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A REVIEW ON "HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY"

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ABSTRACT

The most adaptable technology available is HPTLC, which is renowned for its consistency, purity profile, assay values, and precision and accuracy of outcomes. It can manage several samples with even different compositions and natures. The main objective of this study was to create an analytical method for quantifying polyphenols like chlorogenic acid, which is contained in Calendula officinalis Linn, utilizing high performance liquid chromatography and high performance thin layer chromatography. Combining these two analytical techniques could be helpful for quality control analysis because it would result in a quick, accurate, and straightforward method. To determine the identity of the plant material, a straightforward chromatographic approach HPTLC provides important extra information. High performance thin layer chromatography (HPTLC) is an advanced instrumental technique built on thin layer chromatography's entire range of capabilities.

INTRODUCTION TO CHROMATOGRAPHY[10]

Chromatography is a non-destructive method for isolating the various fractions of a multi-component mixture comprising trace, minor, or substantial elements. Solids, liquids, and gases can all have different variances. Chromatography is a non-destructive method for separating a multi-component mixture of trace, minor, or major ingredients into its distinct fractions, while it can also be used quantitatively. Solids, liquids, and gases can all have different variances. Chromatography is usually used for separation, though it can also be used quantitatively.

Chromatography is a relatively new scientific method that was created by M. a botanist born in Warsaw in 1906. By percolating vegetable extracts through a column of calcium carbonate, he was successful in separating chlorophyll, xanthophyll, and numerous other coloured compounds during that year. The calcium carbonate column served as an adsorbent, and the various compounds were adsorbed to varying degrees. As a result, the column developed coloured bands in various places. However, the name "chromatographic" is misleading because in the vast majority of chromatographic processes, no coloured compounds are produced. The calcium carbonate column utilized in Tswett's approach is referred to as the stationary phase since it does not move. Vegetable extract solution is referred to as the mobile phase since it travels or flows down the column. Chromatography can be thought of as a separation technique in which solutes separate between a stationary phase and a mobile phase.

The stationary phase refers to the calcium carbonate column used in Tswett's method because it is immobile. Since the vegetable extract solution moves or flows down the column, it is referred to as the mobile phase. In chromatography, solutes separate between a stationary phase and a mobile phase as part of a separation process.

Definition of Chromatography

Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases. Essentially, the technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called mobile phase). The chromatographic method of separation, in general, involves the following steps:-

1. Adsorption or retention of a substance or substances on the stationary phase.
2. Separation of the adsorbed substances by the mobile phase.
3. Recovery of the separated substances by a continuous flow of the mobile phase; the method being called elution.
4. Qualitative and quantitative analysis of the eluted substances.

- **Types of chromatographic techniques**

Sr.no	Techniques	Stationary Phase	Mobile Phase
1	Column chromatography or adsorption chromatography	Solid	Liquid
2	Partition chromatography	Liquid	Liquid
3	Thin layer chromatography	Liquid	Liquid
4	Gas liquid chromatography	Liquid or Solid	Liquid
5	Gas solid chromatography	Liquid	Gas
6	Paper chromatography	Solid	Gas
7	Ion Exchange chromatography	Solid	Liquid

The stationary phase in chromatography can be either a solid or a liquid, and the mobile phase can either be a liquid or a gas. Separation happens as a result of a combination of two or more parameters, such as migration rates, capillary action, the amount of adsorption, etc., depending on the stationary and mobile phases utilized. Based on the stationary and mobile phases utilized, chromatographic procedures can be divided into different categories.

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)[3]

The method of TLC that has been enhanced and is more effective is HPTLC. Planar chromatography and flat-bed chromatography are other names for it. The full potential of thin layer chromatography is essentially what high performance thin layer chromatography (HPTLC) depends upon (TLC). Due to the fact that it aids in qualitative technique analysis and combines chromatography's art and speed at a reasonable price. As HPTLC, which can only be used on precoated layers, employing instrumentation, and primarily for quantification purposes, modern TLC is well-known and useful. Therefore, the terms TLC and HPTLC are used synonymously here. TLC is used to teach the basics of chromatography practically everywhere in the globe.

High efficiency, commercially precoated plates, which are smaller (10 x 10 or 10 x 20 cm), have a thinner (0.1-0.2 mm) layer composed of sorbent with a finer mean particle size (5-6 m), and are developed over shorter distances (about 3-7 cm) than classical TLC plates, which are typically 20 x 20 cm with a 0.25 mm layer containing particles with an average particle size of 0.25 mm.

