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सालिम अली पक्षीविज्ञान एवं प्रकृति विज्ञान केंद्र

(भारतीय वन्यजीव संस्थान का दक्षिण भारत केंद्र, पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय, भारत सरकार)

Sálim Ali Centre for Ornithology and Natural History

(South India Centre of Wildlife Institute of India, Ministry of Environment, Forest and Climate Change, Govt. of India)

TWO DAYS WORKSHOP ON BIOPROSPECTING AND TECHNIQUES IN AROMATIC PLANTS RESEARCH

NOVEMBER

21 & 22

2024

Venue: CBNR & SACON

Dr.BGR Publications

Editorial Board

Convener Message

Dr. R. Ragunathan, Ph.D, FSAB

Director and Research Supervisor (CBNR)

Dear Professors and Researchers,

It is my privilege and honor to welcome you all to the **Bioprospecting and Techniques in Aromatic Plants Research**" (**November -21 and 22, 2024**) organized by **Centre for Bioscience and Nanoscience Research** in association with **Sálim Ali Centre for Ornithology and Natural History.** The main goal of this workshop is to provide an extensive platform for researchers, students, industry professionals, teaching staffs and scientist to explore the latest techniques and advancements in aromatic plants research and bioprospecting. We have given a good opportunity for those who have a thirst in knowing the present technology and developments in the field of plants, pharmacology and pharmaceutics. Additionally, this workshop will also facilitate the participants to expose and share various novel ideas and will get ample opportunities to widen your knowledge and network. Plant related research is an emerging approach towards the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or medicine related problem. All medicines and vaccines undergo rigorous testing for safety and efficacy before they are authorized for use. Here this workshop will provide the importance of all the in-vitro tests such as Phytochemical, different antioxidant, antimicrobial, anticancer and characterization with UV- Visible, FTIR, GCMS, LCMS and HPLC studies.

CBNR has hosted various national and international level events and now turning **to 14th** year we planned to give a platform to the interested professionals towards bioprospecting of plant research playing a crucial role in current pharmaceutical industries. I want to thank the workshop committee for extending their valuable time in organizing the program and all the other contributors for their sparkling efforts and their belief in the excellence of **Bioprospecting and Techniques in Aromatic Plants Research.** I cordially invite the entire members to this workshop to learn and share their idea.

My best wishes for successful Workshop

Message from Organizing Committee

Dr. Jesteena Johney, Ph.D.,

Head of the Institute, CBNR

Dr. Thankamariappan, Ph.D.,

Scientist E, Department of Microbiology, CBNR

and

CBNR Team

The aim of the workshop is to provide a comprehensive understanding of Bioprospecting and Research techniques of aromatic plants with the focus of identification and exploration of aromatic plants with therapeutical and commercial values. Scientific passion among young researchers and faculties - Centre for Bioscience and Nanoscience Research Organized workshop on "**Bioprospecting and Techniques in Aromatic Plants Research**" (**November -21 and 22, 2024**). It is our privilege and honor on behalf of the entire organizing committee to welcome all the delegates, invited speakers, panel experts, colleagues, and research scholars to this workshop. Workshop will provide an ideal platform for the plant biological science researchers for knowledge-sharing, brainstorming and networking, thereby facilitating the ignition of new research ideas amongst the participants. Considering the strong requirement the participants can understand the Phytochemical and pharmacological properties of aromatic plants with different antioxidant and characterization study and also it will help to develop skills in modern analytical techniques for plant research. We appreciate the experts for accepting our invitation to share their facilities and interaction with the participants of this workshop. With great pleasure, we would like to thank SACON, join with us to conduct this successful two days workshop. We are extremely thankful to the research scholar volunteers and our colleagues without their tireless efforts this workshop would not have been accomplished. We extend a warm welcome to all the experts and participants to this workshop and hope that it will be scientifically enriching and worth full experience.

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Experiment – I: Soxhlet Extraction

Aim

The Soxhlet extraction process aims to extract bioactive compounds (such as alkaloids, flavonoids, polyphenols, terpenoids, etc.) from plant leaves using ethanol as a solvent. This process ensures efficient extraction by repeatedly cycling the solvent through the plant material, maximizing the extraction yield of target compounds.

Principle

Soxhlet extraction operated on the principle of continuous solvent extraction. In this process, the solvent was heated to its boiling point, with the vapor condensing and repeatedly dripping onto the plant material in a thimble. The solvent extracted soluble compounds from the plant material, and the enriched solution was siphoned back into the boiling flask to be reheated. This cycle repeated multiple times, enhancing extraction efficiency. Gradually, the solvent became saturated with the desired compounds from the plant material, which were then separated by evaporating the solvent.

This method is particularly useful for extracting compounds that are difficult to extract in a single step and ensures a higher extraction yield through the repeated contact between the solvent and plant material.

Materials Needed

- Plant leaves (fresh or dried)
- Ethanol (usually 95% or absolute ethanol)
- Soxhlet extraction apparatus (Soxhlet extractor, condenser, round-bottom flask)
- Heating source (heating mantle or oil bath)
- Thimble (filter paper or cotton for holding the plant material)
- Rotary evaporator (optional for solvent removal)
- Glassware (e.g., beakers, funnel)

Procedure

1. **Preparation of Plant Material:**

Weighing: Weigh about 10–50 grams of dried plant leaves.

Grinding: Grind the plant material into a coarse powder to increase the surface area, allowing better solvent contact.

2. Set Up Soxhlet Apparatus:

Assemble the Apparatus: Set up the Soxhlet extractor by connecting the extractor to a condenser and round-bottom flask.

Insert Plant Material: Line the thimble with filter paper or cotton to hold the powdered plant material. Place the plant material into the thimble and insert it into the Soxhlet extractor.

3. Add Solvent:

Typically 95% ethanol or absolute ethanol was added to the round-bottom flask, with a volume sufficient to cover the plant material in the thimble during extraction, generally around 100–300 mL.

4. Start the Extraction:

Heat the round-bottom flask using a heating mantle or oil bath. The solvent will vaporize, rise through the Soxhlet extractor, and condense in the condenser.

The condensed solvent drips onto the plant material, extracting soluble compounds. After a few cycles, the enriched solvent will siphon back into the flask. This process repeats, maximizing the extraction of bioactive compounds.

5. Completion of Extraction:

The extraction lasts for 4–8 hours, depending on the plant material and desired compounds. Stop the Extraction: After sufficient cycles, stop the heating and allow the system to cool.

6. Solvent Removal (Optional):

A rotary evaporator is used to remove the ethanol from the extract, concentrating the bioactive compounds. Alternatively, the ethanol was allowed to evaporate under a fume hood.

7. Storage and Analysis:

Store the final ethanolic extract in a sealed container, preferably in a cool, dark place, to prevent compound degradation.

Analyze the extract for active compounds using methods such as Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), or Gas Chromatography-Mass Spectrometry (GC-MS).

Safety Considerations:

Flammability: Ethanol is highly flammable. Ensure all equipment is set up in a well-ventilated area away from open flames.

Protective Gear: Always wear gloves, safety goggles, and a lab coat while handling solvents and plant materials.

Proper Ventilation: Conduct the extraction in a fume hood to avoid inhalation of vapors, especially when using solvents like ethanol.

Conclusion

The Soxhlet extraction method is a highly effective way to extract bioactive compounds from plant leaves using ethanol as a solvent. By continuously cycling the solvent through the plant material, the method ensures that the compounds are extracted efficiently, making it suitable for both small- and large-scale extractions. The principle of repeated solvent condensation and evaporation increases the contact between the solvent and plant material, optimizing the yield of the target compounds.

