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THE ANALYSIS OF HERBAL DRUGS AND DIFFERENT METHODS: RECENT REVEW

Name Of 1st Author: **ANUJA GIRISH ALATKAR**

Name Of 2nd Author: **MS. J. R. KAMBLE**

Abstract: Herbal plants have long been used to enhance human health; they have grown increasingly popular across the world as medications, alternative and complementary treatments, meals, vitamins, cosmetics, and, unexpectedly, as healthcare products. The complexity of herbs and extracts, together with their distribution to different markets and compliance with different regulations, has led to an increased demand for analytical methods that can accurately identify and standardize these products while also detecting adulterants and pollutants. Herbal samples raise various kinds of complications for customs laboratories, including worries regarding quality, safety, and even illegality. adopting an appropriate analytical technique between the wide range various options available, such as physical, chemical, biological, microscopic, or macroscopic technologies. There is more discussion of HPLC, GC, photometric techniques, UV, IR, MS, and NMR.

Keywords: Herbal drugs, analysis of herbal drug, Thin layer chromatography, HPLC, gas chromatography, UV visible spectroscopy, IR spectroscopy.

INTRODUCTION

Medicinal herbs have been used for thousands of years in both developed and developing nations due to their natural origins and the fact that they are safer than synthetic alternatives. One distinctive feature of eastern herbal medicine preparation is the use of boiling water during the decoction process to extract the medicinal properties of all herbs, whether they are presented as individual plants or in combination formulae. This may be the fundamental reason why the quality control of eastern herbal drugs is more challenging than that of western drugs. As a source of new, naturally occurring compounds with a wide range of chemical structures, herbal plants are vital to the pharmaceutical industry (1-3). About 85% of the world's population relies on traditional medicines for their health care needs. Keeping the plant effective, safe, and of high quality is crucial for preventing major health issues (4,5). A method that makes use of herbs, herb materials, or herbal preparations that include plant parts, other plant materials, or mixes as active components is considered a herbal medicine according to the World Health Organization (WHO) (6). Herb refers to any unprocessed, whole, or ground aspect of a plant, including but not limited to: leaves, blossoms, fruits, seeds, stems, wood, bark, roots, rhizomes, and so on. Herbal materials encompass a wide range of substances, including not only fresh juices and plants but also gums, essential oils, resins, dried herb powders, and fixed oils. In certain nations, these materials can be prepared in a number of unique ways, including stir-baking, roasting, or steaming with honey, alcohol, or other ingredients. Herbal medicines begin with plant preparations, which might be in the form of tinctures, fatty oils, extracts, or even just pulverized or powdered plant parts. Many physical and biological procedures, including concentration, fractionation, purification, and extraction, go into their production. Also included are concoctions created by simmering or boiling therapeutic herbs with honey, alcohol, or other substances. When you buy a whole herbal product, you're actually getting a combination of herbs. The definition of "mixture herbal product" may also be used when a variety of herb is used. Excipients may be added to finished herbal products and mixture herbal products in addition to the active constituents. Finished products or mixtures of herbal products which include chemically defined active ingredients—such as synthetic compounds or isolated herbal material constituents—are not considered are herbal. Herbal products are commonly used in a variety of traditional health treatments and practices, include homeopathy, naturopathy, ayurveda, Chinese medicine, and Unani (6). Over the past few years, the general people has used more natural substances to treat a variety of diseases. This can be caused by more than their easy availability without a prescription, expensive, or require medical evaluations. professionals, but also due to the perception that natural ingredients have less adverse effects than synthetic medications (7). Because there is always a possibility that the prescription allopathic drugs will have undesirable therapeutic effects, the interaction among herbal and allopathic medicines is an essential problem to investigate. medicinal products also differ in their pharmacokinetic and pharmacodynamic qualities, which ultimately result in a therapeutic 2 response but can also have undesirable effects and/or drug-herbal interactions (8). Herbal remedies frequently have several broad, synergistic, or complimentary impacts on biological systems at simultaneously. These reactions are usually non-specific and follow the same basic therapeutic directions. Further, these actionsusually rarely has adverse effects (9).

Materials and Methods

Methods of analytical evaluation of herbal drugs:

In general, the three pharmacopoeias mentioned following define quality control:

Identify: Is this a suitable herb to be expanding?

Purity: Are there contaminants there such as plant materials that should not be there?

Assay or content: Its active compound content is contained within the prescribed limitations (10). Evaluating content can be likely the most difficult process, since most herbal remedies contain unknown active ingredients. Regardless of any therapeutic effects, chemically defined components can be used as markers. To verify the item's origin and purity, tests must be carried out on moisture, ash content, solvent residue, adulteration, physical characteristics, sensory components, and harmful substances. Identification of the natural herbs or the plant qualitative can be crucial for quality control of herbal medications (11).

Investigating each macro- and microscopically may result to identity. Examples vouchers are reputable sources of information. Plant disease outbreaks have the ability for modifying a plant's appearance causing identification problems (12).

Ash values, contaminants (like other plants), and metallic elements are some of the variables of purity. But due to improved analytical technologies, modern assessment includes pesticide residues, radioactive substances, aflatoxins, and microbial contaminations. Several analytical techniques, including TLC, HPLC, GC, and photometric investigation (UV, IR, MS, and NMR), can be employed to identify the constant amount present in medicinal products (13).

The analysis of methods of herbal drugs:

Chromatography: Chromotherapy, sometimes called "color writing," is a separation technique that allows a mixture of components to be separated, isolated, and purified into discrete molecules based on varied distribution rates, depending on one of two variables: solubility. Strong attraction (to both polar and non-polar compounds). Using stationary objects: There is a mobile phase that follows a predetermined course at different speeds, and a solid phase that we shall describe later. The components of the mixture are then distributed across these two phases (14–15). The identification and quantification of many chemicals, even those contained in complicated matrices, are within the purview of chromatography, an efficient analytical technique. Methods such as CE, TLC, GC, and HPLC (16).

Specific guidelines must be followed to in order to produce an effective herbal medication. Among those are standardization, photochemical screening, and credible botanical identification. Medicinal herb standardization and quality control require various procedures. (17–19).

A] Thin Layer Chromatography:

For separation of various components of non-volatile mixtures, TLC is used as a chromatography technique (20). TLC is most simple, rapid, easy, and inexpensive chromatographic technology available for evaluating separation of organic molecules and their purity, in both quantitative and qualitative. One of these two phases describes TLC, a form of liquid chromatography: A solvent-based mobile phase

A stationary phase (a silica gel-coated glass plate)

The analysis is carried out at room temperature and atmospheric pressure (21).

Principle of TLC:

The process of adsorption is the basis for separation. Through capillary action, compounds that impact mobile phase come into touch with surface of stationary phase. In this process, molecules with a stronger affinity for stationary phase migrate at a slower rate than others. This allows for separation of mixture's constituent parts. The distinct elements appear as spots on plate flowing at a variety of rates once separation occurs effect. Suitable detection techniques have been used to determine their type or qualities (22).

Retention factor (Rf): The compound's travel along the TLC plate can be determined using the retention factor. Rf is the component's travel distance divided by solvent's total travel distance. Its value ranges from zero to one in a continuous fashion. The travel distances of the component and the solvent are multiplied by one another to get Rf.