HPTLC plates offer better in situ quantification, faster analytical times, increased detection sensitivity, and improved resolution. Because of its adaptability for high-throughput screening, sensitivity, and dependability in quantification of analytes at nanogram levels, HPTLC is a potent analytical technology equally applicable for qualitative and quantitative

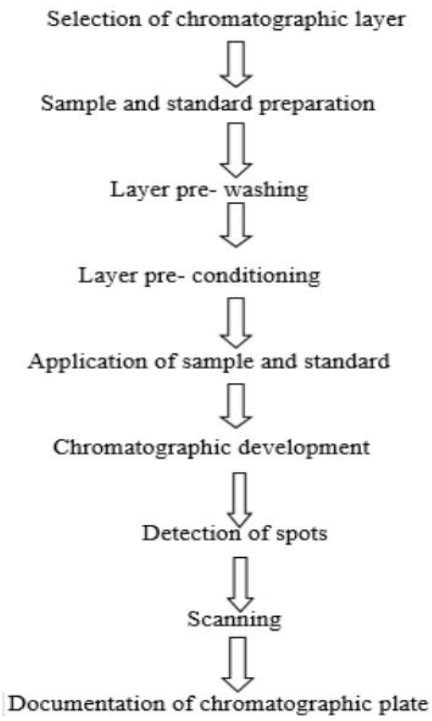
analytical applications. The pharmaceutical industry, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environment analysis, and other fields all use it frequently for analysis.

PRINCIPLE AND WORKING[3]

PRINCIPLE

It is a potent analytical technique that works well for both qualitative and quantitative problems. Depending on the type of adsorbent utilized on the plates and the development solvent system, separation may be caused by partition, adsorption, or both phenomena .Capillary action causes the solvent from the mobile phase to pass through. According to their affinities with the adsorbent, the components migrate. The components that are more affixed to the stationary phase move more slowly, whereas the components that are less affixed move more quickly**.**

❖ **Steps involved in HPTLC**



Selection of chromatographic layer

Precoated plates — different support materials — different sorbents available. 80 % of analysis — silica gel 60F — Basic substances, alkaloids and steroids- Aluminium oxide amino acids, dipeptides, sugars and alkaloids — cellulose. Non-polar substances, fatty acids, carotenoids, cholesterol RP2, RP8 an RP18. Preservatives, barbiturates, analgesic and

phenothiazine-hybrid plates- RPWF24s.[51]

Sample and standard preparation

Methylene chloride to prevent interference from water vapour and contaminants. Straight base line, low signal-to-noise ratio - LOD improvement. There are several other solvents that can be employed, including methanol (1:1), ethyl acetate: methanol (1:1), chloroform: methanol: ammonia (90:10:1), methylene chloride: methanol (1:1), 1% ammonia, or 1% acetic acid. Dry the plates before storing them somewhere dust-free. A excellent solvent system is one that doesn't add anything to the solvent front but instead shifts the equilibrium of the entire mixture. The peaks of interest should be resolved between R_f 0.15 and 0.85. Eluent strength is a factor that affects the mobile phase's elution power.

Pre-Washing

It is the cleansing phase. Pre-washing serves the primary aim of removing pollutants from the atmosphere, such as water vapours and other volatile compounds, before they are exposed to the lab environment. The presence of iron impurities in silica gel 60F is the main drawback of this sorbent. The primary benefit of the pre-washing stage is the removal of this iron using a 9:1 methanol to water solution. Solvents employed in pre-washing are:-

Methanol

Chloroform: Methanol (1:1)

Chloroform: Methanol: Ammonia (90:10:1)