Observation and Result

Experiment – II: Hydrodistillation Using Clevenger Apparatus

Aim

To extract essential oils or volatile compounds from plant material through hydrodistillation using the Clevenger apparatus.

Principle

Hydrodistillation is a technique for extracting essential oils or volatile compounds from plant materials (including leaves, flowers, stems, etc.) using water or steam. This technique involves placing the plant material in a distillation flask and adding water. When the mixture is heated, the volatile compounds in the plant material vaporize and move into the condenser, where they are cooled and collected. The Clevenger apparatus is commonly used for the extraction of essential oils, where it helps in the separation of the distillate from the aqueous phase.

The Clevenger apparatus consisted of a distillation flask where the plant material and water were placed, a condenser to cool and condense the vapors, and a receiver to collect the essential oil and water distillate. As the volatile compounds were immiscible with water, the essential oil floated on the water phase for easy separation.

Materials Required

Fresh or dried plant material (e.g., lavender, peppermint, etc.), Clevenger apparatus (including distillation flask, condenser, receiver), Distilled water, Heating source (e.g., heating mantle), Thermometer, Collection flask or separating funnel, Glassware (beakers, pipettes, etc.)

Procedure

Preparation:

Select the plant material that you desire to distill. The plant material should be finely chopped or shredded to increase surface area for effective extraction. Weigh the plant material (e.g., 50-100 g depending on the plant and the size of the distillation flask).Prepare the Clevenger apparatus by assembling the distillation flask, condenser, and receiver.

Setting up the Apparatus:

Place the plant material into the distillation flask. Add an appropriate amount of distilled water (usually just enough to submerge the plant material). The volume of water should be sufficient to ensure continuous distillation without drying out the flask during the process. Connect the condenser to a cold-water supply to facilitate the condensation of the distillate.

Heating and Distillation:

Start heating the distillation flask using a heating mantle or water bath. Ensure that the temperature is controlled, as excessive heat may cause the decomposition of sensitive compounds. Maintain a steady boiling rate and allow the steam to carry the volatile compounds from the plant material into the condenser. The condensed distillate will drip into the receiver, where the essential oil (which is immiscible with water) will separate and float on top of the water.

Collection of Distillate:

Continue distilling until no more essential oil is collected or the plant material has been adequately exhausted (usually for 2-4 hours, depending on the plant material).After the distillation, stop heating and allow the apparatus to cool before disassembling.

Separation of Essential Oil:

Once distillation is complete, the essential oil can be separated from the water using a separating funnel or collected directly from the receiver if the oil is floating on top of the water. Collect the essential oil in a clean vial or container. Store it in an airtight, dark glass container to prevent degradation due to light or air exposure.

Cleaning the Apparatus:

After use, the Clevenger apparatus was thoroughly cleaned with distilled water and suitable solvents to remove any residual plant material or oils.

Observation and Result

Experiment – III: Phytochemical Analysis

Introduction and Aim of the Study

Phytochemical analysis is a crucial field of study that focuses on the identification and quantification of bioactive compounds produced by plants. These compounds, which include flavonoids, alkaloids, terpenoids, and phenolic acids, play significant roles in plant defense mechanisms against pathogens and herbivores. They are also recognized for their potential health benefits in humans, including antioxidant, anti-inflammatory, and antimicrobial properties. The exploration of phytochemicals is vital not only for understanding plant biology but also for their applications in medicine, nutrition, and agriculture. The primary aim of phytochemical analysis is to systematically evaluate the chemical constituents of plants to uncover their biological activities and potential health benefits.

Principle

The principles of phytochemical analysis involve several key methodologies. Initially, the extraction process is crucial as it determines the efficiency of isolating phytochemicals from plant tissues. Common extraction methods include solvent extraction, steam distillation, and cold pressing, each tailored to specific types of compounds. Following extraction, qualitative tests are conducted to identify the presence of specific classes of phytochemicals using colorimetric reactions or chromatography techniques. Quantitative analysis further evaluates the concentration of these compounds using advanced methods such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS), enabling researchers to establish a comprehensive profile of the phytochemical constituents. The ultimate goal of phytochemical analysis is to elucidate the relationship between plant-derived compounds and their biological activities. This includes exploring their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, which have significant implications for drug development and functional food production. By understanding the phytochemical composition of plants, researchers can identify potential therapeutic agents and contribute to the advancement of natural product chemistry. Additionally, this analysis supports the validation of traditional medicinal practices and promotes the sustainable use of plant resources in various industries.

Procedure for Preliminary Phytochemical analysis

1. Test for Alkaloids

To the 1 mL of extract, 1 mL of Mayer's reagent was added, presence of pale yellow precipitate indicates the presence of alkaloids.

2. Test for Terpenoids

1ml of each sample was combined with 1 mL concentrated $H_2SO_4$and 1 mL chloroform. Reddish-brown at the interphase indicates the presence of terpenoids

3. Test for Phenol

1ml of extract was mixed with three drops of 2% $FeCl_3$. The emergence of greenish-blue forms indicated the presence of phenols.

4. Test for Reducing Sugar

1 mL of plant extract was mixed with 5-8 drops of Fehling's solution A (Copper sulphate solution) and Fehling's solution B (Aqueous solution of Pottasium sodium tartarate) and boiled. The presence of reducing sugar was revealed by the formation of brick red precipitate.

5. Test for Saponins

1 mL of distilled water and 1 mL of extracts were shaken vigorously. The froth persist until 2 minutes suggested the presence of saponins.

6. Test for Flavanoids

1ml of extracts, a piece of magnesium ribbon and concentrated hydrochloric acid were mixed. After a few minutes, the colour changes from red to pink, indicates the presence of flavonoids.

7. Test for Quinines

1ml of sample was treated with 5% of Sodium hydroxide solution. Reddish color formation indicates the presence of quinines

8. Test for Protein

A few drops of concentrated nitric acid were added to the 1ml of plant extract sample. Development of a yellow colour showed the presence of proteins.

9. Test for Steroids

1ml of the extract was dissolved with 1ml of chloroform, and concentrated H_2SO_4 (drop by drop). The top layer becomes red, and the H_2SO_4 layer fluoresces green and turns yellow. This suggests the presence of steroids.

Observation and Result

Experiment – IV: Antioxidant Activity

DPPH

Introduction and Aim of the study

The DPPH assay serves as a valuable tool in research aimed at exploring natural antioxidants from plants. The DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity assay is a widely used method for assessing the free radical scavenging capacity of various substances, particularly plant extracts. The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity.

Principle

The DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay is a widely recognized method used to evaluate the antioxidant capacity of various substances, particularly those derived from natural sources. The principle of this assay is based on the ability of antioxidants to donate hydrogen atoms or electrons to neutralize free radicals. DPPH is a stable free radical that exhibits a deep violet color in solution due to its unpaired electron. When an antioxidant interacts with DPPH, it reduces the radical, resulting in a color change from deep purple to yellow. This change can be quantitatively measured using spectrophotometry at a wavelength of 517 nm, to assess the extent of radical scavenging activity and thus determine the antioxidant capacity of the tested samples.

Procedure

- Prepare the samples to be tested for antioxidant activity in suitable solvents, ensuring they are well-dissolved
- In a test tube add 0.5ml of the test sample and 0.2ml of the 0.1N of DPPH solution. For controls, include a blank with solvent only and mix thoroughly to initiate the reaction
- Incubate the mixture at room temperature for 5 minutes
- After incubation, 0.4 ml of the 50mM Tris HCl was added.
- Allow the mixtures to incubate in the dark at room temperature for about 30 minutes. This period is crucial for the reaction between DPPH and the antioxidants to occur.

