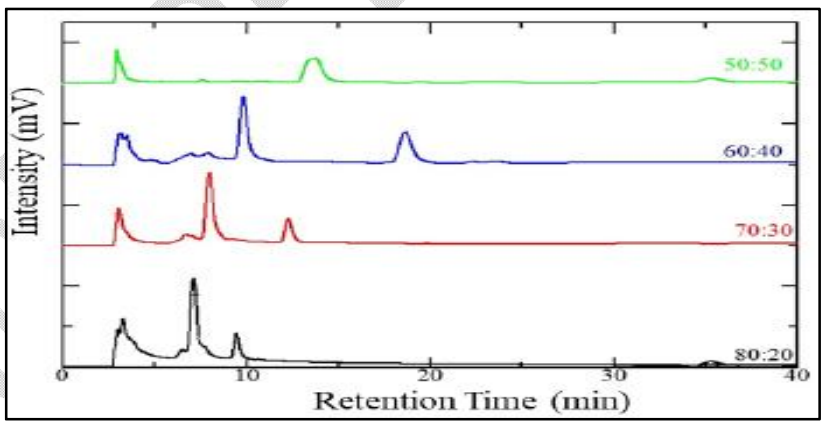


Fig3. Chromatogram Standard of Chlorantraniliprole and Diazinon (effect of extraction volumes on retention time)

The chromatogram of diazinon and chlorantraniliprole separation resulted in four variations of acetonitrile:water composition can be seen in (Fig. 4) that the more acetonitrile solvent used, the faster the retention time of the compound. This was caused by nonpolar columns andpolar solvents so that the more non-polar compounds would be retained longer in the column. At 60:40 of mobile phase compositions, both compounds separated well at the retention time of each

UNDER PEER REVIEW

compound, chlorantraniliprole 9.8 and diazinon 18.64 and a short retention time of fewer than 20 minutes.

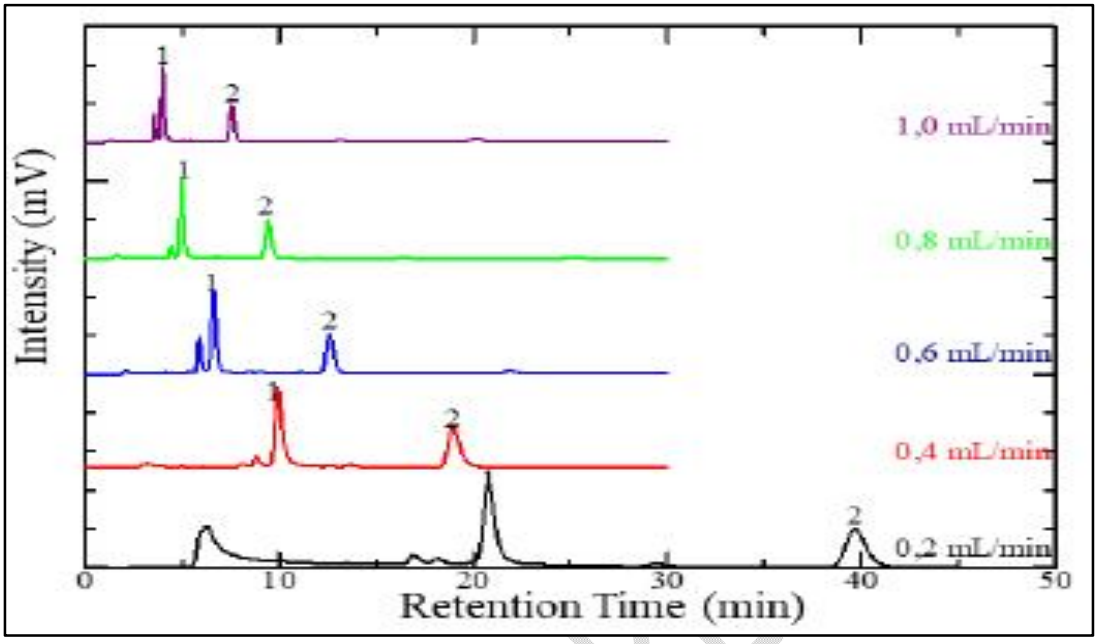


Fig 4. Effect of the flow rate on the retention time of chlorantraniliprole and diazinon compounds.