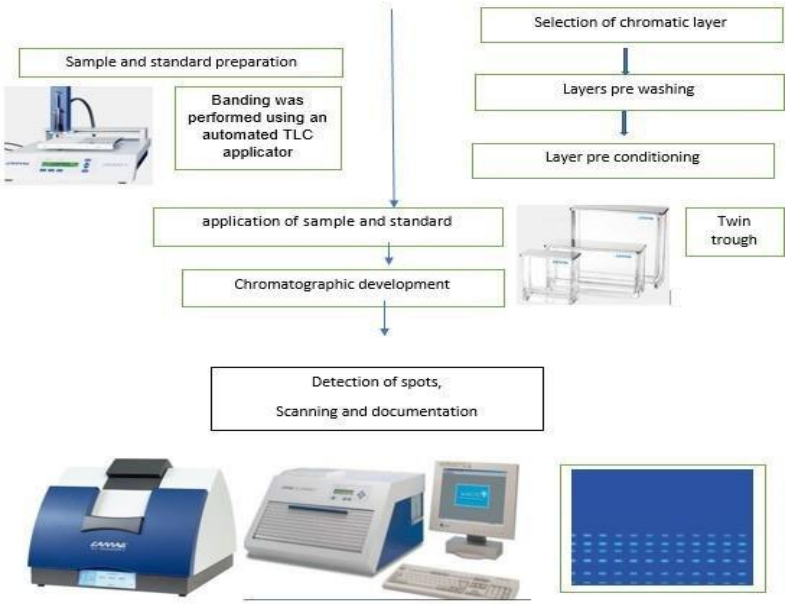


Figure 1: steps involved in hptlc.

Pre-Conditioning (Chamber saturation)

The separation profile is significantly impacted by chamber saturation. The amount of time needed for saturation is dependent on the mobile phase. When plates are placed in an unsaturated chamber throughout the development process, the solvent mostly evaporates from the plate near the solvent front, increasing the Rf values. If the tank is saturated before the development, the solvent vapours quickly spread evenly across the chamber. The plate quickly becomes preloaded with solvent vapours when it is placed in such a saturated chamber, which reduces the amount of solvent needed to cover a given distance and lowers Rf values. However, in some circumstances, chamber saturation and non-saturation are necessary depending on their polarity. Prior to development in the saturation chamber, filter paper lining is applied for 30 min. This causes a consistent distribution of solvent vapours and reduces the amount of solvent needed for the sample to travel.

Application of sample and standard

The typical concentration range is 0.1 g/l; anything higher results in poor separation. Nitrogen gas from an automatic applicator sprays bands of sample and standard onto TLC plates. Better separation and densitometer response with band-wise application. Pharmaceutical preparations are easily dissolved in a suitable solvent that will solubilize the analyte when the analyte concentration is suitably high. 10 It is important to apply the sample correctly and achieve acceptable resolution for HPTLC measurement. Techniques for applying samples are influenced by variables like the workload, sample matrix, and time limitations.

Chromatographic development and drying

Use of various chamber types: 1. A chamber with two troughs 2. A rectangular chamber 3. V-shaped chamber 4. Sandwich Chamber 5. Horizontal development chamber 6. A chamber for automatic development. Ascending, descending, two-dimensional, horizontal, multiple overrun, gradient, radial anti-radial, multimodal, and forced flow planar chromatography are some of the numerous techniques employed for chamber creation. In the developing chambers, plates are dotted with sample, allowed to air dry, and then put. To avoid contaminating the lab environment, the mobile phase must first be removed from the chamber after the development plate. Because the divided components will migrate uniformly to the surface where they may be easily seen when the mobile phase evaporates, the plate should always be positioned horizontally.

Detection and visualization

The first phase in UV light detection is nondestructive. At 254 nm, or short wavelength, fluorescent compound spots can be seen. utilizing a 0.1% iodine solution to visualize non-UV absorbent materials If a particular component is not responsive to UV, derivatization with a visualizing agent is required. Enhancing the identification of separated chemicals on the sorbent layers is quenching fluorescence caused by UV radiation (200-400 nm). Commonly referred to as fluorescence quenching, this process. UV 254 nm visualization: F254 should be characterized as phosphorescence quenching. In this case, the fluorescence persists for a brief time after the source of excitation is removed. Although it lasts for more than ten seconds, it is quite brief. Under 254 nm, it is recommended to look for anthraglycosides, coumarins, flavonoids, propyl phenols in essential oils, and some types of alkaloids like indole, isoquinoline, and quinoline alkaloids. 16 Imagining in white light It is possible to identify a zone that contains distinct chemicals by looking at them in their natural colour in daylight (white light).

Scanning

The peak height or area that the scanner transforms from bands to is related to the drug concentration on the spot or band. Instrument measurements and records are made of the peak height and spot area.

Documentation

Documentation is crucial because labelling each and every chromatogram can help prevent errors in application sequence. You can record the type of plate, chamber system, running time, and detecting technique.