A substance's migration up the TLC plates is inversely proportional to its affinity for the stationary phase adsorbent. Although most TLC adsorbents are polar, non-polar compounds with higher Rf values and faster plate entry times outperform their polar counterparts (23).

Thin layer chromatography procedure:

TLC plates: These are utilized to apply the stationary phase thin layer. In nature, they are uniform and inert. For enhanced analysis, the stationary phase layer remains uniform among these plates. People who conduct experiments usually decide ready-to-use plates (24).

Mobile phase: Strict control should be maintained on both solvents' purity and the amount of combined solvent. If any of the solvents are particularly volatile or hygroscopic, it should be prepared fresh for each run. For example, acetone.

Temperature: The container should be maintained in of direct sunlight, heat sources, and other areas while exact temperature control is not required. Rf values usually decrease slightly as temperatures increase, solvents that are volatile evaporate more quickly, and solvents flow faster (25).

TLC chamber: Thin layer chromatography procedures are carried out in TLC chambers. It prevents the solvent from evaporating and keeps dust particles out of the operation. The chamber is kept in a consistent atmosphere to ensure that the spots develop as required (24).

Layer thickness: For standard plates, a layer thickness approximately 250 micrometres is ideal. R-value variation tailing patch and apparent reduction in the R value might also results from a plate being significantly overloaded. In most cases, you can tell the difference between the two by the brightness of spot (24).

Sample mass: If the medicines that usually tail in the system, consequently raising the amount of sample mass on the plates will likely raise the R-value. Significant plate overflowing can also result in a tailing where the R value appear to be gradually decreasing. The distinct distinction between the two can frequently be seen by the brightness of spot (24).

A piece of glass, plastic, or aluminum foil is usually used for thin layer chromatography. Next, this sheet is coated with a thin coating of an adsorption medium, which is usually cellulose, silica gel, or aluminum oxide. This coated layer is described as being in the constant (stationary) phase. This procedure entails the following steps:

- The sample is first applied to the stationary phase, a glass plate.
- Using capillary action, a solvent or the combination of these solvents that make up the mobile phase is drawn up on that glass plates.
- thus, various analytes begin moving at various a rate on the plate of glass (stationary phase).

The compounds separated as a result of the analytes' traveling. occur as a consequence of different analytes flowing onto the stationary phase plate in the thin layer chromatography process at various rates.

Researchers used the TLC extensively to analyze the course of a reaction, identify the many components that make up any mixture, and verify the quality of any the substance. The solute and the mobile phase in this process are situated opportunities with each other in an effort to bind with the immobility (stationary) phase. The desirable parts separate as a result of their disturbances. For example, attraction occurs due to using of silica gel in the stationary phase. As such, the compound of higher polarity will react more strongly against silica when two compounds with different polarities are employed. This reaction will enable the more polar molecule to extract the mobile phase from The binding comes in place without easy. The compound that is less polar then tends to flow higher on the glass plate, showing that the value of Rf has increased. But the solutes will be distributed from the silica restriction if the mobile stage has been altered to produce with a greater polar combination of solvents. Every mixture on the thin layer chromatography plate will rise to a certain level in this method. For example, if heptane and ethyl acetate are used as the mobile stage, then increasing the amount of ethyl acetic acid leads to in high Rf values for each mixture. But usually, altering the mobile stage's polarity has no effect in the compounds' opposed to movement on TLC plate (26).

Application of TLC:

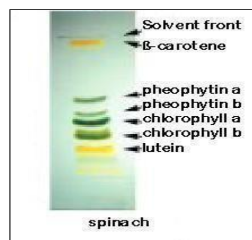
1. **Pharmaceuticals:** TLC can be used in synthetic manufacturing processes including process control, active ingredient identification, purity testing, and concentration determination of active ingredients, auxiliary compounds, and preservative in drugs and drug preparations. The TLC technique has been accepted in certain pharmacopoeias for the purpose to detect contamination in drugs or products. As an example, antibiotics can be identified using penicillin, which are separated on silica gel 'G' using acetone- methanol (1:1) and isopropanol-methanol (3:7) as solvents. Spraying the dried plates with a 0.1% iodine solution containing 3.5% sodium azide performed as the iodine-azide reaction's detecting agent.

2. **Separation of multicomponent pharmaceutical formulations**: Separation multicomponent pharmaceutical formulations is a different application for it.

3. **Qualitative analysis of alkaloids**: It is utilized in control phase of vegetable and pharmaceutical formulations for the qualitative analysis of alkaloids. In toxicology, TLC has been utilized to isolate and identify alkaloids. The 30–60 minute runs of TLC give significant benefits over the 12–24 hour period of paper chromatography. Using silica gel, aluminium oxide, and silicic acid, purine alkaloids have been isolated using TLC. The spots appear by first spraying a solution of alcoholic iodine and potassium iodine, followed 25% HCl and 96% ethanol (1:1).

4. **Cosmetics:** Identification dye raw materials and finished products, and also preservation agents, surfactants, fatty acids, and perfume materials.

5. **Food analysis:** To determine prevented additives in Germany to identify the presence of pesticides and fungicides in drinking water, to identify residues in vegetables, salads, and meat, to test for aflatoxins in milk and milk products, and to determine the presence of vitamins in soft drinks. The information that follows represents a typical separation of the dyes in spinach: (25,27,28,29,30,31,32,33,34).



B] High performance liquid chromatography:

Columns containing packing material and pumps that circulate mobile phase(s) through them are three main parts of HPLC system. Detectors show retention periods of molecules. A number of factors, including interactions between the investigated compounds, stationary phase, and solvent(s), affect retention time. The process is delayed by adding a small amount of material to the mobile phase stream, which interacts with the stationary phase in certain physical or chemical ways. The composition of the mobile and stationary phases, along with the properties of the analyte, determine the level of retardation. A substance's retention time is the time required to go up the column from its base. Most people think of methanol and acetonitrile when they hear "solvent," but any combination of water and organic liquids will do. During an investigation, gradient elution—a separation technique—can be employed to alter the mobile phase's composition. Analyte affinity for mobile phase determines the analyte-to-solvent ratio in a gradient. The kind of analyte, stationary phase, and gradient additives needed are determined by these factors (35, 36, 37).