- After incubation, measure the absorbance at 517 nm using a spectrophotometer. Record the absorbance values for all samples, including blanks and controls.
- Calculate the percentage of DPPH scavenging activity using the formula:

$$\text{Percentage of Scavenging activity} = \frac{\text{Absorbance Control} - \text{Absorbance of Sample}}{\text{Absorbance Control}} \times 100$$

Observation and Result

Total Phenolic Content estimation

Introduction and Aim of the study

Total phenols are known for their significant antioxidant properties, which help in neutralizing free radicals and reducing oxidative damage in biological systems. The assessment of total phenolic content (TPC) is crucial for understanding the health benefits associated with plant-based foods and herbal products, as higher phenolic content is often correlated with enhanced antioxidant capacity. The primary aim of the total phenol assay is to quantify the concentration of phenolic compounds in various samples, such as plant extracts.

Principle:

The principle of the Total Phenolic Content (TPC) assay, particularly the Folin-Ciocalteu (F-C) method, revolves around the redox reaction between phenolic compounds and the Folin-Ciocalteu reagent. The F-C reagent is a mixture of phosphomolybdic and phosphotungstic acids, which, in an alkaline environment, undergoes reduction when reacting with phenolic compounds. This reaction results in the formation of a blue-colored complex that can be measured spectrophotometrically. The intensity of the blue color, which has a maximum absorbance at around 765 nm, is directly proportional to the concentration of total phenols present in the sample. In this assay, a sample containing phenolic compounds is mixed with the Folin-Ciocalteu reagent and sodium carbonate. The sodium carbonate provides an alkaline medium necessary for the reduction reaction to occur. After a specified incubation period, the absorbance of the resulting solution is measured using a spectrophotometer. The concentration of total phenols is then calculated based on a standard curve generated from known concentrations of gallic acid, which is commonly used as a reference compound. The results are typically expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract.

Procedure:

- To 1 ml of extract, 0.2 ml of 0.2 N Folin–Ciocalteau’s reagent was added, mixed by gentle shaking.
- Incubate the mixture for 10 minutes in room temperature

- To the mixture, 1ml of Na_2CO_3 (20% w/v) was added and incubated at 45°C for 45 min.
- Measure the absorbance of sample at 765 nm using a spectrophotometer. Ensure that a blank (solvent only) is included in measurements to calibrate the instrument.
- Calculate the total phenolic content using a standard curve generated from known concentrations of gallic acid (or another standard phenolic compound). The results are typically expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry extract.

Observation and Result

Total Flavonoid Content Estimation

Introduction and Aim of the study

Total flavonoid content (TFC) refers to the amount of flavonoids present in a given plant or food sample, which are important phytochemicals known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Flavonoids are polyphenolic compounds that contribute significantly to the color, flavor, and health benefits of many fruits, vegetables, and beverages. The quantification of total flavonoids is essential for evaluating the nutritional value of plant-based products and understanding their potential health benefits. The primary aim of the total flavonoid content assay is to accurately measure the concentration of flavonoids in various samples. This is typically achieved using colorimetric methods, such as the aluminum chloride colorimetric assay.

Principle

The principle behind the total flavonoid content assay typically involves a colorimetric method using aluminium chloride ($AlCl_3$). Flavonoids react with aluminium ions to form a stable complex that exhibits a characteristic color. In this assay, a sample containing flavonoids is mixed with aluminium chloride and other reagents, resulting in a colour change that can be quantitatively measured using a spectrophotometer. The intensity of the color produced is directly proportional to the concentration of flavonoids in the sample. This relationship allows for the determination of TFC by comparing the absorbance readings to a standard curve created using known concentrations of a flavonoid standard, such as quercetin.

Procedure

- In clean test tubes or cuvettes, add 0.5 mL of the sample extract.
- Add 0.1 mL of 10% aluminium chloride solution to the tube.
- Follow this by adding 0.1 mL of potassium sodium tartrate solution and 2.8 mL of distilled water.
- Allow the mixture to stand at room temperature in the dark for about 30 minutes to facilitate color development.

- Measure the absorbance at 415 nm using a spectrophotometer. Ensure that a blank (solvent only) is included in your measurements for calibration.
- Calculate the total flavonoid content based on a standard curve generated from known concentrations of quercetin or another flavonoid standard. The results are typically expressed as milligrams of quercetin equivalents (mg QE) per gram of dry extract.

Observation and Result

SOD (Superoxide Dismutase) Assay

Introduction and Aim of the study

The primary aim of the Superoxide Dismutase (SOD) antioxidant activity assay is to quantify the enzymatic activity of SOD in various biological samples. SOD is a crucial enzyme that catalyzes the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide, thereby protecting cells from oxidative damage caused by reactive oxygen species (ROS). By measuring SOD activity, it can assess the antioxidant capacity of different tissues or extracts, evaluate the oxidative stress levels in various conditions, and understand the role of SOD.

Principle

Superoxide radicals are generated in the reaction mixture, typically through the auto-oxidation of hydroxylamine. These superoxide radicals then reduce the yellow-colored Nitro Blue Tetrazolium (NBT) to the blue colored formazan. SOD present in the sample can scavenge the superoxide radicals, thereby inhibiting the reduction of NBT. The degree of inhibition of NBT reduction is proportional to the SOD activity in the sample. By measuring the absorbance of the blue formazan product, the SOD activity can be quantified.

Procedure

- In a cuvette or test tube, mix 0.5ml of sample with 1ml of SOD reaction mixture I prepared by adding 1 ml of 50m phosphate buffer solution, 0.075 ml of EDTA, 20M L-Methionine and 0.04 ml of 10mM Hydroxy amide hydrochloride.
- Then the mixture was kept at pre-incubation at 37°C for 10 mins.
- 50µl of 50mM Riboflavin was added after incubation.
- The tubes were mixed well and exposed for 5 mins under UV fluorescent light.
- After the exposure time, 1 ml of SOD reaction mixtures II was added which was freshly prepared by mixing 1% sulphanilamide in 5% phosphoric acid.
- Measure the absorbance at 560 nm using a spectrophotometer.
- Calculate the percentage inhibition using the formula:

$$\text{Percentage of Scavenging activity} = \frac{\text{Absorbance Control} - \text{Absorbance of Sample}}{\text{Absorbance Control}} \times 100$$

Observation and Result

Experiment – V: UV- Visible Spectroscopy

Introduction and Aim of the study

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state. The primary aim of UV spectrometry, (ultraviolet-visible spectrophotometry), is to measure the absorption of ultraviolet and visible light by chemical substances. This technique is widely used to determine the concentration of analytes in a solution, identify unknown compounds, and analyze the physical and electronic structures of materials.

Principle

UV-visible spectroscopy is a quantitative technique used in analytical chemistry to measure the amount of light absorbed by a substance. When light falls upon a substance it absorbs and reflects a certain amount of radiation. As the light passes through the sample, the amount of radiation absorbed by the substance is the difference between the incident radiation (I_o) and the transmitted radiation (I). The amount of radiation absorbed is called absorbance (A) and transmittance (T), which is a fraction (I/ I_o) indicating the amount of light that has passed through the sample.

Procedure

- Prepare a solution of the analyte with an appropriate concentration. Ensure that it falls within the linear range for accurate measurements.
- Choose an appropriate cuvette made from quartz or glass (for visible light). Ensure it is clean and free from scratches to avoid interference with measurements.
- Before measuring samples, calibrate the spectrophotometer using a blank solution (solvent without analyte) to set a baseline for absorbance measurements.
- Measure and record the absorbance of the blank at the desired wavelengths.
- Place the cuvette containing the sample into the spectrophotometer.

- Select the desired wavelength (s) for measurement based on known absorption characteristics of the analyte.
- Record the absorbance values displayed by the spectrophotometer.