FACTORS AFFECTING HPTLC

- Types of stationary phase
- Mobile Phase
- Layer Thickness
- Temperature
- Mode of development
- Amount of Sample
- Dipping zone, etc.

Stationary Phase[5]

The most technologically advanced and contemporary TLC is HPTLC. To achieve homogeneous layers with a smooth surface, it uses HPTLC plates with microscopic particles with a narrow size distribution. Smaller plates (10 10 or 10 20 cm) are used in HPTLC. HPTLC plates are utilized for industrial pharmaceutical densitometric quantitative chemical analysis because they offer increased resolution, higher detection sensitivity, and improved in place quantification. 90% of reported analyses of drugs and medicines have utilized normal phase adsorption TLC on colloid with a less polar mobile phase, like chloroform-methanol. Reversed-phase TLC is performed using lipophilic C-18, C-8, and C-2, phenyl chemically modified colloid phases, hydrocarbon impregnated colloid plates, and a more polar aqueous mobile phase, such as methanol-water or dioxane-water. Alumina, magnesium silicate, periclase, polyamide, cellulose, kieselguhr, ion exchangers, and polar modified colloid layers with bound amino, cyano, diol, and thiol groups are some other precoated layers that are employed.

Mobile Phase[5]

The choice of mobile phase depends on the analyte's physical and chemical characteristics as well as the adsorbent utilized as the stationary phase. As the strength-adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran combined with water for strength adjustment in reversed phase TLC, respectively, ether, dichloromethane, and chloroform are used as mobile-phase systems to support their various selectivity properties. A mobile phase like methanol-0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentane sulfonate is used in ion pairing separations on C-18 layers.

CLASSIFICATION OF HPTLC[8]

HPTLC techniques may be classified into four classes i.e. Classical, High performance, Ultra and Preparative thin layer chromatography. They differ with classical TLC in the particle size distribution and thickness of the sorbent layers. The mean particles sizes are 12, 5, 25 µm for classical, high-performance and preparative thin-layer chromatography, respectively, whereas Ultra- thin layer chromatography does not have particles but a monolithic layer with 1–2 um macropores. Another difference is the thickness of the sorbent layers which is 250 um, 200 um, 10 um and 0.5–2 mm, for classical, high-performance, ultra-thin and preparative sorbent layers, respectively.

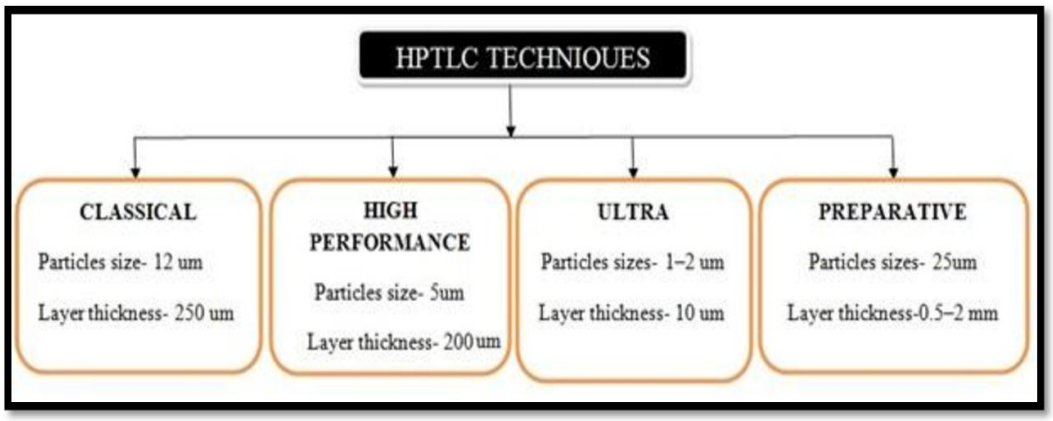


Figure 2: techniques of hptlc.