Different types of HPLC

The following HPLC variations exist, each tailored to a specific stationary phase system:

1. **Normal phase HPLC:** With this approach, analytes are separated according to their polarity. Use of a polar stationary phase and a non-polar mobile phase are essential components of NP-HPLC. Typical components of the mobile phase are hexane, chloroform, methylene chloride, diethyl ether, or a mix of these two, with silica serving as the stationary phase. Thus, polar samples, in contrast to less polar material, remain on the polar surface of the column packing for an extended duration. (38).
2. **Reversed phase chromatography:** Phase reversal An approach that employs a non-polar stationary phase and a moderately polar mobile phase is known as hydrogen peroxide gas chromatography (HPLC). Because of the attractive and repulsive forces between the analyte, the stationary phase, and the polar eluent, RPC is able to function. When the analyte molecules' non-polar parts interact with the ligand in the water eluent, the contact surface area around these molecules determines how much of the analyte is bound to the stationary phase (39).
3. **Ion exchange chromatography:** The attraction between the solute's ions and the charged ions connected to the stationary phase forms the basis of the retention principle in ion-exchange chromatography. Here you won't find any ions that share a charge. This type of chromatography is widely used in several applications, including purification, protein ion-exchange chromatography, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other methods (40).
4. **Size exclusion chromatography:** The main method for particle size separation is size exclusion chromatography, which is also known as gel filtration chromatography or gel permeation chromatography. It can also be used to determine the three-dimensional structures of proteins and amino acids, which is useful for both research and everyday life. This method is usually used to find the molecular weight of polysaccharides. (41).
5. **Bio-affinity chromatography:** fragmentation dependent on a distinct, reversible protein-ligand interaction. Proteins interact with the ligands bound to the bio affinity matrix's solid support, which is itself bound to the ligands by covalent bonding.
6. Proteins bound to a bio affinity column can be eluted using a mix of two techniques: In bio-specific elution, unbound ligand is introduced to the elution process in order to compete with ligand bound to the column. If the protein-bound substrate interacts weakly with the column-bound substrate due to changes in salt, pH, etc., then a specific elution has occurred. (42).

PARAMETERS:

Some parameters are used as guidelines for a specific compound so as for the accurate compound analysis. A change in the parameters might create a major effect on results. The properties that are usually utilized consist of pump pressure, pore size, internal diameter, the particle size. Internal diameter:

An HPLC column's sensitivity and the maximum amount of analyte that may be loaded onto the column are both affected by its internal diameter (ID), a key parameter. Isolating pharmaceuticals before use is one example of how larger columns are put to use in production. An alternate to high-ID columns with reduced solvent usage and enhanced sensitivity is low-ID columns with cross-loading capacity.

Particle size: Conventional HPLC often involves using tiny spherical silica particles (beads) as the stationary phase. Although smaller particles offer greater surface area and improved separations, the pressure needed for optimal linear velocity grows in direct proportion to the square of the particle diameter.

Pore size: Many stationary phases make use of porous materials to boost surface area. For larger analytes in particular, larger pores provide better kinetics, whereas smaller pores provide more surface area. The size of the particle's pores determines the analyte molecules' capacity to enter and interact with its internal surface. This is of paramount importance due to the fact that the exterior and inner surfaces of the particles are nearly 1:1000.

Interactions between molecules typically take place on the inner surface of particles.

Pump pressure: Although a pump's pressure capacity varies, its effectiveness is determined by how well it can produce a steady and repeatable flow rate. Higher pressures may now be obtained by modern HPLC systems, allowing for the use of much smaller particle sizes (less than 2 micrometres) in the columns (37, 43).

Principle: When a mobile phase and a stationary phase are utilized together, a separation column is employed for the purifying process. Granular materials with extremely tiny pores are common for use as the stationary phase in separation columns. A solvent or mixture of solvents, known as the mobile phase, is subjected to high pressure as it is pushed through the separation column. The sample is transported from the pump to the separation column via a sample loop, which consists of a tiny tube or stainless-steel capillary, and an attached valve. Once there, it is integrated into the mobile phase flow. The sample's constituents move at different rates due to interactions with the stationary phase, which in turn lead them to be retained to different degrees. The chemicals are detected by an appropriate detector once they have been removed from the column, and this information is then sent to the computer's HPLC software. In this process, computer's HPLC software generates a chromatogram that allows for the identification and quantification of various chemicals (44, 45, 46).

Instrumentation:

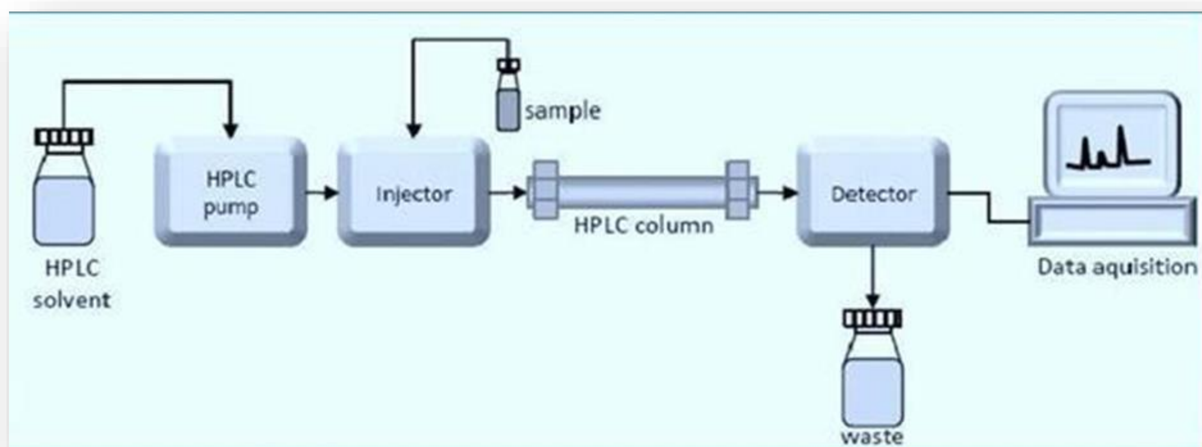


Fig: Instrumentation flow chart solvent

1. **Reservoir:** Within a glass reservoir are substances that are used for mobile stages. The dissolvable or flexible stage of HPLC often consists of a combination of polar and non-polar liquid segments, the exact fixations of which depend on the specimen's arrangement.
2. **Pump:** The flexible stage is pumped out of the dissolvent reservoir and guided through the framework' detector and column by a pump. Column measurements, molecule size in the stationary stage, stream rate in the versatile stage, and synthesis are among the variables that affect the maximum working weights that may be generated, which can reach 42000 kPa (about 6000 psi).
3. **Sample Injector:** An automated infusion framework or a single infusion can serve as the injector. A high weight (up to 4000 psi) and very consistent infusion of the liquid specimen within the range of 0.1–100 mL of volume should be provided by an injector for an HPLC system.
4. **Columns:** Columns usually have a width of 2–5 mm and a length of 50–300 mm. Clean stainless steel is the standard material for these. Typically, they are fed a stationary stage with molecules that are 3 to 10 µm in size. An example of a column design is the microbore HPLC column, which typically has an inner separation of less than 2 mm. Assuming all goes according to plan, the portable stage and column temperatures should stay the same during the test.
5. **Detector:** Towards the very end of the chromatographic column sits the HPLC indicator, whose job it is to reveal which analytes have eluted. Several common types of detectors are electrochemical indicators, mass spectrometry, fluorescence, and ultraviolet spectroscopy.
6. **Data System:** Electronic integrators and outline recorders are two options for capturing the indicator's signals; these devices differ in the depth and breadth of their analysis, storage, and reprocessing capabilities for chromatographic data. The

chromatograph is incredibly user-friendly, and the PC coordinates the identifier's reaction with each component. (43,47, 48).

Sample preparation for HPLC

Liquid samples can be analyzed immediately, following an appropriate extraction to eliminate matrix interferents or after a proper clean-up to remove any particle contaminants. To identify polyaromatic hydrocarbons (PAH) in wastewater, for example, a first extraction using CH_2Cl_2 concentrates the analytes and isolates them from matrix interferents.

