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Recent Herbal Technology

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Abstract: *Natural medicines were the only option for the prevention and treatment of human diseases for thousands of years. Natural products are important sources for drug development. The amounts of bioactive natural products in natural medicines are always fairly low. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of those bioactive natural products. This paper intends to provide a comprehensive view of a variety of methods used in the extraction and isolation of natural products. This paper also presents the advantage, disadvantage and practical examples of conventional and modern techniques involved in natural products research. HERB: Herb can be defined as, " any plant which has leaves, stem, flowers, roots and rhizomes, fruits, bark, tubes and seeds; used for different purpose like flavoring, food, medicine or perfume." HERBAL DRUGS: These consist of plants or any part of the plants, usually in unprocessed or crude forms (crude drugs) which have medicinal value. They include different parts of plants like entire aerial part, flowers, fruits, seeds, bark, leaves, roots, rhizomes etc. The constituents and their therapeutic activity may be known or unknown. Examples: Senna, Ginseng, Ginkgo, Ashwagandha and Hypericum.*

Keywords: Herbal Technology

I. INTRODUCTION

1.1 History

Natural medicines, such as traditional Chinese medicine (TCM) and Ayurveda, were formed and developed in the daily life of ancient people and in the process of their fight against diseases over thousands of years, and they have produced a positive impact on the progress of human civilization.

Today, natural medicines not only provide the primary health-care needs for the majority of the population in developing countries but have attracted more and more attention in developed countries due to soaring health-care costs and universal financial austerity.

In the USA, approximately 49% of the population has tried natural medicines for the prevention and treatment of diseases [1]. Chemicals known to have medicinal benefits are considered to be “active ingredients” or “active principles” of natural medicines.

Natural products have provided the primary sources for new drug development. From the 1940s to the end of 2014, nearly half of the FDA approved chemical drugs for the treatment of human diseases were derived from or inspired by natural products [2, 3].

Natural products offer more drug-like features to molecules from combinatorial chemistry in terms of functional groups, chirality, and structural complexity [4, 5].

The amounts of active ingredients in natural medicines are always fairly low. The lab-intensive and time-consuming extraction and isolation process has been the bottle neck of the application of natural products in drug development.

There is an urgent need to develop effective and selective methods for the extraction and isolation of bioactive natural products. This review intends to provide a comprehensive view of a variety of methods used in the extraction and isolation of natural products.

II. ISOLATION AND PURIFICATION TECHNIQUE

2.1 General Isolation Technique

1. Maceration
2. Percolation
3. Decoction
4. Extraction

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5. Reflux extraction
6. Soxhlet extraction
7. Pressurized liquid extraction
8. Microwave assisted extraction

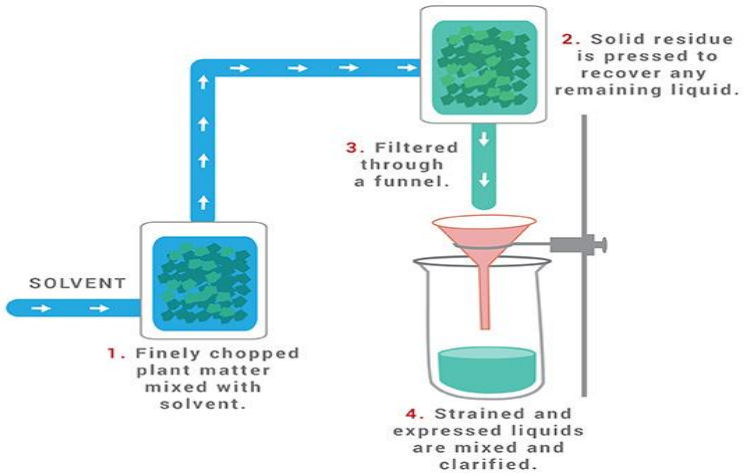
2.2 Chromatographic Technique

A. Maceration

Definition: A process for tincture made from organised drug e.g. roots,stem,leaves etc. This process is called 'Simple Maceration'.

(11) This is a very simple extraction method with the disadvantage of long extraction time and low extraction efficiency.