INSTRUMENTATION OF HPTLC[11,12]

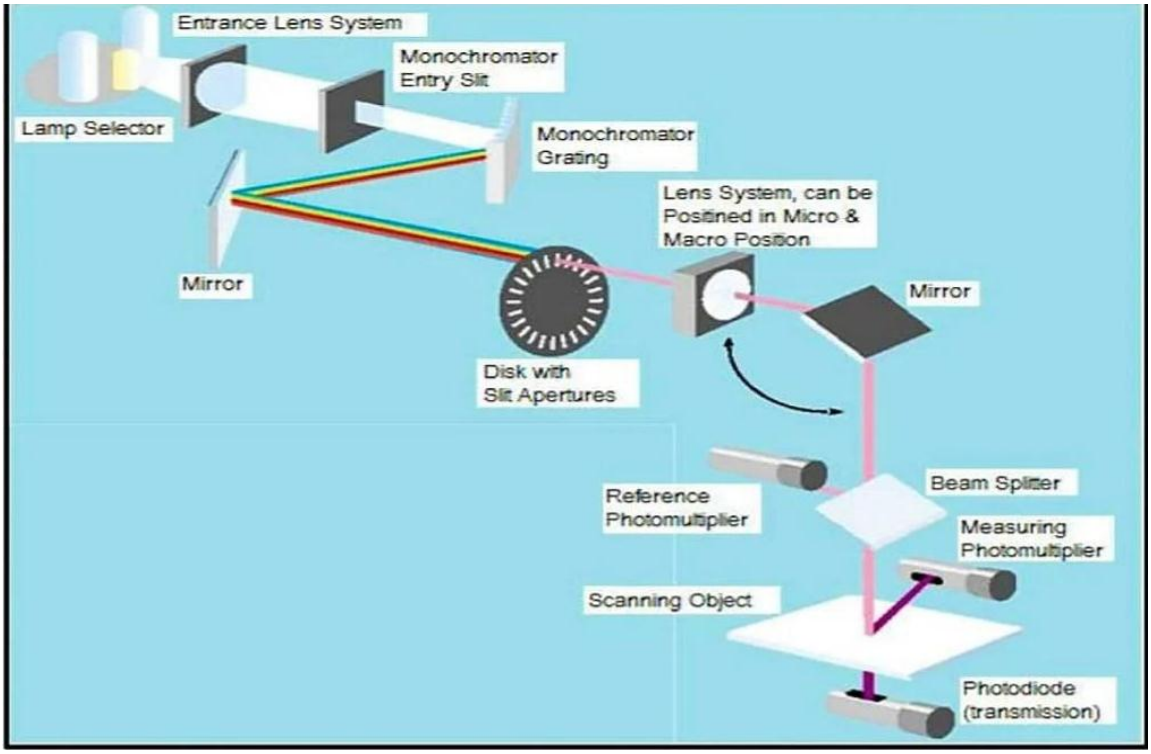


Figure 3: instrumentation of hptlc.

It consists of the following components:

- Lamp selector
- Entrance lens slit
- Monochromator entry slit
- Grating
- Mirror
- Slit aperture disc

- Beam splitter
- Reference photo multiplier
- Measuring photo multiplier
- Photo diode for transmission measurement

1. Lamp Selector

1) Hydrogen lamp

Hydrogen lamps are reliable, steady, and continuously emit radiation between 160 and 380 nm. It consists of hydrogen gas at high pressure, which causes an electrical discharge. The excited hydrogen molecules produce radiation.

2) Deuterium lamp

A gas discharge lamp called a deuterium lamp is frequently employed as a UV source. It emits radiation in the 160–450nm range. It costs more than a hydrogen lamp.

3) Tungsten lamp

The most typical light source utilized in spectrophotometers is the tungsten lamp. With a wavelength range of roughly 330 to 900 nm, it comprises of a tungsten filament encased in a glass envelope and is utilized for the visible spectrum.

4) Xenon discharge lamp

A xenon lamp is a discharge light source that contains xenon gas inside a bulb. Radiation from xenon ranges from 250 to 600 nm.

2. MONOCHROMATOR

By filtering out undesirable wavelengths from the radiation source light, a monochromator creates monochromatic light. Through the entrance slit, multi-wavelength polychromatic light enters the monochromator. Following collimation, the beam is directed at an angle toward the dispersion component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit when the dispersing element or the exit slit are moved.

Types of monochromators

1) Prism monochromator
2) Grating monochromator

All Monochromator contain the following component parts:

- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

Radiation with many wavelengths, or polychromatic radiation, enters the monochromator through the entrance slit. After being collimated, the beam angles toward the dispersing component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit by changing the dispersing element or the exit slit.