It is necessary to dissolve solid samples in a suitable solvent before extracting the analytes of interest into solution. When conducting an HPLC analysis on pharmaceutical tablets, for instance, the initial step is often to extract the powdered tablet using a portion of the mobile phase. This allows for a better understanding of the tablet's active ingredients and degradation products. Gathering gases is as simple as bubbling them through a trap containing the right solution. By passing air through a 1-(2-methoxyphenyl) piperazine, this technique can be used to detect organic isocyanates in industrial settings.in a toluene solution.

Before HPLC analysis, the isocyanates are stabilized against degradation by reacting with 1-(2-methoxyphenyl) piperazine. This reaction produces a derivative that can be identified by UV absorption (37).

Applications of High-Performance Liquid Chromatography (HPLC)

One of the many pieces of information that can be retrieved by HPLC is the drug's identity, concentration, and resolution. To separate and purify compounds, a process known as "preparative HPLC" is employed. On the other hand, analytical HPLC seeks to gain a better understanding of the sample material. Here are some of the most common uses.

1. **Pharmaceuticals:**
   In a single run, High-Performance Liquid Chromatography can determine API and related compounds because to its high linear dynamic range, consistent quantitative precision, and accuracy. Dispersing samples in water or aqueous media that has been modified with methanol or acetonitrile is a useful procedure for preparing samples for solid dosage forms. There are a number of HPLC-based methods for isolating the enantiomers of chiral substances. One of these is diastereomer formation via precolumn derivatization. Alternatively, stationary phases such as cyclodextrins or columns designed with specific chiral moieties could be used. For quantitative analysis, high-performance liquid chromatography (HPLC) is most often used in the pharmaceutical industry, particularly in reverse phase HPLC.

2. **Foods:**
   There have been some positive outcomes from the use of high-performance liquid chromatography in food analysis. The extraction of analytes from food matrices is often challenging due to their complexity. It doesn't help that trace amounts of both desired and unwanted components are often found. Furthermore, the required degrees of accuracy and precision cannot be provided by traditional extraction and analysis procedures. With HPLC, you have options because there is a large variety of stationary phases and mobile phase alternatives.

3. **Research:**
   Research can utilize similar assays to determine concentrations of potential clinical candidates, like antifungals and asthma medicines. Obviously, this approach is useful for studying a wide range of specimens in the collected samples; nonetheless,standard solutions are required for the purpose of identifying a species. This method is used to verify the outcomes of synthesisreactions because purity is very important in this field of study.

4. **Medical:**
   While HPLC has several medical applications, one of the most intimately related is nutritional analysis. Urine is the most common medium for drug concentration measurement, although most medical HPLC studies use blood serum as their sample. Several different molecular detection methods have been tested against HPLC in clinical research, including immunoassays. Here, we looked at how well HPLC and CPBA detected vitamin D as an example of how sensitive these methods are. Although this CPBA was useful in detecting vitamin D insufficiency in kids, it was found that its specificity only accounted for 60% of theHPLC's capacity, and its sensitivity only 40%. HPLC is an expensive tool, yet it's precision is second to none (49).

C] GAS CHROMATOGRAPHY

Gas chromatography is a unique and versatile method. Initially, it was employed for the purpose of studying gases and vapours produced by very volatile components1. To identify the individual components of a sample, an analytical device known as a gas chromatograph (GC) is employed (50,51). Popular among analytical scientists, gas chromatography (GC) separates and studies substances that may be evaporated without dissolving. Finding the individual components of a mixture or checking if a product is clean are two common applications of GC (52, 53,54,55). In the realm of chemical separations and analysis, gas-liquid chromatography (GLC) was born out of the groundbreaking work of Martin and Synge, who were succeeded by the equally groundbreaking contributions of James and Martin. (56).

PRINCIPLES OF GAS CHROMATOGRAPHY:

A carrier gas, typically helium or nitrogen, slows down the components as they are carried down a long column, which is the foundation of the separation process. The column is made up of a glass or steel tube that is packed with inert materials, including ceramic or glass beads (see to Figure 1). To increase the amount of liquid surface area that comes into touch with the gas, gas-liquid chromatography (GLC) involves coating them with a volatile liquid. When the packing is solid rather than liquid, the process is known as gas-solid chromatography (GSC), but it is not as common as gas-liquid chromatography (GLC). A stream of carrier gas is introduced to the sample by injection. The molecules of each substance in the sample will be distributed between the gas and the liquid as it passes through the column with the carrier gas. The equilibrium between the gas and liquid states is dynamic, and individual molecules are in a constant state of flux between the two. Molecules move up the column when they're in the gas phase but stay still when they're in the liquid phase. The time it takes for a substance to emerge from the column is directly proportional to its volatility; the more volatile the substance, the longer its molecules will spend in the carrier gas. That manner, by the end of the column, each substance will have emerged in its own distinct state, determined by the passage of time. Under all circumstances, every substance has a unique retention time (Rt), which is the duration it takes for an injection to cause an outward manifestation of the material. Considerations like as the substance's volatility, column temperature, and column dimensions (diameter, length, and thickness) are essential. To get around the problem of chemicals having an annoyingly lengthy retention time at room temperature, the column can be heated in an oven. Now that we have separated the components in the column, we need a way to identify and measure them independently. Thermal conductivity and flame ionization detectors are the two most typical kinds. Whenever gas passes through a column, thermal conductivity detectors (TCDs) pick up on any changes in the gas's thermal conductivity. A gas that carries just helium cools a

tungstenrhenium filament that is heated as it travels through it, because to helium's extremely high thermal conductivity. There will be less cooling and a rise in filament temperature when a chemical material emerges with the carrier gas. You may measure and record the rise in electrical resistance with temperature, as is the case with most metals. Due to the high sensitivity of flame ionization detection (FID) compared to thermal conductivity detection for organic compounds—which are numerous in the food industry— FID is more commonly used in food applications. A mixture of hydrogen and air is used to burn the gas that emerges from the column. A chart recorder can be used to amplify and record the electric current that is conducted by the ions that are formed. Even though this process only produces a tiny fraction of the carbon ions in a sample—maybe as little as 0.0001%—the percentage remains constant. This indicates that the quantity of the chemical component has a direct correlation with the overall signal recorded on the chart recorder (57).

Types of Gas Chromatography :

Two main gas chromatography types are:

1. **Gas-Solid Chromatography:** This technique uses a solid stationary phase (such as absorbance materials like silica, alumina, active carbon, etc.). This technology yields an important column life duration, but it also exhibits catalytic changes.

2. **Gas-Liquid Chromatography:** This technique uses an immobilized liquid released on a solid substrate as the stationary phase. The disadvantage of this approach is that the liquid bleeds off gradually (58).