Faster the flow rate caused smaller standard peak area. So it may be determined the optimum flow rate conditions for the separation of diazinon and chlorantraniliprole was 0.4 mL/min. Where the fast analysis time is below 20 minutes and the large peak compound area was 550134 for chlorantraniliprole and 427586 for diazinone.

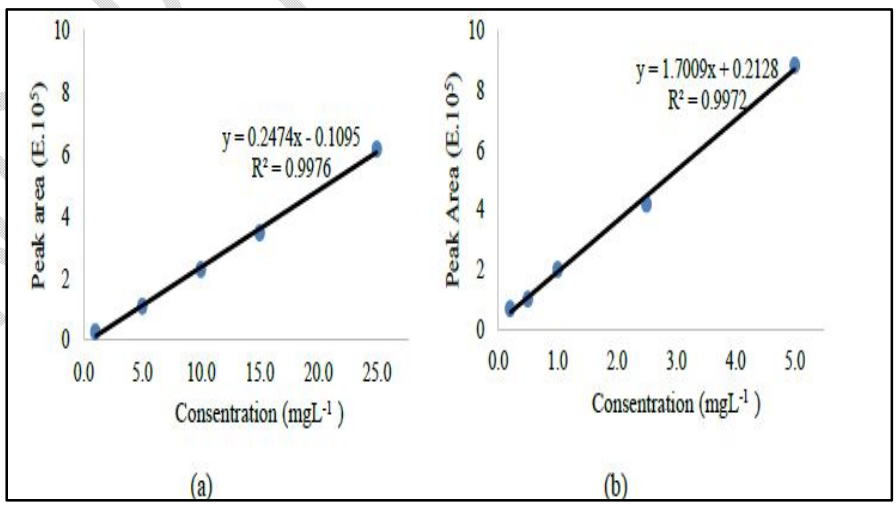


Fig 5. Standard Curve Diazinon (a) and Chlorantraniliprole (b)

Standard curve diazinon and chlorantraniliprole may be seen in (Fig. 5). Method validation obtained diazinon linearity was in the range from 1-25 mgL^{-1} with R2 of 0.9976, 1.19 mgL^{-1}

LOD, and 3.98 mgL^{-1} LOQ; whilst the linearity of chlorantraniliprolewas in the range from 0.2-5 $mgL^{-1}$with R2 of 0.9972, 0.39 mgL^{-1}LOD, and 1.29 mgL^{-1}LOQ. When the method was applied to the soil sample, both pesticides showed acceptable recoveries for real sample of more than 85%. thus, the developed method met the validation requirement.

Conclusion

Critical comparison is established between the proposed subcritical water extraction and the conventional Soxhlet extraction method, the time of analysis is decreased from 20 hr to less than 2 hr and the organic solvent used in the extraction procedure can be decreased to less than 2 percent. Dispersive liquid-liquid microextraction provides good repeatability, recovery and has the advantage of simplicity, fastness and lower consumption of organic solvents. Gas chromatography-mass spectrophotometer detection and quantification limits achieved with this method allow its application to monitoring of pesticides residues in soil. A simple, rapid, and sensitive DLLME method combined withGC–FPD has been developed for the determination of OPPs in soil samples. Compared with conventional methods, DLLME provides good repeatability, recovery, and has the advantage of simplicity, fastness, and lower consumption of organic solvents.

References

ANASTASSIADES, M., LEHOTAY, S. J., STAJNBAHER, D. AND SCHENCK, F. J., 2003, Fast and easy multiresidue method employing acetonitrile extraction/partitioningand "dispersive solid-phase extraction" for the determinationof pesticide residues in produce. *J. AOAC Int.,* **8**6:412–431.

MARSELINA, T. D. T., AKHMAD,S., AND HERMIN S., 2017,Method development for pesticide residue analysis in farmland soil using High Perfomance Liquid Chromatography.*International Conference on Chemistry and Material Science (IC2MS*).

ZHONGHUA, Y., YU LIU, DONGHUI LIU AND ZHIQIANG, Z., 2012,Determination of organophosphorus pesticides in soil by Dispersive Liquid–LiquidMicroextraction and Gas Chromatography.*J. Chromatographic Sci*,**50**:15–20.

UNDER PEER REVIEW