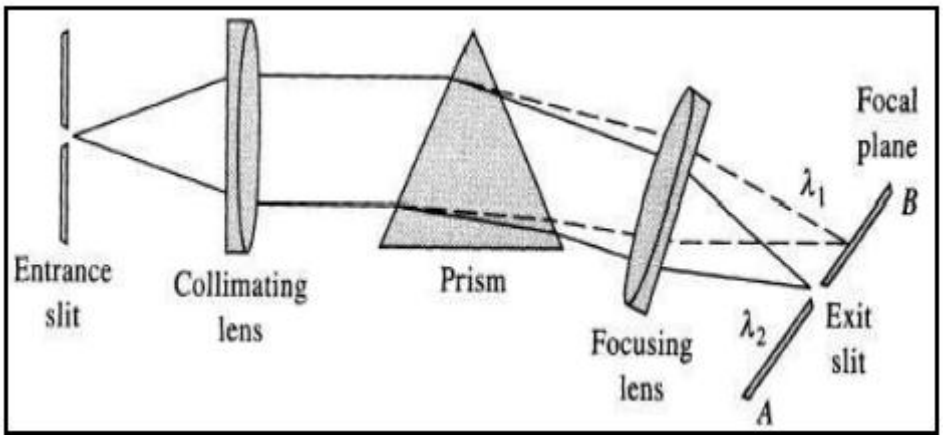


Figure 4: Prism monochromator.

Grating

The grating is a dispersive element used to select the wavelengths required to probe the electronic transitions of a sample's chromophores. It is rotated to the wavelengths selected and diffracts the light into several beams. The direction that light is diffracted depends on the angle and wavelength of the incident beam, and the grating's groove (or line) frequency, or the number of grooves on the grating per millimeter.

The spacing between the grooves determines the diffraction order of the light, or how many beams are diffracted at that particular wavelength, as well as the spectral resolution. A diffraction order of 0 means the incident light angle and the diffracted angle are roughly the same while a diffraction order of 1 is twice the incident wavelength. Wider groove spacing

means fewer orders of diffraction, resulting in higher the light throughput. Aside from using a grating with large groove spacings, filters are commonly used to remove any higher orders of diffraction from the grating. Light diffracted from the grating is refocused by another mirror onto the exit slit which is adjusted to accommodate for the dispersive properties of different wavelengths of light. There are two types of gratings:

1. Transmission Grating

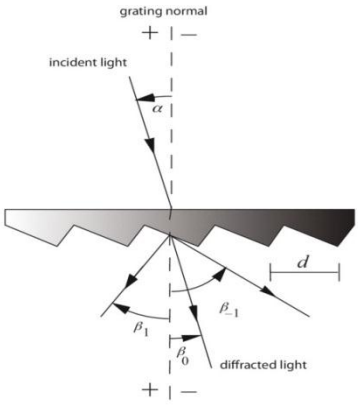


Figure 5: transmission grating.

2. Reflection Grating

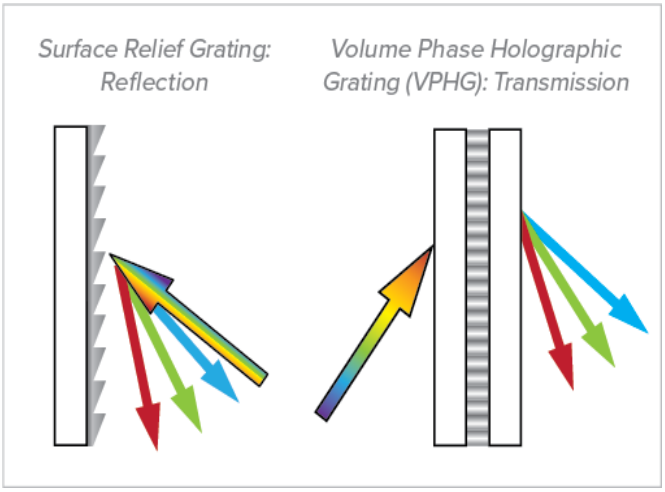


Figure 6: reflection grating.

PHARMACEUTICAL APPLICATIONS OF HPTLC[3]

Numerous qualitative and quantitative methodological applications, including those involving herbal and dietary supplements, neutraceuticals, and a range of medications, utilize the HPTLC technique. Forensic applications include toxicity trials, assaying radio chemical contaminants in radio medicines, and detecting and identifying prescription raw ingredients, products, and their metabolites in biological medium. Scientific usage include metabolism assays and drug screening. Numerous lipids have also been examined and investigated using

HPTLC; 20 different lipid sub classes were identified with repeatable and encouraging results. Many papers on investigations related to clinical medicine have previously been published in numerous journals. HPTLC is now highly advised for the analysis of medicines in serum and other tissues.

