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HPTLC In The Pharma Industry

***Pranjal Batwal*[+1], *Tanaya Wakchaure*[+2]**

[+1]VIDYA NIKETAN INSTITUTE OF PHARMACY AND RESEARCH CENTRE, BOTATAL-SANGAMNER, DIST-AHMEDNAGAR, MAHARASHTRA 422602, INDIA.
[+2]DEPARTMENT OF PHARMACEUTICS VIDYA NIKETAN INSTITUTE OF PHARMACY AND RESEARCH CENTRE, BOTA, TAL-SANGAMNER, DIST-AHMEDNAGAR, MAHARASHTRA 422602, INDIA.
Email Id- pranjalbatwal123@gmail.com

ABSTRACT:

Chromatography is commonly used in the pharmaceutical industry for the quantification of drugs and other pharmaceuticals. Among the various chromatographic techniques, High Performance Thin Layer Chromatography (HPTLC) has gained popularity due to its speed, versatility, applicability, and ability to analyze more than 100 samples simultaneously. Given its potential applications, researchers worldwide have utilized this technique for pharmaceutical analysis. This review summarizes research conducted on 63 formulations, of which 38 contain one active ingredient, while 25 contain two active substances. This review aims to provide a comprehensive understanding of the utility of this technique in pharmaceutical analysis and to assist scientists interested in using HPTLC for their analytical purposes.

KEYWORDS: HPTLC, Pharmaceutical analysis, Method development, Active ingredient,Pharmaceutical formulation.

INTRODUCTION :

Planar chromatography, also known as flat-bed chromatography, is another term for this technique. HPTLC is safe and allows for multiple detections without the need to repeat chromatograms. It is significantly faster and more economical than HPLC, another liquid chromatography technique. HPTLC is used for the analysis of non-volatile organics such as pharmaceuticals, botanicals, forensics, foods, chemicals, and active pharmaceutical ingredients. It is employed for establishing purity, reverse engineering, detecting impurities, and fingerprinting. HPTLC is a stable, quick, and effective method for the quantitative analysis of compounds. Common criteria for drug estimation include the quality and therapeutic value of the pharmaceutical product, content, purity, content uniformity, and chemical and biological availability2. Table 1 lists the different performance characteristics of the silica gel plate betweenTLC and HPTLC.

Table 1: Performance features of HPTLC and Thin layer chromatography

PARAMETER (Features of HPTLC versus classicalTLC)	**Classical TLC**	**HPTLC**
Mean particle size	10-12 µm	10-12 µm
Particle size distribution	**5 - 20 µm**	**4 - 8 µm**
Pore diameter	**40,60,80,100A0**	**60 A0**
Plate dimensions	**5×10,5×20,10×20,20×20 cm**	**10×10,10×20,20×20 cm**
Plate height	**30 µm**	**12 µm**
Layer thickness	**0.20-0.25mm ((250 µm)**	**0.20-0.25 mm**
No of samples per plate	**< 10**	**< 36 (72)**
Spot size recommended	**2-5mm**	**1mm**
Sample volume	**2-5mm**	**1mm**
Band size recommended	**1-5µl**	**0.1 -0.5 µl**
Band loading	**10-15mm**	**5-10 mm**
Typical separation time	**5-10 µl**	**1-4 µl**
Chromatographic plateused	**20 - 200 minutes**	**3 – 20 minutes**

HPTLC Benefits:

- **Speed:**

HPTLC allows for rapid analysis, significantly reducing the time required for sample preparation and processing compared to other chromatographic techniques.

- **Versatility:**

It can be used to analyze a wide range of substances, including pharmaceuticals, botanicals, forensics, foods, chemicals, and active pharmaceutical ingredients**.**

- **Cost-Effectiveness:**

HPTLC is more economical than High Performance Liquid Chromatography (HPLC),making it a cost-effective option for many laboratories.

- **Simultaneous Analysis:**

The technique enables the analysis of more than 100 samples in parallel, increasingthroughput and efficiency.

- **Safety:**

HPTLC is a risk-free method that allows for multiple detections without the need torepeat chromatograms, ensuring safety and reliability in results.

- **Stability:**

The method provides stable and reproducible results, which are crucial for quantitativeanalysis.

- **Comprehensive Analysis:**

HPTLC is effective for establishing purity, reverse engineering, detecting impurities, andfingerprinting of compounds.

- **Environmental Friendliness:**

The technique uses smaller amounts of solvents and reagents, making it moreenvironmentally friendly compared to other methods.

- **Ease of Use:**

HPTLC is relatively easy to perform and does not require highly specialized equipmentor extensive training.

- **Wide Applicability:**

It is suitable for both qualitative and quantitative analysis, making it a versatile tool invarious fields of research and industry.

Steps involved in HPTLC:

Sample Preparation:

Extraction: Isolate the analytes from the sample matrix using appropriate solvents.
Filtration: Filter the extract to remove any particulate matter.
Concentration: Concentrate the extract if necessary to achieve the desired detectionlimits.