Working of Gas Chromatography

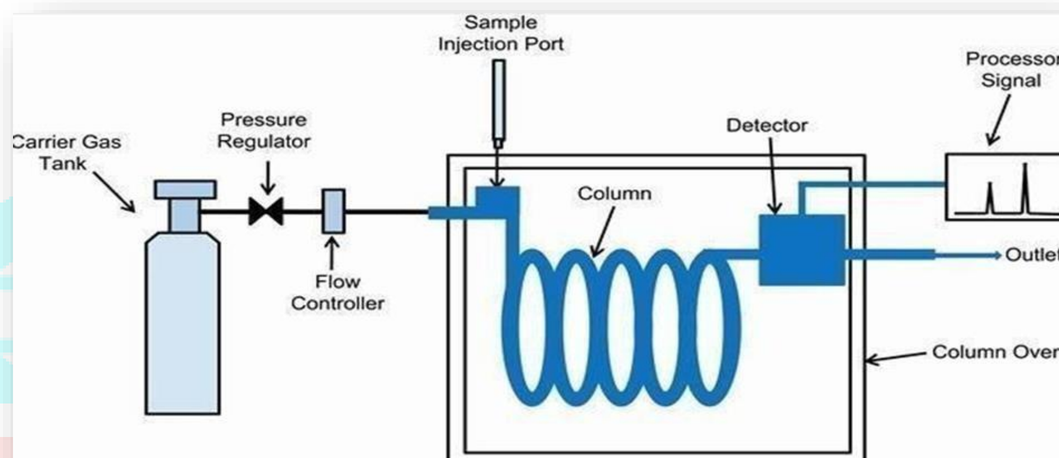


Fig: Flow chart of gas chromatography

Using chromatographic methods, you'll need a mobile phase and a stationary phase. The mobile phase is made up of a non-reactive gas, such as nitrogen, helium, or argon. Coated with liquid stationary phase or serving as the stationary phase itself, the packing or solid support makes up the stationary phase in a packed column. A capillary column, in which the stationary phase coats the inside of a narrow tube, is the most typical type of instrument. Because of the interaction between the chemicals and the stationary phase, this method is able to separate various compounds. It takes more time for the compound to pass through the column when the interaction is strong because it stays connected to the stationary phase for a longer period of time.

Gas chromatography instrumentation consists of the following components.

1) Carrier gas provided rapidly and consistently while maintained at a high pressure
2) Injector sample
3) Separator columns
4) detector
5) Amplifier and recorder system.
6) Thermostat chambers to regulate the temperature of the column and detectors.

Gas chromatography uses a stationary phase and an adsorbent to separate components in a glass, metal, or Teflon tubular column. Before the liquids are packed into the column with the adsorbent, which is packed as fir size graded powder, they are either sprayed as a fine film on the column wall or coated over an inert size graded porous support, like firebrick powder. A mobile phase, or carrier gas, is continuously pumped through the column to distribute the sample components uniformly.

Through the opening in the gas container, the vapour sample is drawn into the column. The distribution coefficients of the sample components define how much of the stationary phase each component absorbs. Every gas phase component is immediately swept up by the carrier gas. A portion of the adsorbed quantity needs to desorb before the distribution coefficient can remain constant. To keep the distribution coefficient value constant, some of the amount swept away is replaced at the same time by some that enters the adsorbent at the next point in the column. Each component's band moves down the column until it resembles a Gaussian distribution as a result of this procedure.

1. **Carrier gas:**

The four most common carrier gases are hydrogen, nitrogen, helium, and air. Hydrogen offers more advantages than other gases, however using it is potentially dangerous. Despite its low density, fast flow rates, inertness, and great thermal conductivity, helium—the second-best gas—is less common due to its costly price. Nitrogen is inexpensive and dulls the senses. Only when atmospheric oxygen is present does air help with separation or detection.

In establishing a carrier gas, the following factors need to be taken into considered:

1) It must not react with the sample, stationary phase, or touched hardware; in other words, it must be inert.

2) It must be appropriate for both the kind of material being analyzed and the detector being employed.

3)It need to be accessible in a highly pure form.

4)It need to provide the most dependable column performance at the necessary analysis speed.

5)The cost shouldn't be high.

6)It shouldn't present a risk of explosion or fire.

2. **Flow Controllers:**

Even when the column is heated and subjected to constant pressure, the chromatographic system is not guaranteed a continuous flow of mobile phase. A gas's viscosity rises as its temperature rises, and a constant inlet pressure causes the flow rate to fall.Both the temperature gradient and the temperature program's restrictions will have an impact on the reduced flow rate. A consistent mass of movable travels through the column per unit time regardless of the system temperature; this is achieved by using mass controls, which prohibit the flow rate variation.

3. **Injection Devices:**

Two primary sampling methods are used in gas chromatography: packed column and open tubular column. It is also necessary to have a variety of sample injectors that can accommodate various column configurations. Note that a gas chromatography (GC) analysis can only be as accurate and precise as the data produced by the sample injector, regardless of the design of the related equipment. A well-designed and regularly serviced sample injector is an essential component of any chromatographic machine.

spilt injections: One way it does this is by using a needle valve, which creates two uneven flows in the sample stream: one that goes into the atmosphere and another that goes through the columns. If extreme caution is necessary, this method is not the wayto go.

Spitless injectors: Loading occurs when the full sample is allowed to travel through the column. A substantial dilution of the sample is required to prevent the column from overlanding. A high capacity column, such as a SCOT or a WCOT with a heavy coating, is ideal.

On Column injectors: A syringe is a device that uses an extremely fine needle made of quartz. After cooling to 20 degrees Celsius below the boiling point, warmer air is introduced to evaporate the sample.

Automatic injectors: When operating without an attendant is required for the analysis of several samples, automatic injectors are utilized to improve reproducibility. To crush the solid samples in the gas steam, either a solution or a sealed glass ampoule containing the samples is inserted and a gas tight plunger is utilized. Upon interaction with the carrier gas, the sample vapors make their way into the column.

4. **Separation of column:**

The 4.8-millimeter-diameter columns are tubular structures constructed of glass or metal. They can range in width from a few centimeters to one hundred meters or more. They may be straight, curving, or coiling.

Gas chromatography makes use of six distinct kinds of analytical columns:

Packed Columns: Columns like these are made by packing granular stationary phase into metal or glass tubes. Substrates for gas liquid chromatography are made by applying the liquid phase on inert solids of varying sizes. Columns of open tubular, capillary, or golay construction consist of lengthy tubes with consistently small internal diameters. Their construction involves a variety of materials, including copper, nylon, glass, and stainless steel, the latter of which being the most common. The liquid phase covers the inside of the capillary tube in a thin, consistent layer that is between half a micron and one micron thick. Carrying gas encounters less resistance in these columns due to the absence of packing.

Support Coated Open Tubular Columns: A thin film coating is applied to the inner wall of a capillary column using a liquid phase. Then, a porous layer of support material, measuring microns in size, is applied.

One step in making wall-coated open tubular columns is to apply the liquid stationary phase to the tube's smooth inner wall, which has not been changed.

A porous substance makes up the interior wall of porous-layer open-tubular (PLOT) columns. Chemical etching or the application of a dispersion of porous particles can make a wall appear porous. The liquid stationary phase is either supported by or serves as the stationary phase itself in the porous layer. Columns with a porous covering made of support particles that developed from a suspension are known as support-coated open-tubular (SCOT) columns (58).

Detector:

Many different types of detectors are accessible for use in gas chromatography. Detectors vary in the selectivies they produce. Whereas a specific detector responds to a single chemical compound, a non-selective detector reacts to all chemicals (with the exception of the carrier gas), and a selective detector responds to many compounds sharing a common physical or chemical feature. Another way to categorize detectors is by whether they rely on mass flow or concentration. There is typically no sample loss when using a concentration-dependent detector, as the signal is proportional to the solute concentration within the detector.