HPTLC in quality control of pharmaceuticals

Pharmaceutical formulations including dutasteride, nabumetone, and primates have all undergone routine quality control using HPTLC. For the simultaneous quantitative evaluation of sulpiride and mebeverine hydrochloride in the presence of their reported impurities and hydrolytic degrades, whether in pure form or in pharmaceutical formulation, validated sensitive and highly selective stability indicating procedures were reported. HPTLC is also a great screening tool for adulterations and is highly suitable for evaluating and monitoring of cultivation, harvesting, and extraction processes testing of stability. A stability-indicating HPTLC method for the analysis of ropinirole HCL was developed and validated for precision, accuracy, ruggedness, robustness, specificity, recovery, and limit of detection (LOD) and limit of quantification (LOQ). For the creation of a quality assurance programme, HPTLC has been reported**.**

HPTLC as biomarker in pharmacognostical research Micheliachampaca

Numerous plants utilized in Indian medical systems have undergone HPTLC study for a variety of pharmacological properties like CNS and hepatoprotective, among others. Quercetin in Michelia champaca (leaves and stembark) has been detected and quantified using the HPTLC method, and the estimated values show that the leaves constitute the plant's richest source of quercetin. With strong reliability and reproducibility, the HPTLC method can be used regularly to estimate the amount of curcumin in commercial turmeric powder.

HPTLC in other fields

The developing world has recently demonstrated HPTLC as an internationally recognized, workable approach for the characterization of small compounds in quality evaluation. It is used to analyze vitamins, water-soluble flood dyes, pesticides in fruits and vegetables, as well as other things, and to check the purity of chemicals, pesticides, and steroids. Applications for HPTLC include the analysis of herbal items, drugs, and fingerprints. The HPTLC technique is also extremely helpful for routine analyses of pharmaceutical and clinical data, analyses of traditional medicines and medicinal plants, analyses of foods and dietary supplements, analyses of environmental factors, analyses of cosmetics and toxicology, analyses of plants

and herbs, and analyses of food and food supplements.

Clinical applications

- Lipids
- Metabolism studies
- Drug screening
- Doping control, etc.

Cosmetics

- Identity of raw material
- Preservatives, colouring materials, etc.
- Screening for illegal substances, etc.

Herbal medicines and botanical dietary supplements

- Identification
- Stability tests
- Detection of adulteration
- Assay of marker compounds, etc.

Food and feed stuff

- Quality control
- Additives (e.g. vitamins)
- Pesticides
- Stability tests (expiration), etc.

ADVANTAGES OF HPTLC[3]

- Technically, learning and operating is easy.
- Many analysts work together on the system.
- Low Analysis Time and Low-cost Analysis.
- Low maintenance cost compared to other instruments.
- Visual recognition is possible because it is an open method.
- Availability huge ranges of stationary phases.
- UV-absorbing and corrosive mobile phases can be working on this system.
- Filtration and degassing for solvents are not necessary.

- Due to the use of the freshly prepared mobile phase and stationary phase, this does not have any chance of interference or contamination.
- Samples cleaning not often require.
- Sensitivity range of analysis usually occurs at the pictogram (pg) and Nanogram (ng).
- It uses less solvent, and has the ability to simultaneously run multiple samples thus saving time and cost.
- Enormous flexibility.
- Parallel separation of many samples with minimal time requirement.
- Unsurpassed clarity and simultaneous visual evaluation of all samples and sample components.
- Simplified sample preparation due to single use of the stationary phase.
- Required less mobile phase and sample amount

CONCLUSION

To recapitulate it can be said that the method is found to be sensitive, accurate, precise, repeatable, cost-effective, and time-efficient. The suggested method can be regarded as one that indicates stability because it can selectively quantify. As an alternative to the previous methods, the proposed method can be used to regularly check the quality of pharmaceutical formulations that contain medications, either alone or in combination. HPTLC has become a powerful analytical tool in the field of analysis thanks to applications in phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis, and HPTLC future to combinatorial approach, HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser. It is interesting that instrumental HPTLC will increasingly be used to analyze pharmacological formulations, bulk medications, natural items, clinical samples, foods, environments, and other pertinent materials.

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