- **Plate Selection and Preparation:**

Plate Selection: Choose the appropriate HPTLC plate (e.g., silica gel, alumina) based onthe nature of the analytes.
Plate Activation: Activate the plate by heating it in an oven to remove any adsorbedmoisture.

- **Sample Application:**

Spotting: Apply the sample solutions onto the HPTLC plate using a micropipette or anautomated applicator.
Banding: Ensure the samples are applied as narrow bands to improve resolution.

- **Chromatographic Development:**

Mobile Phase Preparation: Prepare the mobile phase (solvent system) based on thepolarity of the analytes.
Development Chamber: Saturate the development chamber with the mobile phase toensure a uniform environment.
Development: Place the HPTLC plate in the chamber and allow the mobile phase toascend the plate by capillary action, separating the analytes.

- **Detection and Visualization:**

Visualization: Visualize the separated analytes using appropriate detection methods (e.g.,UV light, iodine vapor).
Derivatization: Apply derivatizing agents if necessary to enhance the visibility of theanalytes.

- **Documentation:**

Scanning: Scan the developed plate using a densitometer or a digital camera to recordthe chromatogram.

Data Analysis: Analyze the chromatogram to quantify the analytes using software tools.

- **Interpretation and Reporting:**

Quantification: Calculate the concentration of the analytes based on the intensity of thespots or bands.
Reporting: Compile the results into a comprehensive report, including chromatograms,calibration curves, and quantitative data.

HPTLC IN THE PHARMA INDUSTRY AND OTHER FIELDS Pharmaceutical Industry

1. **Drug Development:**
- **Purity Testing:** HPTLC is used to ensure the purity of active pharmaceutical ingredients (APIs) by detecting impurities and degradation products.
- **Formulation Analysis:** It helps in analyzing the composition of pharmaceuticalformulations, ensuring consistency and quality.
2. **Quality Control:**
- **Content Uniformity:** HPTLC is employed to verify the uniform distribution ofactive ingredients in tablets and capsules.
- **Stability Testing**: It is used to monitor the stability of drugs under variousenvironmental conditions, ensuring their efficacy over time.
3. **Regulatory Compliance:**
- **Validation:** HPTLC methods are validated to meet regulatory requirements,ensuring reliable and reproducible results.
- **Documentation:** Comprehensive documentation of HPTLC analysis is essentialfor regulatory submissions and audits.

Botanical and Herbal Products

1. **Identification and Authentication:**
- **Species Identification:** HPTLC is used to authenticate botanical species bycomparing their chromatographic fingerprints.
- **Adulteration Detection:** It helps in detecting adulteration and contamination inherbal products.
2. **Quality Assurance:**
- **Active Compound Analysis**: HPTLC quantifies active compounds in herbalextracts, ensuring their therapeutic efficacy.
- **Standardization:** It aids in the standardization of herbal products by establishingreference standards.

Food Industry

1. **Nutritional Analysis:**
- **Vitamin and Mineral Content:** HPTLC is used to analyze the vitamin andmineral content in food products.
- **Additive Detection:** It helps in detecting food additives and preservatives,ensuring compliance with food safety regulations.
2. **Contaminant Detection:**
- **Pesticide Residues:** HPTLC is employed to detect pesticide residues in fruits,vegetables, and other food products.
- **Mycotoxin Analysis:** It is used to identify and quantify mycotoxins, which areharmful fungal metabolites in food.

Forensic Science

1. **Toxicology:**
- **Drug Detection:** HPTLC is used to detect and quantify drugs and theirmetabolites in biological samples.
- **Poison Analysis:** It helps in the identification of toxic substances in forensicinvestigations.
2. **Document Examination:**
- **Ink Analysis:** HPTLC can analyze the composition of inks, aiding in theexamination of questioned documents.
- **Paper Analysis**: It is used to compare the chemical composition of paper samplesin forensic cases.

Cosmetic Industry

Quality Control

1. **Ingredient Verification:**
- **Authenticity**: HPTLC is used to verify the authenticity of natural ingredients in cosmetic products, ensuring they meet the required standards.
- **Purity Testing**: It helps in detecting impurities and contaminants in raw materialsand finished products.
2. **Consistency:**
- **Batch-to-Batch Consistency**: HPTLC ensures that each batch of cosmeticproducts maintains consistent quality and composition.
- **Stability Testing**: It is used to monitor the stability of cosmetic formulations overtime, ensuring their efficacy and safety.

Formulation Development

1. **Active Ingredient Analysis:**
- **Quantification**: HPTLC quantifies active ingredients in cosmetic formulations,ensuring they are present in the correct amounts.
- **Efficacy Testing**: It helps in evaluating the efficacy of active ingredients indelivering the desired cosmetic benefits.

2. **Complex Mixtures:**

- **Separation and Identification**: HPTLC is effective in separating and identifyingcomplex mixtures of ingredients in cosmetic formulations.
- **Optimization**: It aids in optimizing formulations by analyzing the interactionsbetween different ingredients.

Safety and Compliance

1. **Regulatory Compliance:**

- **Adherence to Standards**: HPTLC ensures that cosmetic products comply withregulatory standards and guidelines.
- **Documentation**: Comprehensive documentation of HPTLC analysis is essentialfor regulatory submissions and audits.