It could be used for the extraction of thermolabile components(1)



Types of Maceration

- Simple maceration
- Unorganized maceration
- Multiple maceration

Example of Maceration

- Tincture of Orange
- Tincture of Lemon
- Tincture of Squill

Advantages

- For more effective extraction multiple maceration can be done.
- Both organized and unorganized drug can be extracted.
- Thermolabile constituents of the drug can be extracted with ease.

Limitation

- This extraction process is time consuming. [3]

2.3 Percolation

Definition: The process in which a comminuted drug is extracted of its soluble constituents by the slow passage of suitable solvent through a column of drug. Percolation is more efficient than maceration because it is a continuous process in which the saturated solvent is constantly being replaced by fresh solvent.

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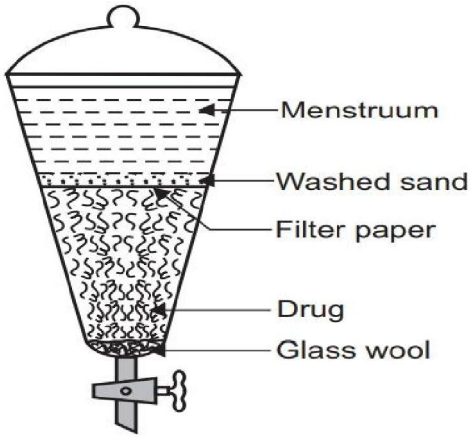

Figure: Percolation technique [4]

It is continuous downward displacement of the solvent through the bed of crude drug material to get extract.
Most frequently used to extract active ingredients in the preparation of tinctures and fluid extracts.
It is the method of short successive maceration or process of displacement. (5)

Types of Percolation

- Reserved percolation
- Continuous hot percolation
- Continuous cold percolation

Advantages:

- It is comparatively less time consuming.
- The process can be modified to increase the efficiency.

Limitations:

- The process requires a particular apparatus, percolator.
- The process is not suitable for soft drugs which may block the percolator. (6)

2.4 Decoction

The process is mainly used for vegetable drugs of hard and woody nature having thermostable water soluble contents.
The extract from decoction contains a large amount of water-soluble impurities.
Decoction cannot be used for the extraction of thermolabile or volatile components. In this process crude drug is boiled in specified volume of water for a defined time; it is then cooled and filtered.
This procedure is suitable for extracting water soluble, heat stable constituents.
It is a liquid preparation obtained by boiling the herbal material with water(7)

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Advantages:

- Hard and woody drugs can be extracted.
- Less time consuming.

Limitations:

- Only water soluble and heat stable constituents are extracted.
- Only freshly prepared Decoction is dispensed and consumed.

2.5 Extraction

These are preparations of liquid (liquid extracts and tinctures), semi-solid (soft extracts and oleoresins) or solid (dry extracts) consistency, obtained from herbal drugs (or animal matter) which is usually in a dry state.

Extraction is the first step to separate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method.

The extraction of natural products progresses through the following stages: (9) the solvent penetrates into the solid matrix; the solute dissolves in the solvents; (10) the solute is diffused out of the solid matrix; (11) the extracted solutes are collected.

Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency [12- 18].

Reflux Extraction:

Reflux extraction is more efficient than percolation or maceration and requires less extraction time and solvent.

It cannot be used for the extraction of thermolabile natural products.

The reflux method was found to better than the decoction method and the highest yields of baicalin and puerarin were obtained from the reflux method with 60% ethanol as the extraction solvent.(19)

Soxhelt Extraction

The Sohxlet extraction is an automatic continuous extraction method with high extraction efficiency that requires less time and solvent consumption than maceration or percolation.

The high temperature and long extraction time in the Sohxlet extraction will increase the possibilities of thermal degradation (20)

Advantages:

1. Efficient and continuous extraction.
2. It needs less solvent to yield concentrated extract.
3. We can use modified Sohxlet extractors to meet different needs and increase efficiency further.
4. By modifying certain things, we can use the Sohxlet extractor on the industry level.

Limitations:

1. In general, the drug must be powdered.
2. The method is unsatisfactory with drugs having thermolabile constituents.
3. The method is restricted to pure boiling solvent or to azeotropes.