Observation and Result

Experiment – VI: Fourier Transform Infra Red Spectroscopy (FT-IR)

Introduction and Aim of the study

The primary aim of Fourier Transform Infrared (FTIR) spectroscopy in the analysis of plant extracts is to identify and characterize the functional groups and chemical compounds present in the extracts. This technique provides valuable information about the molecular structure of phytochemicals, helping researchers to understand the bioactive components that contribute to the medicinal properties of plants. By analyzing the spectral data, scientists can correlate specific functional groups with potential health benefits, aiding in the development of herbal medicines and functional foods.

Principle of FTIR Spectroscopy

FTIR spectroscopy is based on the principle that molecules absorb infrared radiation at specific wavelengths corresponding to their vibrational modes. When a sample is exposed to infrared light, certain frequencies are absorbed by the chemical bonds within the molecules, causing them to vibrate. This absorption results in a spectrum that displays peaks at various wavelengths, each representing a different functional group or bond type (e.g., O-H, C=O, N-H). The position and intensity of these peaks can be used to identify the presence of specific compounds in the sample. The resulting FTIR spectrum serves as a fingerprint for the chemical composition of the plant extract, allowing for qualitative and quantitative analysis.

Procedure

- Collect fresh plant material and dry it in a shaded area or an oven at low temperature to prevent degradation of sensitive compounds.
- Grind the dried plant material into a fine powder using a mortar and pestle or a grinder.
- Weigh approximately 10 mg of the powdered sample.
- Preparation of KBr Pellets:
- Mix the powdered sample with approximately 100 mg of potassium bromide (KBr) powder to form a homogeneous mixture.

- Compress this mixture into a pellet using a hydraulic press at high pressure to create a translucent disc.
- Place the KBr pellet into the sample holder of the FTIR spectrophotometer.
- Set the instrument parameters, including scan range (typically from 400 cm^{-1} to 4000 cm^{-1}) and resolution (commonly 4 cm^{-1}).
- Run the FTIR analysis to obtain the spectrum of the sample.
- Analyze the resulting FTIR spectrum by identifying peaks corresponding to specific functional groups.
- Compare these peaks with standard reference values to determine which compounds are present in the plant extract.
- Interpret the data to assess potential bioactive compounds based on their functional groups.

Observation and Result

Experiment – VII: Chromatography

Chromatography is a separation technique, used to separate an individual compounds from a complex mixture. Separations are extremely important in synthesis and analytical chemistry. Chromatography is a widely used method that allows separation, identification and determination of chemical compound in a complex mixture. No other separation method is as powerful and generally applicable as chromatography. Chromatographic separation will take place between two phases namely, stationary and mobile phase based on their chemical nature. Compounds of a mixture are separated based on the differences in the rate at which they are carried through a stationary phase by a mobile phase. The stationary phase in chromatography is fixed in place either in a column or on a planar surface. The mobile phase moves over or through the stationary phase carry with it the analyte mixture. The mobile phase may be a gas, a liquid or a supercritical fluid. Chrom methods are of two basic types, column and planar. In column chrom the stationary phase is held in a narrow tube, and the mobile phase is forced through the tube under pressure or gravity (eg. HPLC & GC). In planar chrom, the stationary phase is supported by a plate or in the pores of paper. Here, the mobile phase moves through the stationary phase by capillary action or under the infukence of gravity (eg, TLC & paper chrom). Although, many types of chromatographic techniques are available, High performance liquid chromatography (HPLC) and Gas Chrom (GC) are the commonly used for analysis. Here we deal HPLC & GC combined with Mass spectroscopy as the scope of the workshop.

Column Chromatography

Introduction and Aim of the study

To determine different compounds of a solution using column chromatography. This technique enables the separation, identification, and purification of the components from a mixture.

Principle

Column chromatography is adsorption of the solutes of a solution through a stationary phase and separates the mixture into individual components. This is based on the affinity towards the stationary phase.

Procedure

- 1g of plant extract leaf was taken.
- It was powered and grinded with 20 ml of distilled water/ solvents.
- It was transferred into conical flask and kept it in shaker for 1 hour at 37℃ from 75 to 80 rpm.
- The solution was filtered using filter paper.
- Simultaneously silica gel was prepared.
- Cotton was placed in the column chromatography setup and distilled waster was poured in it.
- Excess water is eluted after 15 minutes.
- Silica gel was added and kept undisturbed for 1 hour.
- Again, excess water eluted.
- The filtered solution of leaf extract was added in the column chromatography.
- Elute in Eppendorf tubes and the reading was taken by using UV spectrometer at 480, 560 and 640nm

Mobile phase - contain solvents and the sample mixture can be introduced in the column. Which helps for the separation of components in the sample to form bands. Elutent may help the components that are separated during the experiment are removed from the column. Some examples of solvents used as mobile phases based on their polarity are – methanol, ethanol, acetone, water, n-hexane etc.

Stationary phase - is a solid material – gel, Particles should have a uniform shape and size, with high mechanical stability and chemically inert. Which has no reaction with acids or bases or any other solvents. Which should be colourless, which may help for the free flow of mobile phase. It should be suitable for the separation of mixtures of various compounds.

Observation and Result

Thin Layer Chromatography

Introduction and Aim of the study

Chromatography is the process through which biomolecules are separated and analysed from a complex mixture. This separation process consists of two phases: a stationary phase and a mobile phase. The mobile phase consists of the mixture to be separated which percolates through the stationary phase. These two phases can be solid-liquid, liquid-liquid or gas-liquid. Thin Layer Chromatography (TLC) is a solid-liquid form of chromatography where the stationary phase is a polar absorbent and the mobile phase can be a single solvent or combination of solvents. Thin layer chromatography is a kind of chromatography used to separate and isolate mixtures that are non-volatile in nature.

Principle

The separation in TLC relies on the interaction between the stationary phase, typically thin layer of silica gel or alumina coated on a plate, and the mobile phase, which is a solvent or a mixture of solvents. When a sample mixture is applied to the plate and the mobile phase is allowed to move up through capillary action, different compounds in the mixture migrate at different rates based on their affinities for both phases. Compounds that have a higher affinity for the stationary phase will travel more slowly, while those with a greater affinity for the mobile phase will move faster. This differential migration results in the separation of components within the mixture, which can be visualized as distinct spots on the TLC plate after development.

Procedure

- Take a TLC plate (usually made of aluminium foil, plastic, or glass coated with silica gel or alumina).
- Using a pencil draw straight line approximately 1-2 cm from the bottom of the plate. This line serves as the baseline for spotting samples.
- Mark equidistant points along this line where apply the samples.
- Using a toothpick or capillary tube, spot approximately 10µL of sample solution onto the marked points on the baseline. Ensure that spots are small and well-defined.
- Allow the spots to air dry for a few minutes to ensure that the solvent has evaporated.

- Carefully place the spotted TLC plate into the prepared TLC chamber, ensuring that the solvent level is below the baseline to prevent dissolving the samples directly into the solvent pool.
- Cover the chamber with its lid or watch glass to create a closed environment.
- Allow the solvent to rise up the plate by capillary action until it reaches about 0.5 cm from the top of the plate.
- Once the solvent front has reached the desired height, carefully remove the TLC plate from the chamber using forceps.
- Immediately mark the solvent front with pencil before allowing it to dry completely.
- The sample spots can be observed under a UV light chamber.
- Observe and record the positions of different spots on the plate.
- Calculate the retention factor (R_f) using:

$$\mathrm{Rf} = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent front}}$$

Observation and Result

Experiment – VIII: High performance liquid chromatography (HPLC)

HPLC is an abbreviation for High Performance Liquid Chromatography. HPLC is the most versatile and widely used type of elution chrom. The technique is used by chemist to separate and determine species in a variety of organic, inorganic, and biological samples. Only compounds dissolved in solvents can be analyzed with HPLC. HPLC separates compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component are contained in the sample.