2. **Contaminant Detection:**

- **Heavy Metals**: HPTLC is used to detect heavy metals and other harmfulcontaminants in cosmetic products.
- **Microbial Contaminants**: It helps in identifying microbial contaminants,ensuring the safety of cosmetic products.

Research and Development

1. **Innovation:**

- **New Ingredients**: HPTLC is used in the research and development of new cosmetic ingredients, helping to identify their properties and potential benefits.
- **Product Improvement**: It aids in improving existing products by analyzing andoptimizing their formulations.

2. **Comparative Analysis:**

- **Benchmarking**: HPTLC allows for the comparison of different cosmeticproducts, helping to benchmark against competitors.
- **Market Research**: It provides valuable insights into market trends and consumerpreferences by analyzing popular cosmetic products.

Environmental Analysis

1. **Pollutant Monitoring:**

- **Water Quality:** HPTLC is used to detect pollutants and contaminants in watersamples.
- **Soil Analysis**: It helps in analyzing soil samples for the presence of pesticides andother environmental pollutants.

2. **Air Quality:**

- **Volatile Organic Compounds (VOCs):** HPTLC is employed to monitor VOCsin air samples, contributing to air quality assessments

Biotechnology

Genetic Engineering

Gene Expression Analysis:

- **Quantification:** HPTLC is used to quantify nucleotides and other smallmolecules involved in gene expression.
- **Purity Testing:** It helps ensure the purity of DNA and RNA samples used ingenetic engineering.

Recombinant Protein Analysis:

- **Protein Purity:** HPTLC is employed to analyze the purity of recombinantproteins produced through genetic engineering.
- **Impurity Detection:** It helps in detecting impurities and degradation products inprotein samples.

Microbial Biotechnology

Metabolite Profiling:

- **Secondary Metabolites:** HPTLC is used to profile secondary metabolites produced by microorganisms, which are often used in pharmaceuticals andagriculture.
- **Fermentation Monitoring:** It helps monitor the fermentation process byanalyzing the metabolites produced.

Strain Improvement:

- **Mutant Screening:** HPTLC is employed to screen for beneficial mutations inmicrobial strains used for industrial applications.
- **Genetic Stability:** It helps in assessing the genetic stability of microbial strainsover multiple generations.

Environmental Biotechnology

1. **Bioremediation:**

- **Pollutant Degradation:** HPTLC is used to monitor the degradation of pollutantsby microorganisms in bioremediation processes.
- **Enzyme Activity:** It helps in analyzing the activity of enzymes involved in thebreakdown of environmental contaminants.

2. **Waste Treatment:**

- **Effluent Analysis:** HPTLC is employed to analyze the composition of industrialeffluents before and after treatment.
- **Toxicity Testing**: It helps in assessing the toxicity of treated waste to ensureenvironmental safety.

Agricultural Biotechnology

1. **Pesticide Residue Analysis:**

- **Residue Detection**: HPTLC is used to detect pesticide residues in crops and soil,ensuring food safety.
- **Degradation Studies**: It helps in studying the degradation of pesticides in theenvironment.

2. **Plant Metabolomics:**

- **Metabolite Profiling:** HPTLC is employed to profile metabolites in genetically modified plants, aiding in the assessment of their nutritional and safety profiles.
- **Stress Response**: It helps in analyzing the metabolic response of plants to variousstress conditions.

Medical Biotechnology

1. **Pharmacokinetics:**

- **Drug Metabolism:** HPTLC is used to study the metabolism of drugs in biologicalsystems, providing insights into their pharmacokinetics.
- **Bioavailability:** It helps in assessing the bioavailability of drugs by analyzingtheir presence in biological fluids.

2. **Diagnostic Applications:**

- **Biomarker Detection:** HPTLC is employed to detect biomarkers in biologicalsamples, aiding in the diagnosis of diseases.
- **Pathogen Identification**: It helps in identifying pathogens by analyzing theirmetabolic profiles.

CONCLUSION :

The pharma industry has observed a rise in the use of HPTLC alone or in combination with other techniques such as FTIR, MSfor the determination of formulations and bulk drugs and flavonoids content in some leaf extracts of syzygiumcumini and phytocmecalscreeing and HPTLC fingerprinting of extracts of Thujaoxidentalis and some classical Ayurvedic Preparations. HPTLC is also being utilized magnificently in fields of biomedicine, biochemistry with a growing trend in its application in modern agriculture for estimating pesticide residues in fruits and vegetables. HPTLC is also often seen in pharma field and clinical research, analysis of medicinal plants and conventional medicines, analysis of food, feed, commodities, and dietary supplement, environmental, cosmetic, toxicological, and forensic aspects, herbal and plant analysis, and detection of free radical scavenging Activity. This HPTLC Method was developed

and validate and validated as per the guidelines OF ICH[53-60] The critical challenges concerned with column-based hyphenations are capital costs, approaches for coping with massive amounts of information produced by the application, which increases the complexity of instrumentation, and operational difficulties.

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