Pressurized Liquid Extraction:

Pressurized liquid extraction (PLE) has also been described as accelerated solvent extraction, enhanced solvent extraction, pressurized fluid extraction, accelerated fluid extraction, and high pressure solvent extraction by different research groups.

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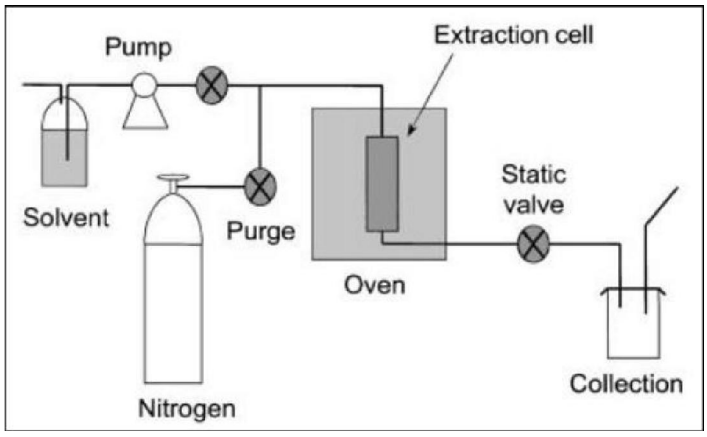
PLE applies high pressure in extraction. High pressure keeps solvents in a liquid state above their boiling point resulting in a high solubility and high diffusion rate of lipid solutes in the solvent, and a high penetration of the solvent in the matrix



Supercritical Fluid Extraction (SFE):

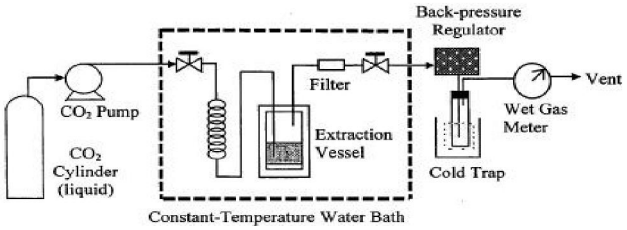
Supercritical fluid extraction (SFE) uses supercritical fluid (SF) as the extraction solvent.

SF has similar solubility to liquid and similar diffusivity to gas, and can dissolve a wide variety of natural products.

Their solvating properties dramatically changed near their critical points due to small pressure and temperature changes.

Supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. It has a unique ability to diffuse through solids like a gas and dissolve materials like a liquid.

Moreover, it can readily change in density upon minute change in temperature or pressure. These properties make it suitable as a substitute for organic solvents in SFE. Carbon dioxide and water are the most commonly used supercritical fluid(21)



Advantages:

1. Low viscosity.
2. Lower operating temperature.
3. High diffusion co efficient.
4. High resolution at low temperature.

Disadvantages:

1. SFC is pressure operating conditions. High-pressure vessels are expensive and bulky.
2. Maintaining pressure in SFC is difficult.
3. Supercritical fluids are highly compressible
4. Physical properties change with pressure.
5. Cleaning will be time consuming.

Microwave Assisted Extraction

Microwaves generate heat by interacting with polar compounds such as water and some organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms.

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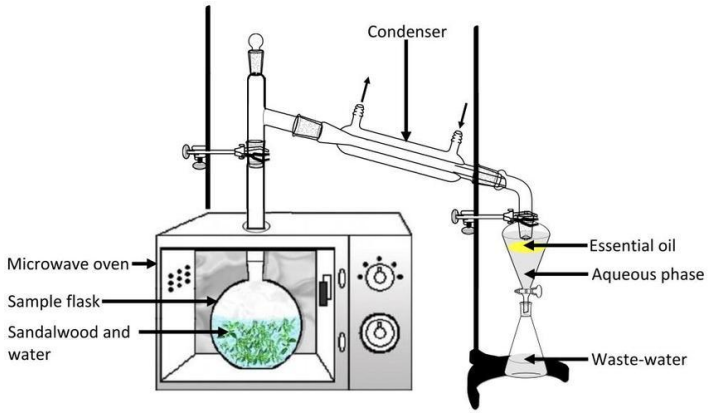
The transfers of heat and mass are in the same direction in MAE, which generates a synergistic effect to accelerate extraction and improve extraction yield.