The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy. HPLC is thus basically a highly improved form of column liquid 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. In the liquid chrom, the mobile phase is a liquid solvent containing sample as a mixture of solutes. Among the various technologies developed for chromatography, devices dedicated for molecular separation called columns and high-performance pumps for delivering solvent at a stable flow rate are some of the key components of chromatographs. As related technologies became more sophisticated, the system commonly referred to as High Performance Liquid Chromatography, simply became referred to as "LC". Nowadays, Ultra High-Performance Liquid Chromatography (UHPLC), capable of high-speed analysis, has also become more wide-spread.

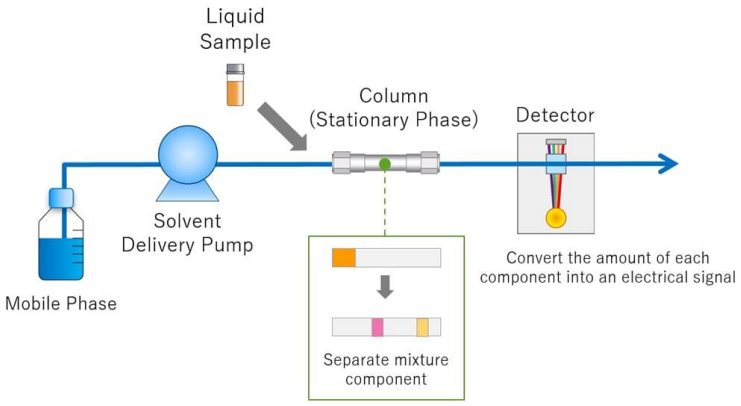


Fig. HPLC process

Fig.1 shows a basic overview of the HPLC process. The solvent used to separate components in a liquid sample for HPLC analysis is called the mobile phase. The mobile phase is delivered to a separation column, otherwise known as the stationary phase, and then to the detector at a stable flow rate controlled by the solvent delivery pump. A certain amount of sample is injected into the column and the compounds contained in the sample are separated. The compounds separated in the column are detected by a detector downstream of the column and each compound is identified and quantified.

HPLC Principle

- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

HPLC Separation

HPLC can separate and detect each compound by the difference of each compound's speed through the column. Fig.2 shows an example of HPLC separation.

There are two phases for HPLC: the mobile phase and the stationary phase. The mobile phase is the liquid that dissolves the target compound. The stationary phase is the part of a column that interacts with the target compound.

In the column, the stronger the affinity (e.g.; van der waals force) between the component and the mobile phase, the faster the component moves through the column along with the mobile phase. On the other hand, the stronger the affinity with the stationary phase, the slower it moves through the column. Fig. 3 shows an example in which the yellow component has a strong affinity with the mobile phase and moves quickly through the column, while the pink component has a strong affinity with the stationary phase and moves through slowly. The elution speed in the column depends on the affinity between the compound and the stationary phase.

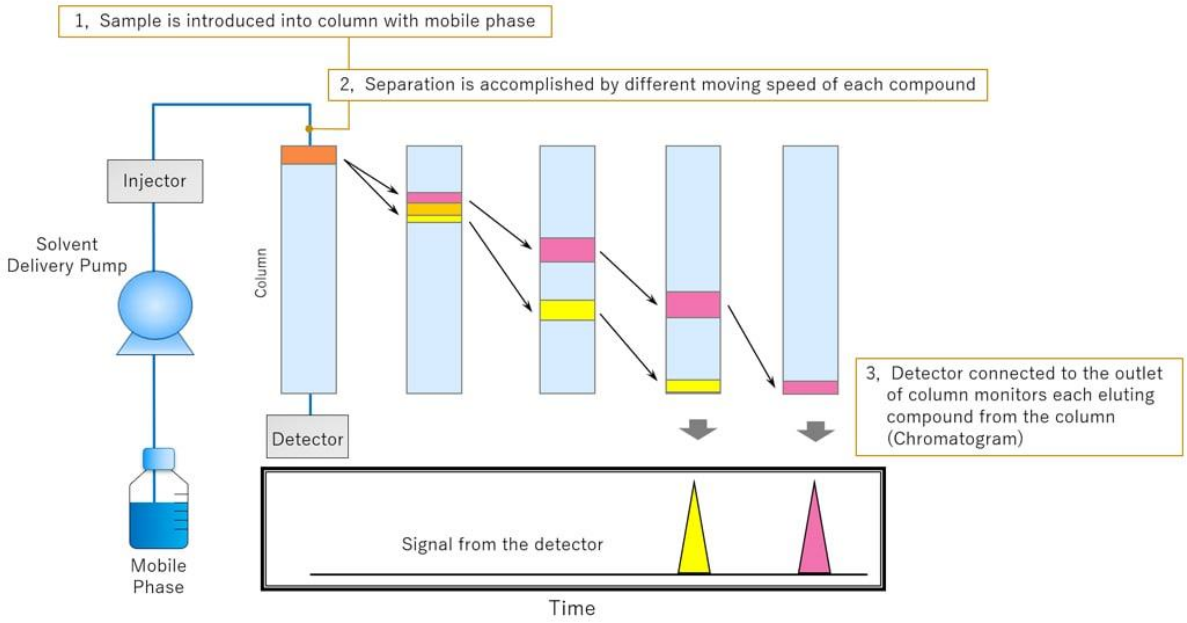


Fig. HPLC separation.

Chromatogram

The word "chromatogram" means a plot obtained via chromatography. Fig.3 shows an example of a chromatogram. The chromatogram is a two-dimensional plot with the vertical axis showing concentration in terms of the detector signal intensity and the horizontal axis representing the analysis time. When no compounds are eluted from the column, a line parallel to the horizontal

axis is plotted. This is called the baseline. The detector responds based on the concentration of the target compound in the elution band. The obtained plot is more like the shape of a bell rather than a triangle. This shape is called a "peak".

Retention time (tR) is the time interval between sample injection point and the apex of the peak. The required time for non-retained compounds (compounds with no interaction for the stationary phase) to go from the injector to the detector is called the dead time (t0).

The peak height (h) is the vertical distance between a peak's apex and the baseline, and the peak area (A) colored in light blue is the area enclosed by the peak and baseline. These results will be used for the qualitative and quantitative analysis of a sample's components.