The detector's reactivity will be reduced by diluting it with make-up gas. In mass flow dependent detectors, the sample is typically destroyed, and the signal is related to the solute molecular transit time to the detector. The use of make-up gas has no effect on the response of a mass flow dependent detector. Here is a table displaying the most popular GC detectors:

1. Flame Ionization Detectors:

The column's effluent is burned after being mixed with air and hydrogen. When organic molecules burn in a flame, they release ions and electrons, which can transfer electricity from one area to another. The burner tip is subjected to a high electrical potential, and an electrode for collecting electricity is placed above the flame. A measurable current is produced during the pyrolysis of any organic substance. Since the detector's response is unaffected by changes in the mobile phase flow rate, FIDS's mass sensitivity is preferable to concentration sensitivity. An ideal general-purpose detector for organic

compound detection, the FID boasts a wide linear response range, minimal noise, and exceptional sensitivity. Unfortunately, it eliminates the sample, but it is also dependable and easy to use.

1. **Thermal conductivity detectors:**
   For preparative applications, this is great since the sample is not destroyed, even if it is less sensitive than the FID (105-10g/s, linear range: 103-10). This is based on the comparison of two gas streams: one that contains the chemical and the other that merely contains the carrier gas. An increase in the temperature differential (and, by extension, the resistance difference) between two thin tungsten wires is achieved by naturally using a carrier gas with high thermal conductivity, like helium or hydrogen. The rapid stabilization is made possible by the huge surface-to-mass ratio. A Wheatstone bridge circuit is used to monitor the
   temperature differential between the filaments of the reference and sample cells.
2. **Electron Capture Detectors:**
   A radiation source that emits Ẑ-radiation, such as "Ni, 3H," and two electrodes are housed in a cavity that constitutes the detector. Plasma comprising electrons and positive ions is created when electrons collide with the carrier gas, which is a mixture of methane and an inert gas. Whenever an electronegative atom-containing chemical is in the vicinity,
   The procedure for collecting electrons slows down when they are "captured" and replaced by negative ions.The detector has a narrow linear range of around 102 to 103 cm^2, but it is highly selective for compounds containing atoms with a high electron affinity (10-14 g/s). The detection of chlorinated chemicals, such as pesticides and polychlorinated biphenyls, is a common application of this detector due to its great sensitivity.
3. **Flame photometric:**
   In the flame photometric (FPD) method, spectral lines of burning mixtures are measured using a photomultiplier tube. It is the eluted compounds that supply the hydrogen energy for the fire. This fire excites specific molecular components (e.g., P, S, halogens, and a few metals) and causes them to emit light with wavelength characteristics. The light is separated and detected by a photomultiplier tube. Between 510 and 536 nm, phosphorus emission is noted, while around 394 nm, sulfur emission is recorded (59).

APPLICATIONS:

Gas chromatography (GC) is frequently used for food analysis. Some common uses include testing for pesticides, fumigants, environmental pollutants, natural toxins, veterinary medications, packaging materials, flavor and aroma components, natural products, food additives, and food composition (60).

Fatty acid measurements using gas chromatography (GC) have shown to be an invaluable analytical tool in oilseed plant

breeding, biosynthesis, and human metabolism. alongside chromatographic separations and spectroscopic identification, and the characterization of complex mixtures of geometric isomers (61). When researching oilseed crops like rapeseed, plant breeders employ GC because it is a faster and more accurate way to analyze fatty acid variation and inheritance (62).

To study the transmission and evolution of fatty acid profiles in oilseed crops, such as rapeseed, plant breeders employ GC, a more accurate and efficient technique (62).

D] UV-visible spectroscopy

Optical spectroscopy employs visible, ultraviolet, and near-infrared light; this physical subfield is known as ultraviolet spectroscopy. The Beer-Lambert law states that the amount of light absorbed by a sample is dependent on the concentration of absorbing species and the length of the solution's path. The absorber concentration in a solution can be determined using this method, provided that the path length remains constant. Knowing how quickly absorbance varies with concentration is crucial since, in the past 37 years, UV-VIS spectroscopy has surpassed all other analytical techniques in importance in modern laboratories. While alternative methods may be used in certain situations, none can compare to the versatility, accuracy, speed, and practicality of UV-VIS spectroscopy (63, 64).

PRINCIPLE:

The Principle of UV Visible Light Spectroscopy is based on the unique spectra that are formed when chemical compounds absorb either visible or ultraviolet light. The basis of spectroscopy is the connection between matter and light. A spectra is the result of light absorption, which includes both excited and de-excited states.the third Multiple processes, such as transmission, absorption, reflection, and scattering, can occur when an electromagnetic wave interacts with a material. The observable spectra show the interactions between atomic, molecular, and macromolecular entities at different wavelengths. Light is absorbed when the energy gap between a molecule's excited and ground states is equal to the frequency of the light that is entering the material. Make the transition to electronic (65).

Instrumentation:

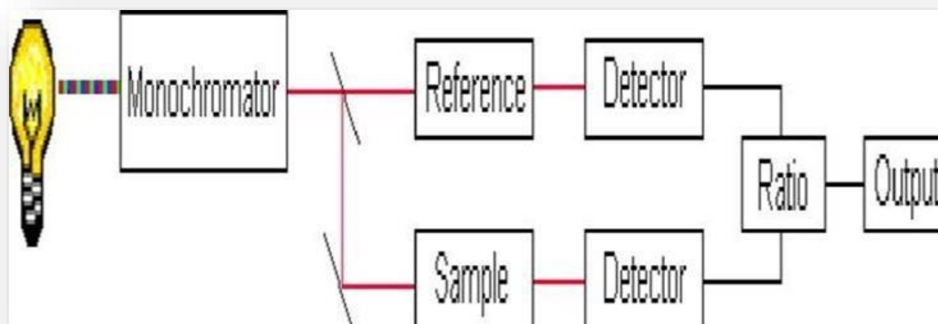


Fig. 4. Instrumentation of UV-Spectroscopy.

All of these parts come together to form instruments that can measure the absorption of visible or ultraviolet radiation. (63,66-70).

1. Sources:

1. Sources of UV radiation:

The radiation source's power must be kept somewhat constant across its wavelength range. A continuous ultraviolet spectrum is produced by electrically stimulating deuterium or hydrogen at low pressure. An excited molecular species is formed, which then disintegrates into two atomic species and an ultraviolet photon, causing this. The corresponding formula is D2 + electrical energy ▪ D2* D' + D" + hv.

Deuterium and hydrogen lamps emit radiation with a wavelength range of 160-375 nm. This type of lamp requires quartz cuvettes and windows since glass absorbs light with wavelengths less than 350 nm.

2. The Monochromator (Wavelength selector)

Polychromatic radiation, or light with many wavelengths, enters the monochromator through the entrance slit. Prior to making an angle contact with the dispersion element, the beam is collimated. The wavelengths of the beam are separated into their component parts by the prism or grating. The only way to get radiation of a specific wavelength out of a monochromator is to change either the dispersing element or the exit slit.