The application of MAE provides many advantages, such as increasing the extract yield, decreasing the thermal degradation and selective heating of vegetal material.

MAE is also regarded as a green technology because it reduces the usage of organic solve



There are two types of MAE methods:

- Solvent-free extraction (usually for volatile compounds)
- Solvent extraction (usually for non-volatile compounds)

Advantages:

- Decreased in extraction time.
- Loss of volatile substances is avoided.
- Less Solvent.
- Is required because no evaporation occurs.

Disadvantage:

- High pressure used pose safety risks
- The usual constituent material of the vessel does not allow high solution temperatures
- Addition of reagents is impossible since it is a single step procedure(22)

2.5 Chromatographic Techniques (23)

Chromatography

Chromatography is the technique for the separation, purification, and testing of compounds.

The term “chromatography” is derived from Greek, Chroma meaning, “color,” and graphein meaning “to write.”

In this process, we apply the mixture to be separated on a stationary phase (solid or liquid) and a pure solvent such as water or any gas is allowed to move slowly over the stationary phase, carrying the components separately as per their solubility in the pure solvent.

Chromatography Principle

Chromatography is based on the principle where molecules are transferred between the mobile phase and the stationary phase.

It occurs due to the absorbance or partition of molecules present in the mixture which we want to separate.

The rate of travel of individual solute molecules through a column or thin layer is not uniform.

It is directly related to the partition of the molecules between the mobile phase and stationary phase.

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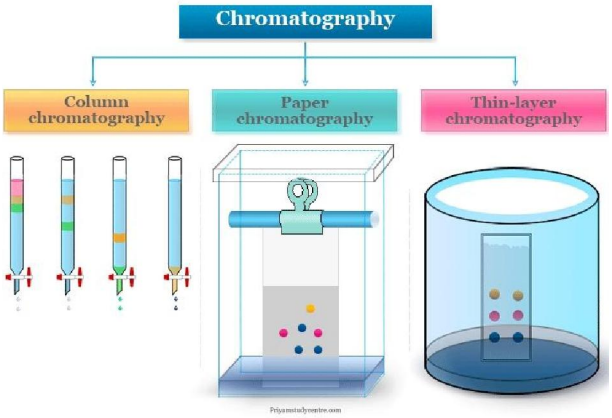
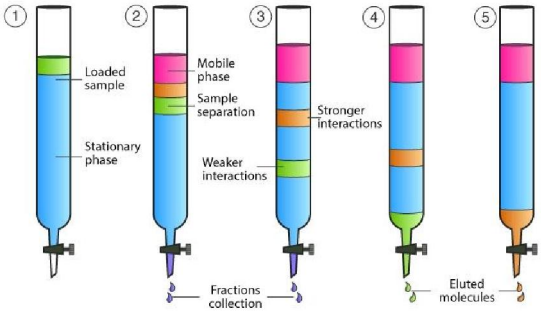


Types of Chromatography

Column Chromatography

Column chromatography is the technique used to separate the components of a mixture using a column of suitable adsorbent packed in a glass tube, as shown in the figure below. The mixture is placed on the top of the column, and an appropriate eluent is made to flow down the column slowly.

Depending upon the degree of adsorption of the components on the wall adsorbent column, the separation of the components takes place. The component with the highest absorptivity is retained at the top, while the other flow down to different heights accordingly.



Advantages

1. All different kinds of complex mixtures can be separated by column chromatography.
2. The mobile phase has a wide range.
3. There is no limit for quantity as any amount of mixture can be separated by this technique.
4. It is a robust method.
5. The separated analytes can be reused.
6. This process can be automated.

Disadvantage

1. It is a time-consuming process for the separation of compounds.
2. It Is expensive as higher quantities of solvents are required.
3. The automated process becomes complicated and therefore costly.
4. It has a low separation power.

Thin Layer Chromatography: (24)

In the process of thin-layer chromatography (TLC), the mixture of substances is separated into its components with the help of a glass plate coated with a very thin layer of adsorbent, such as silica gel and alumina, as shown in the figure below.