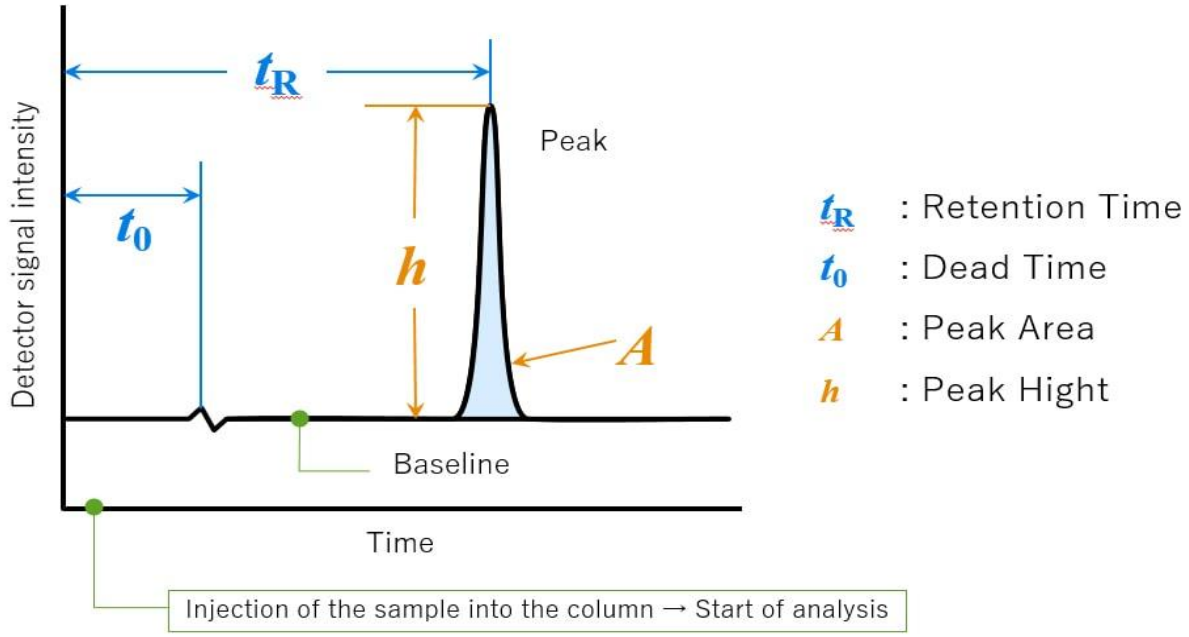


Fig. Chromatogram

Instrument

The Pump

- The development of HPLC led to the development of the pump system.
- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.

- High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.
- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector

- An injector is placed next to the pump. The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in a stainless-steel housing, instead of glass columns.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents. The packing material generally used is silica or polymer gels compared to calcium carbonate.
- The eluent used for LC varies from acidic to basic solvents.

Column Heater

- The LC separation is often largely influenced by the column temperature.
- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater).

Detector

- Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation.
- The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
- This difference is monitored as a form of an electronic signal. There are different types of detectors available.
- Different types of detectors are, UV, Fluorescence, Refractive Index (RI), Evaporative light scattering detector (ELSD), Mass Detector…

Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Degasser

- The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.
- When gas is present in the eluent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.
- The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.

Different types of detectors

To quantify and identify the compounds separated within a HPLC column, High Performance Liquid Chromatography (HPLC) requires the use of a suitable detector. Detectors

serve the crucial role of analysing the component of the eluted mixture once it has passed through the HPLC column. While there exists a range of HPLC detection methods, it is essential to acknowledge that no single detector can effectively detect all compounds or analytes. For liquid chromatography analysis, it is customary to employ two or more detection methods to facilitate comprehensive sample characterisation. The selection of the appropriate HPLC detector depends on the specific compounds of interest. In this blog, we will elaborate on some of the various types of detectors that can be used in conjunction with a liquid chromatograph.

HPLC UV Detector

Ultraviolet (UV) detectors stand as the most commonly, if not one of the most commonly employed, detections methods in liquid chromatography. UV detectors are non-destructive instruments employed in chromatography to quantify the absorption of ultraviolet or visible light by the component of an eluted mixture from the chromatography column. The UV detection process begins by passing the sample through a transparent flow cell, typically made of glass. Subsequently, UV light is directed onto the flow cell, and the sample interacts with this light, absorbing a portion of it. The quantity of the sample can be determined by calculating the variation in UV light intensity between the mobile phase (absence of sample) and the phase with the sample. The appropriate UV wavelength selection is contingent on the specific analyte present in the sample, as it significantly influences UV absorbance.

HPLC Fluorescence Detector (FLD)

HPLC fluorescence detectors are renowned for their exceptional sensitivity and specificity when compared to other types of HPLC detectors. These detectors function by measuring the emission of light from excited atoms within an analyte, allowing researchers to gather valuable information from a solution collected from a HPLC column.

While it provides a substantially higher level of sensitivity compared to UV/VIS detectors (ranging from 10 to 1000 times greater), its applicability is limited to the analysis of fluorescent molecules, making it a less commonly employed detection method. In cases where a solution lacks natural fluorescence, it can still be measured using a fluorescence derivative. Fluorescence detectors prove particularly valuable in the analysis of pharmaceuticals (especially when dealing

with samples with substantial levels of contaminants), food analysis, environmental monitoring, clinical research, and oil analysis (specifically petroleum products).

Types of HPLC

Normal phase:

Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds.

Reverse phase:

The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable, and ionic samples.

Ion exchange:

Column packing contains ionic groups and the mobile phase is buffer. It is used to separate anions and cations.

Size exclusion:

Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later.

HPLC Applications

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy. Identification and quantification of organic compounds. Analysis of drugs, Analysis of synthetic polymers , Analysis of pollutants in environmental analytics , Determination of drugs in biological matrices , Isolation of valuable products, Product purity and quality control of industrial products and fine chemicals , Separation and purification of biopolymers such as enzymes or nucleic acids

Advantages

Accuracy, Efficiency, Versatile and extremely precise when it comes to identifying and quantifying chemical components.

Disadvantages

- Cost: Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics.

- Complexity
- HPLC does have low sensitivity for certain compounds,
- Volatile substances are better separated by gas chromatography.

Observation and Result

Experiment – IX: Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid chromatography/mass spectrometry (LC/MS) is an analytical technique that combines the separation power of liquid chromatography with the direct mass measurement of a mass spectrometer as the detector. Liquid chromatography can separate a wide range of compounds, while the mass detector provides valuable information about molecular weight, structure, identity, quantity, and purity. Mass spectrometry (MS) is used to determine the mass of gas phase ions and their fragments. Analytes are ionized and filtered based on their mass-to-charge ratio; they're detected as ions, and their neutral mass is calculated.

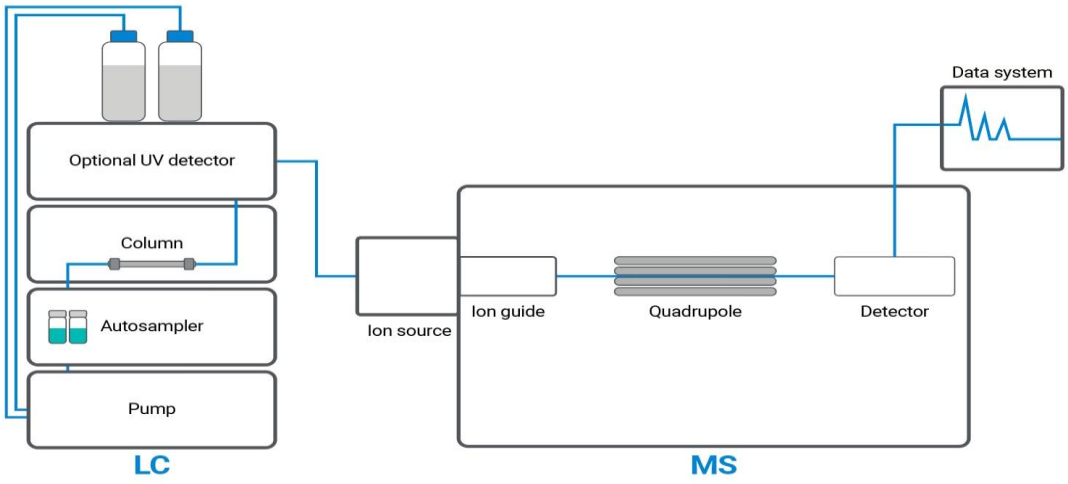


Fig. Liquid chromatography/mass spectrometry (LC/MS)

After compounds are separated by LC, the first component they encounter in the mass spectrometer is called the ion source. Electrospray ionization (ESI) is a common ionization technique that generates analyte ions in solution before the analyte reaches the inlet capillary of the mass spectrometer. LC eluent is sprayed (nebulized) into a chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The heat causes further stripping of solvent from the analyte molecules.