3. Sample cell:

Radiation must be able to seep through the containers holding the sample and reference solutions. Cuvettes made of quartz or fused silica are necessary for ultraviolet spectroscopy. These cells are transparent in the visible spectrum as well. One may produce cuvettes in the 350–2000 nm wavelength range using silicate glasses.

4. Detectors:

An electrical signal is generated from a light signal by means of a detector. It should react linearly over a wide range of high sensitivity and low noise. 1. Photomultiplier tube detector 2. Photodiode detector (71).

Applications of UV Spectroscopy QualitativeAnalysis:

UV absorption spectroscopy can be employed to assess any material that is capable of absorbing UV light. It is useful for locating specific functional groups that are chromophoric.

In polar liquids, the weak absorption band of carbonyl compounds, which is located between 280 and 290 nm, is pushed towards shorter wavelengths. As previously suggested based on possible conjugation effects on absorption wavelength, it would be possible to differentiate different molecules.

- Vitamin A1 shows only one band, but vitamin A2 shows two. Many ketosteroids have strong UV absorption due to their presence of a conjugated enol system; so, max is the same for all compounds without extended conjugation.
- A bischromic shift is seen, with Vitamin A1 showing a shift from 326 nm to 351 nm.

Quantitative Analysis:

- UV absorption spectroscopy measures the intensity of absorption at a certain wavelength to quantitatively identify substances that absorb UV radiation.(72).

E] Infrared spectroscopy

Sir William Herschel (73) found infrared radiation in 1800. The energy levels linked to visible light wavelengths were the focus of Herschel's research. The use of a prism allowed sunlight to display the visible range of colors, which is generally recognized as a rainbow. This visible spectrum is made up of comparable wavelengths or frequencies that range from blue to red (74, 75). Figure No.1

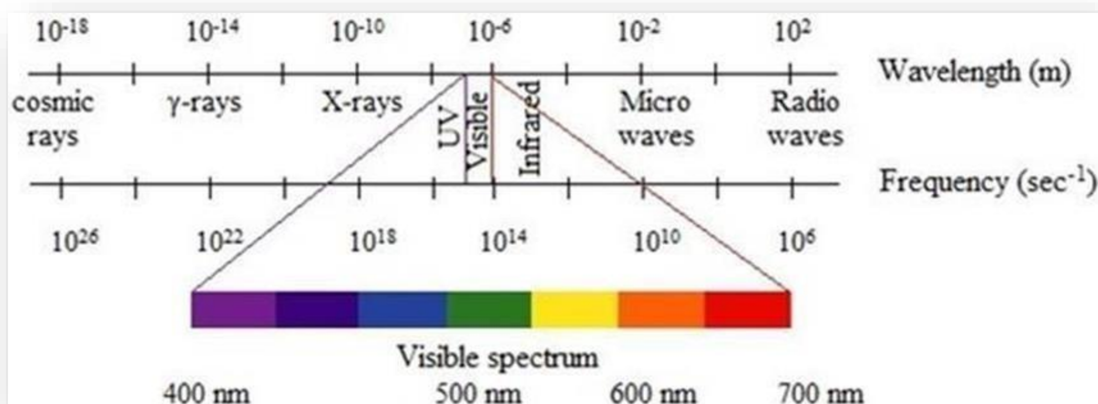


Fig. The electromagnetic spectrum

INSTRUMENTATION OF INFRARED (IR) SPECTROSCOPY

Because common optical materials like glass and quartz absorb a lot of light in the infrared spectrum, equipment for detecting infrared spectra differs significantly from that used to measure visible UV light. An IR spectrometer's primary components are as follows:

The IR Radiation Sources:

Incandescent lamp: A regular incandescent light is typically employed in near-infrared equipment. But because it is glass enclosed and has low spectral emissivity, this fails in the far infrared.

Nernst Glower: It is made out of a hollow rod that is 30 mm long and 2 mm in diameter. Zirconia, yttria, and thoria are examples of rare earth oxides that make up the glower. When nernst glowers are left at room temperature, they become non- conductive and need to be heated externally to become conducting. The typical temperature range for a heater is 1000– 18,000C. The radiation reaches its peak at approximately 7100 cm-1 (1.4μ). The primary drawback of the Nernst glower is that it emits infrared radiation throughout a large wavelength range, with long-term stability and consistent radiation intensity.

Mercury Arc: In the far infrared (wave number <200 cm-1), the previously mentioned sources lose their effectiveness, and instead, special high-pressure mercury arc lamps are used. Beckman also made several unusual quartz mercury lamps for that location. Mercury plasma travels through heated quartz, which emits radiation at lower wavelengths, while the quartz itself emits radiation at longer wavelengths (76).

Monochromators:

Single Pass Monochromator:

By holding it at or near the beam's focus, the sample is brought to rest just in front of the monochromator's entrance slit "A." Radiation travels from the source, passes through the sample and entrance slit, and then hits the off-axis parabolic Littrow mirror B, which makes the radiation parallel and sends it in a straight line into the prism C. Here we see Mirror D making a triumphant return, finally making it into the detector section **Double Pass Monochromators:**

Double pass monochromators produce higher resolution in the radiation than monochromators do before it reaches the detector. In mono-pass and double-pass monochromators, the sodium chloride (rock-salt) prism is utilized throughout the entire 4000 to 650cm-2 (2.5 to 15.4 μ) range. The region containing the significant stretching vibrations can be more precisely imaged using lithium fluoride or calcium fluoride prisms.

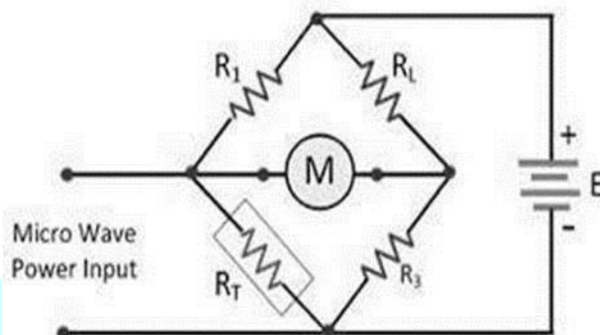
Sample cells and sampling of substances.

Since samples of solid, liquid, or gas have been characterized using infrared spectroscopy, it is clear that handling samples in diverse phases is necessary. However, these samples require a different approach. All phases of sampling have one thing in common, though: the material holding the sample needs to be transparent to infrared radiation. Our selection is limited to specific salts such as NaCl or KBr due to this limitation.

Nevertheless, the wavelength range to be examined will determine the ultimate salt selection.

Detectors:

Bolometer: The majority of bolometers are made from very thin metal conductors. Infrared and other forms of radiation can alter the temperature of this material. Since the resistance of a metal conductor changes with temperature, the amount to which the resistance changes is thought to signal the amount of radiation that has reached the bolometer. A Bolometers are housed in an arm of the Wheatstone Bridge. The bridge's balance arm is a similar metal strip. Nobody is putting infrared light on this stretch. The bolometer maintains its equilibrium when radiation shielding is applied. As a result of changes in the bolometer's electrical resistance caused by infrared light, the bridge's equilibrium is disturbed, and current flows through the galvanometer (G). The amount of current flowing through the galvanometer is a direct correlation to the radiation intensity that reaches the detector. The reaction time of a bolometer is four milliseconds.