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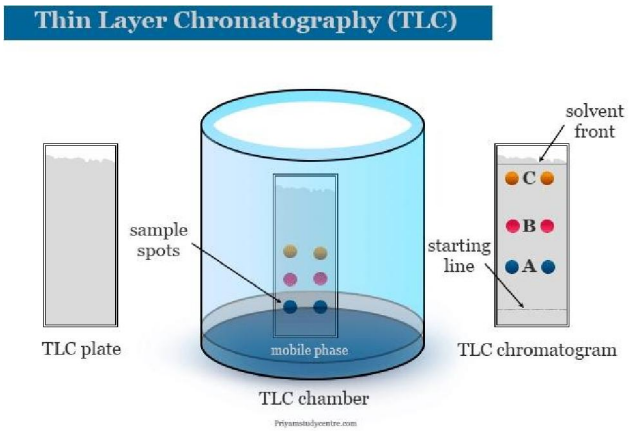


The plate used for this process is known as chrome plate.
The solution of the mixture to be separated is applied as a small spot at a distance of 2 cm above one end of the plate.
The plate is then placed in a closed jar containing a fluid termed as an eluent, which then rises up the plate carrying different components of the mixture to different heights

Advantages

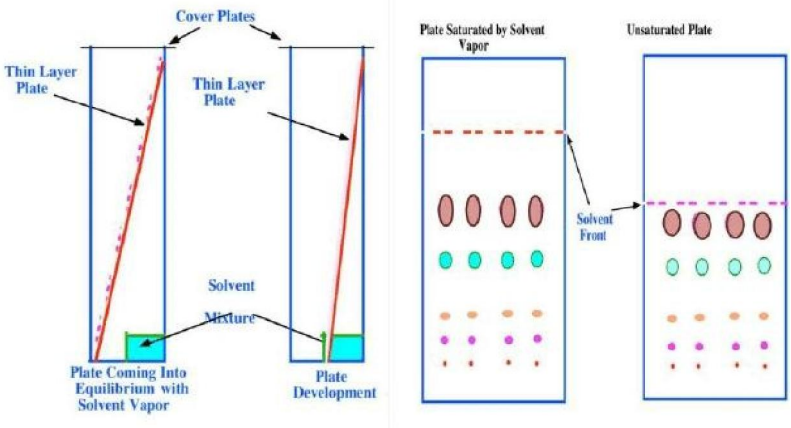
1. A simple method of component separation. Fewer types of equipment are used in this technique.
2. As the components elute rapidly, the separation is achieved in a very short time.
3. It is possible to visualize all elements of UV light.

Disadvantages

1. It is difficult to reproduce the findings obtained from the experiment.
2. Applicable for components of soluble mixtures only.
3. Qualitative analysis, not the analysis in quantitative terms.
4. It is not an automatic mechanism.

High Performance thin Layer Chromatography

HPTLC have similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e. the principle of separation is adsorption.
The mobile phase solvent flows through because of capillary action.
The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower.
The component with lesser affinity towards the stationary phase travels faster.
Thus the components are separated on a chromatographic plate.



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Advantages:

1. Sample requirement is very less.
2. No risk of involvement of costlier stationary phase like HPTLC, Columns.
3. Less amount of mobile phase is required.
4. Skilled people are not required like HPTLC

Limitations:

1. Limitation of separation of more compounds in small plate.
2. Blockage of needle occurs when concentrated sample is used
3. The whole setup of equipment cost is more

Paper Chromatography

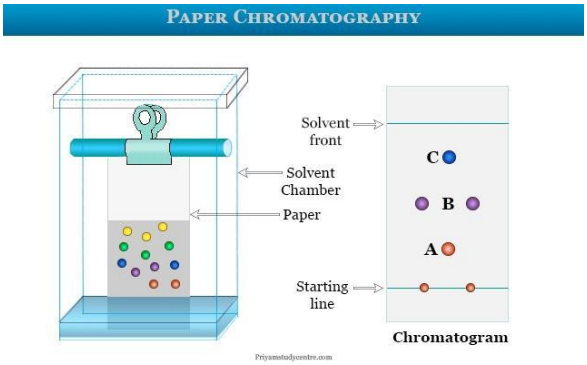
The principle involved can be partition chromatography or adsorption chromatography.