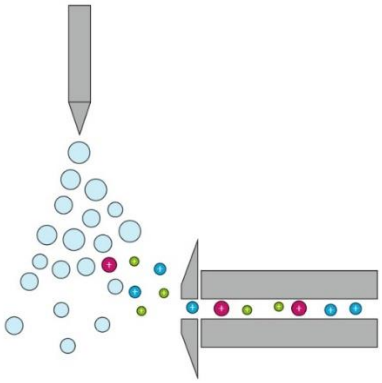


Fig. Nebuliser at MS

After entering the mass spectrometer through the inlet capillary, a series of electrodes known as lenses direct the charged molecules away from the source toward the quadrupole mass analyzer (or mass filter). A quadrupole consists of four rods to which a direct current voltage and radio frequency are applied. Various combinations of these forces ensure that only fragments of a specific mass—called a mass-to-charge ratio or m/z—will travel down the quadrupole's electric field toward the detector at a given time. This feature dramatically decreases noise and increases sensitivity.

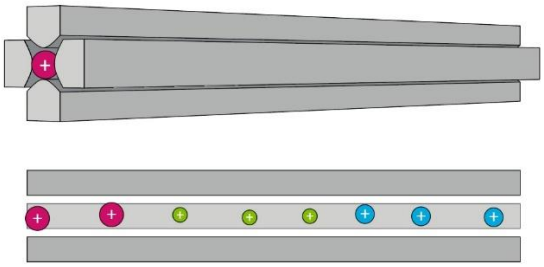


Fig. Quadrupole mass analyser

To produce the mass spectrum, the detector records the signal intensity from ions arriving at each given time. The pattern of this mass spectrum can be used for identification, much like a

fingerprint. In addition, the response recorded for the different ion species can be calibrated for quantitative purposes.

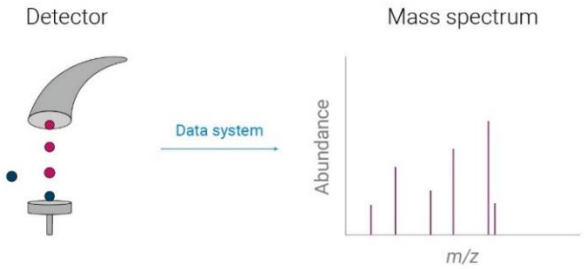


Fig. Detector

Observation and Result

Experiment – X Gas Chromatography – Mass Spectrometer (GC-MS)

Gas chromatography (GC) is one of the popular chromatography techniques to separate volatile compounds or substances. The mobile phase is an inert gas such as helium, nitrogen, and the stationary phase is a high-boiling liquid that is adsorbed on a solid. In GC, the chromatographic separations take place by the difference in partitioning behavior between mobile and stationary phases. Because of its simplicity, high sensitivity, and the ability to effectively separate mixtures, gas chromatography has become one of the most important tools in analytical chemistry to analyse volatile compounds.

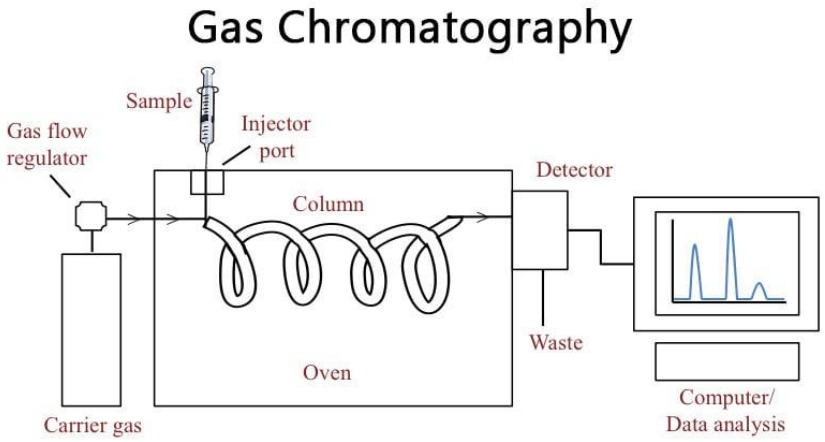


Fig. Gas chromatography (GC) – diagram

When a mixed solution sample is injected into the GC system, the compounds contained in the sample, including the solvent components, are heated and vaporized within the sample injection unit. With GC system, the mobile phase, referred to as the carrier gas, always flows in sequence from the sample injection unit to the column, and then to the detector. The target components that were vaporized in the sample injection unit are transported by the carrier gas to the column. Once in the column, the mixture of compounds is separated into the various components, and the amount of each compound is then measured by the detector.

The detector converts the amount of each compound into an electrical signal, and sends these signals to a data processing unit. The data obtained enables determination of the compounds contained in the sample, and in what amounts

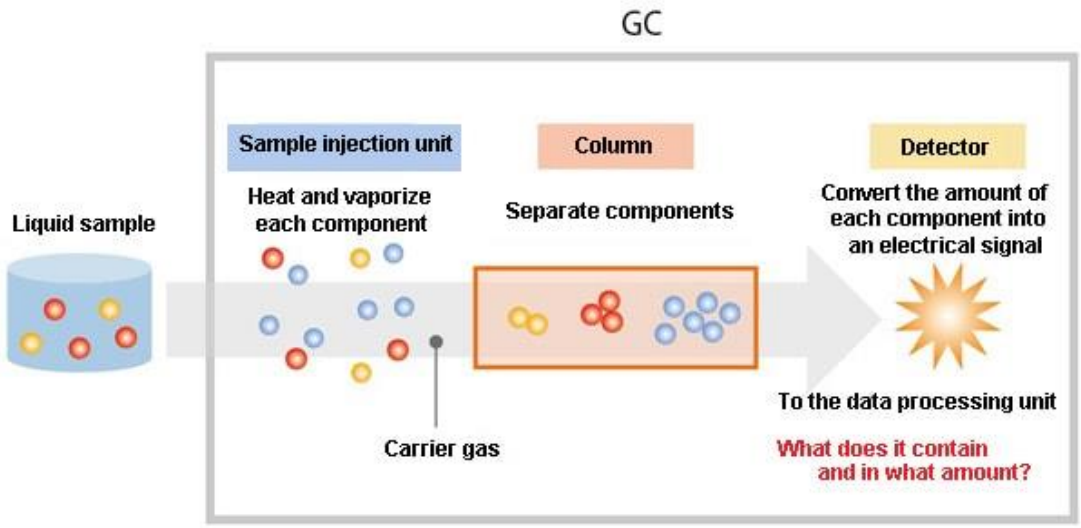
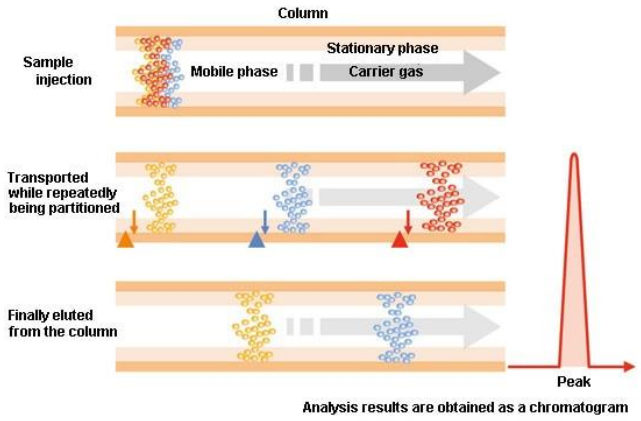