Thermocouple:

The basic principle behind a thermocouple detector is that an electrical current will flow when two separate metal wires are linked at both ends and there is a difference in temperature between them. "Hot junction" describes the part that is directly exposed to the infrared beam. Energy gathering is usually enhanced by a "black body." The second connection, the "cold junction," is thermally insulated and features finely screened edges to prevent light from penetrating. There is a direct correlation between the amount of electricity and the energy differential between the two connections. A tiny electrical potential can be created between two welded connections by maintaining them at different temperatures. a thermocouple with a KBr (or Csl) window to prevent convectional energy loss in a steel housing. Two welded joints are used in this process; one is called the cold junction and is kept at a constant temperature and shielded from infrared radiation; the other is called the hot junction and is heated by infrared radiation. What determines the potential difference between the two junctions is the amount of infrared radiation that strikes the hot junction, which causes a temperature differential. A thermocouple typically has a response time ofabout 60 ms.

Thermistors: A fused combination of metal oxides makes up a thermistor. In contrast to the bolometer, the mixture's electrical resistance drops as temperature rises. Thermistors can be utilized in the same manner as bolometers as infrared detectors due to the relationship that exists between temperature and electrical resistance. Usually, the thermistor alters resistance by 5% for every degree Celsius. It responds slowly as well.

Golay Cell: It consists of a little metal cylinder with a blackened metal plate sealing one end and a flexible metalized diaphragm sealing the other. After the xenon has been supplied, the cylinder is sealed. When ionizing radiation hits the oxidized metal plate, the gas increases due to thermal expansion. The resulting rise in gas pressure distorts the metallized diaphragm that separates the two chambers. A photocell is illuminated by light shining on a diaphragm, which in turn is reflected off of the lamp. When the diaphragm moves, it changes the cell's yield.

Pyroelectric Detector: When an electrical field is applied to a dielectric, the dielectric constant determines how polarized the material becomes. The polarization often vanishes when the field is removed, with the exception of ferroelectric materials, which retain a significant amount of polarization. On very rare cases, the residual polarization could be temperature dependent. Such substances are known as pyroelectrics. The surface of the pyroelectric detector is a thin dielectric flake that, in response to variations in temperature, acquires an electrostatic charge A. This happens as a result of infrared dilatation exposure. Electrodes attached to the flake collect charges, which is then used to generate a voltage. In the process of cutting a single crystal, a little pyroelectric flake is created. In terms of area, it can be anything from 0.25 to 12.0 mm2. It is sometimes difficult and inconvenient to focus the radiation of the infrared instrument on such a little flake, yet this is necessary for operation. The most popular pyroelectric is triglyceride sulfate (TGS), although its reaction rapidly degrades and disappears around 45°C, and it becomes completely ineffective above the Curie limit (49°C). It is typically chilled in liquid N2 as a result. Deuterated TGS detectors that operate at ambient temperature have recently become accessible. Their Fourier transform infrared technology has replaced Golay detectors as the go-to detector. They can be applied to multiplex scanning, a popular method in infrared (IR) Fourier transform, due to their fast response (77).

Applications:

In Industry: IR spectroscopy is used for three main objectives. It can be used to detect contaminants in raw materials initially. In order to ensure high-quality products, this is necessary. Secondly, quality control refers to the continuous assessment of the tie's composition and the proportion of required product that is present. This is the most affordable way to manufacture high- quality products. The third goal is to identify compounds produced by competitors or at industrial research focuses (78).

Future perspective:

Technological developments and an increasing focus on standardization and quality control will probably influence the future of herbal medication analysis. Observations on upcoming patterns and techniques are as follows:

1. **Advanced Analytical Techniques:** Herbal medicine analysis will be more sensitive and precise if advanced analytical methods like mass spectrometry, nuclear magnetic resonance, and chromatography are included.

This makes it possible to comprehend the chemical makeup of herbal items in more depth.

2. **Metabolomics and Systems Biology:** A comprehensive understanding of the relationships between herbal substances and biological systems can be obtained by utilizing metabolomics and systems biology techniques. This may help us comprehend the therapeutic effects and possible side effects better.
3. **DNA Barcoding and Authentication:** Herbal products will be greatly aided by DNA barcoding techniques, which will help to detect and stop adulteration or substitute. In order to guarantee the security and effectiveness of herbal medications, thisis very crucial.
4. **Quality-by-Design (QBD):** Quality-by-Design practices will be applied more frequently. This method focuses on comprehending and managing the key factors that influence the quality of herbal drugs, building quality into the product from the outset of creation.
5. **Omics Technologies:** Although examination of the properties of herbal drugs, such as genetic variants and protein interactions, will be made possible by the integration of genomics, proteomics, and other omics technologies. This may help with focused and customized herbal medicine approaches**.**
6. **Blockchain for Traceability:** The authenticity and integrity of herbal goods will be further ensured by using blockchain technology to provide traceability and transparency in the supply chain. Records documenting the product's route from manufacturer to market are safe, indestructible, and accessible to both consumers and regulatory bodies.
7. **Portable Analytical Devices:** On-site testing of herbal items will be possible with the development of portable and user-friendly analytical instruments. This is especially helpful for quality control at different stages of the supply chain, since it addresses storage and transportation-related problems.
8. **Data Integration and Artificial Intelligence (AI):** Gathering information from several sources and employing AI algorithms allows for the examination of complex herbal collections. Pattern recognition, efficacy prediction, and potential safety concern identification are all made simple with the help of AI.
9. **Global Harmonization of Standards:** Harmonization of standards for the analysis of herbal drugs on a global scale will make quality evaluation techniques more consistent. This will boost consumer confidence and facilitate international trade.
10. **Regulatory Evolution:** Regulatory bodies will probably update their standards and specifications for the analysis of herbal drugs in order to stay up with the latest developments in technology. Strong standards will require cooperation between industry, regulators, and researchers.

The future of herbal drug analysis involved a multidisciplinary approach, integrating technological innovations.

Conclusion:

The rapid separation time, high sensitivity, low cost, and ease of use of thin-layer chromatography (TLC) make it a popular analytical instrument. The rate of migration of a chemical is determined by its affinity for the stationary and mobile phases, the same principle that regulates all chromatography and is also applicable to thinlayer chromatography. The thorough investigation proves that high-performance liquid chromatography (HPLC) is a versatile and repeatable chromatographic technique for pharmaceutical product assessment. It finds extensive use across many fields for both quantitative and qualitative assessment of active substances. Gas chromatography (GC) is a well-liked method of chromatography used in analytical science for the separation and study of compounds that may be vaporized without dissolving. In order to analyze the product based on retention time, various detectors are used. In order to create functional materials with important technological uses, researchers are analyzing polymer nanocomposites with optically sensitive nanofillers, like semiconductor nanocrystals and metals, using a UV- visible spectroscopy technique. Infrared is a qualitative method that is widely used in the chemical, fertilizer, and pharmaceutical sectors to identify the functional groups. These methods are discussed in this review.

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Jyothirmaie *Department of Pharmaceutical Analysis, SreeVidyanikethan College of Pharmacy, Tirupathi517102, Andhra Pradesh, India.

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