Partition chromatography because the substances are partitioned or distributed between liquid phases.

The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place.

The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper.

Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.



Advantages:

1. Separation of compounds in a short time.
2. Analysis requires a low amount of sample.
3. Easy to handle and setup.
4. The less sample quantity required for the analysis.
5. Cost-effective method.
6. It requires quantative material

Disadvantages:

1. Volatile substances cannot be separated using paper chromatography techniques.
2. Paper chromatography cannot be compatible with large amounts of sample.
3. Quantitative analysis is not useful in paper chromatography.
4. Paper chromatography cannot be separated complex mixture.
5. As compared to the HPLC, TLC or HPTLC, paper chromatography has less accuracy.
6. Data cannot be saved for long periods

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High Performance Liquid Chromatography

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.

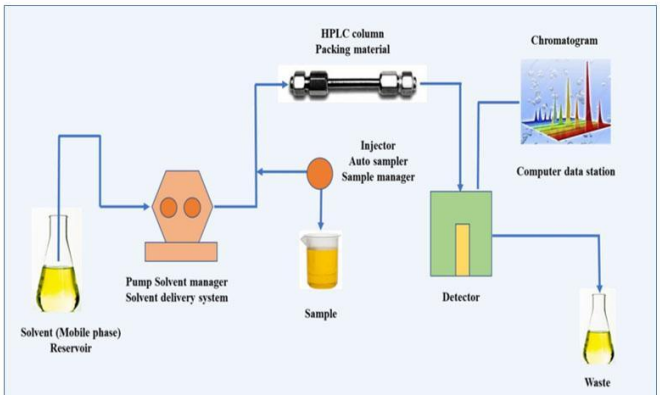
The mixture Is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.

Advantages

1. HPLC offers a rapid, automated and highly precise method to recognize certain chemical components in a sample.
2. High-performance liquid chromatography offers a fast and precise quantitative analysis.
3. A gradient solvent system can be applied in certain methods.
4. It is highly reproducible.
5. HPLC can be upgraded to mass spectroscopy (MS).

Disadvantage:

1. HPLC can be an expensive method, it required a large number of expensive organics, needs a power supply, and regular maintenance is required.
2. It can be complicated to troubleshoot problems or develop new methods.
3. The lack of a universal detector for HPLC, however, the UV-Vis detector only detects chromophore compounds.
4. The separation in High-performance liquid chromatography has less efficiency than GC.
5. It is more difficult for the beginner



Application of Chromatography (25)

Pharmaceutical Analysis

- Chromatography is used for characterizing and isolating organic compounds such as amino acids, alcohol, amines, acids, antibiotics, etc.
- Chromatography is used to find the amount of chemicals in each pharmaceutical product.

Herbal Analysis

- HPTLC is widely used for the herbal analysis. Herbal extracts which are known to contain more chemical constituents are difficult to be analysed by other techniques. HPTLC is a reliable technique to analyse herbal extracts and poly herbal formulations. Multidimensional chromatography is often a good choice in case of very complex samples, offering many advantageous features in the analysis of medicinal plants.
- Herbal drug standardization.

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Forensic Science

- In Forensic studies paper chromatography is used in crime scene investigation and DNA and RNA sequencing along with other studies.
- Chromatography is used in forensic labs to identify components in the samples collected from the suspects

DNA Sequencing

- Isolation, Cloning Analyzation of DNA in forensic investigation.

Taxonomical and Genetic Study

- It has been used for the taxonomical and genetic study example, Taxonomic study in the molluscan genus lymnaea.

Food Industry:

- Chromatography is also used in the food industry to identify the nutritive value of the food additives and their components in the food.
- It is also used in hospitals to detect the alcohol level in the patient's bloodstream.
- It is used in clinical tests like urine analysis, antibiotic analysis, etc.

Bioanalysis:

- It is used to determine the drugs in biological matrix such as blood, plasma, urine, serum & feces.

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