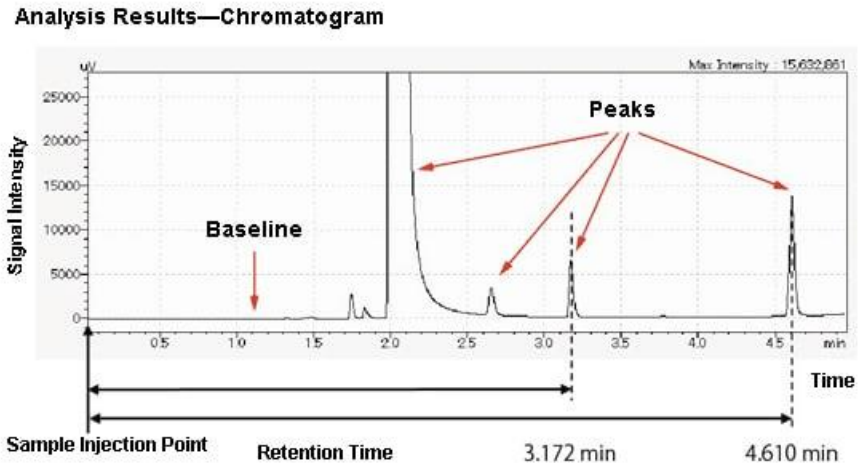
Fig. GC-Separation

Separation by GC occurs within the column. The sample containing multiple compounds is injected into the column together with the mobile phase. (In GC, the mobile phase is a gas referred to as the carrier gas. He is frequently used). Both the sample and the mobile phase travel through the column, but the rate of progression within the column differs depending on the compound. Accordingly, differences arise in the times at which the respective compounds arrive at the column outlet. As a result, a separation between each compound occurs.

The row of peaks drawn when the electrical signals output from the GC detector are plotted on the vertical axis and the elapsed time after sample injection is plotted on the horizontal axis is called a chromatogram. The components passing through the column are transported by the mobile phase (gas phase) while being partitioned from and adsorbed into the stationary phase (liquid phase and solid phase).



A typical chromatogram is shown here. The horizontal axis shows the time until the component reaches the detector. The vertical axis shows the signal intensity. The part at which nothing is detected is called the baseline, and the part where a component is detected is called a peak. The time from when the sample is injected into the system until the peaks appear is called the retention time. As the elution times for each component differ, each component can be separated and detected.



Gas chromatography is mainly composed of the following parts

1. **Carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters**
    - Helium, N2, H, Argon are used as carrier gases.
    - Helium is preferred for Mass detector.
    - N2 is preferable when a large consumption of carrier gas is employed.
    - Carrier gas from the tank passes through a toggle valve, a flow meter, (0.01-99 ml/min), capillary restrictors, and a pressure gauge (1-4 atm).
    - Carrier gas flow fully controlled through ought the analysis.
2. **Sample injection system**
    - Liquid samples are injected by a micro syringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater.
    - Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves.
    - Typical sample volumes range from 0.1 to 0.2 ul.

3. **The separation column**
    - Liquid stationary phase coated inside the walls of the Copper capillary tubes are act as a column in GC.
    - GC columns are placed inside the column oven with temperature controlled.
    - It can withstand the temperature ranged -50 to 450 ^{o}C.
    - Several sizes of columns are used depending upon the requirements.
4. **Liquid phases**
    - An infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support.
    - No single phase will serve for all separation problems at all temperatures.
    - Non-Polar – Parafin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.
    - Intermediate Polarity – These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and non-polar solutes. For example. diethyl hexyl phthalate is used for the separation of high boiling alcohols.
    - Polar – Carbowaxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.
    - Hydrogen bonding – Polar liquid phases with high hydrogen bonding e.g. Glycol.
    - Specific purpose phases – Relying on a chemical reaction with solute to achieve separations. e.g $AgNO_3$ in glycol separates unsaturated hydrocarbons.

Detector

- Detectors sense the arrival of the separated components and provide a signal.
- These are either concentration-dependent or mass dependent.
- The detector should be close to the column exit and the correct temperature to prevent decomposition.

Types of Detectors

- Similar to HPLC, different types of detectors are available
- Based on the chemical nature need to use the detectors
- Notable are

- Flame ionization detector (FID)
- Flame photometric detector (FPD)
- Thermal conductivity detector (TCD)
- Electron capture detector (ECD)
- Nitrogen Phosphorus Detector (NPD)
- Photoionization detector (PID)
- Mass detector (MS)

Recorder

- A specific software with controller is used to control and generate the data based on the signal produced by the detector.

Compounds Suitable for GC Analysis

Components that can be analyzed with GC have the following three main features.

- Compounds with a boiling point up to 400 °C
- Compounds that are not decomposed at their vaporization temperature
- Compounds that decompose at their vaporization temperature, but always by the same amount. This is called pyrolysis GC.

Compounds That Cannot Be Analyzed

- Compounds that do not vaporize (inorganic metals, ions, and salts)
- Highly reactive compounds and chemically unstable compounds
- (hydrofluoric acid and other strong acids, ozone, NOx and other highly reactive (compounds)

Compounds That Are Difficult to Analyze

- Highly adsorptive compounds (compounds containing a carboxyl group, hydroxyl group, amino group, or sulfur)
- Compounds for which standard samples are difficult to obtain (Qualitative and quantitative analyses are difficult.).

Advantages of gas chromatography

- High separation efficiency and analysis speed: for example, gasoline samples can obtain more than 200 chromatographic peaks in 2 hrs. A general sample analysis can be completed in 20 minutes.
- Small sample consumption and high detection sensitivity: 1 ml of gas sample consumption, 0.1 µl of liquid sample consumption, a few mg of solid sample consumption. Proper detectors can detect impurities in the tens to a few parts per million.
- Gas chromatography has good selectivity and can be used to analyze azeotropic mixtures and samples with close boiling points. For example, some isotopes, cis-trans isomers, adjacent or intertrain isomers, optical isomers, etc.
- Wide range of applications, although mainly used to analyze gases and volatile organic substancesunder certain conditions, it can also be used to analyze high boiling point substances and solid samples.

Disadvantages of gas chromatography

- It can only be used to analyze volatile substances.
- Some highly polar substances can be derived to increase their volatility for GC analysis, but the process can be complex and may introduce errors in quantitative analysis.

Observation and Result

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Day 1 - 21st Nov 2024

9.00 am to 9.30 am	: Registration
9.30 am to 10.00 am	: Inauguration and About the Workshop **Prof.Dr.R.Ragunathan., Ph.D.,FSAB** Director & Research Supervisor Centre for Bio science and Nano science Research (CBNR)
10.00 am to 1.00 pm	: Key note address and Practical **Mr.S.Satheesh Kumar** Assistant Professor, Dept. of Biotechnology (FoE) Karpagam Academy of Higher Education , Coimbatore - 21
1.00 pm to 2.00 pm	: Lunch Break
2.00 pm to 5.00 pm	: Laboratory Practical **Dr.Jesteena Johney and team** Centre for Bio science and Nano science Research (CBNR)
5.00 pm	: Panel discussion, Tea Break and Wrap Up of day 1

Day 2 - 22nd Nov 2024

8.30 am	: Departure to SACON from CBNR
9.30 am to 10.00 am	: Introduction
10.00 am to 3.30 pm (Including Lunch Break)	: Sophisticated Instrumentation Lab (All Chromatography Techniques) : **Dr.P.R.Arun** Principal Scientist & Head, Environmental Impact Assessment (EIA) Division, Salim Ali Centre for Ornithology and Natural History (SACON), Coimbatore. and **Dr. K. Nambirajan,** Scientist, Division Ecotoxicology Salim Ali Centre for Ornithology and Natural History (SACON), Coimbatore.
3.30 pm	: Departure from SACON to CBNR
5.00 pm	: Valedictory function & Certificate Distribution

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