



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

HPTLC In The Pharma Industry

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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.

I. 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. High-performance thin layer chromatography (HPTLC) is a sophisticated analytical technique used in the pharmaceutical industry for a variety of purposes. 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 between TLC and HPTLC.

Table 1: Performance features of HPTLC and Thin layer chromatography

PARAMETER (Features of HPTLC versus classical TLC)	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	1-5 μl	0.1 -0.5 μl
Band loading	10-15mm	5-10 mm
Typical separation time	5-10 μl	1-4 μl
ographic plate used	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, increasing throughput and efficiency.
- **Safety:**
HPTLC is a risk-free method that allows for multiple detections without the need to repeat chromatograms, ensuring safety and reliability in results.
- **Stability:**
The method provides stable and reproducible results, which are crucial for quantitative analysis.
- **Comprehensive Analysis:**
HPTLC is effective for establishing purity, reverse engineering, detecting impurities, and fingerprinting of compounds.
- **Environmental Friendliness:**
The technique uses smaller amounts of solvents and reagents, making it more environmentally friendly compared to other methods.

- **Ease of Use:**
HPTLC is relatively easy to perform and does not require highly specialized equipment or extensive training.
- **Wide Applicability:**
It is suitable for both qualitative and quantitative analysis, making it a versatile tool in various 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 detection limits.
- **Plate Selection and Preparation:**
Plate Selection: Choose the appropriate HPTLC plate (e.g., silica gel, alumina) based on the nature of the analytes.
Plate Activation: Activate the plate by heating it in an oven to remove any adsorbed moisture.
- **Sample Application:**
Spotting: Apply the sample solutions onto the HPTLC plate using a micropipette or an automated 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 the polarity of the analytes.
Development Chamber: Saturate the development chamber with the mobile phase to ensure a uniform environment.
Development: Place the HPTLC plate in the chamber and allow the mobile phase to ascend 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 the analytes.
- **Documentation:**
Scanning: Scan the developed plate using a densitometer or a digital camera to record the 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 the spots or bands.
Reporting: Compile the results into a comprehensive report, including chromatograms, calibration curves, and quantitative data.

II. 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 pharmaceutical formulations, ensuring consistency and quality.

2. Quality Control:

- **Content Uniformity:** HPTLC is employed to verify the uniform distribution of active ingredients in tablets and capsules.
- **Stability Testing:** It is used to monitor the stability of drugs under various environmental 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 essential for regulatory submissions and audits.

Botanical and Herbal Products

1. Identification and Authentication:

- **Species Identification:** HPTLC is used to authenticate botanical species by comparing their chromatographic fingerprints.
- **Adulteration Detection:** It helps in detecting adulteration and contamination in herbal products.

2. Quality Assurance:

- **Active Compound Analysis:** HPTLC quantifies active compounds in herbal extracts, ensuring their therapeutic efficacy.
- **Standardization:** It aids in the standardization of herbal products by establishing reference standards.

Food Industry

1. Nutritional Analysis:

- **Vitamin and Mineral Content:** HPTLC is used to analyze the vitamin and mineral 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 are harmful fungal metabolites in food.

Forensic Science

1. Toxicology:

- **Drug Detection:** HPTLC is used to detect and quantify drugs and their metabolites in biological samples.
- **Poison Analysis:** It helps in the identification of toxic substances in forensic investigations.

2. Document Examination:

- **Ink Analysis:** HPTLC can analyze the composition of inks, aiding in the examination of questioned documents.
- **Paper Analysis:** It is used to compare the chemical composition of paper samples in 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 materials and finished products.

2. Consistency:

- **Batch-to-Batch Consistency:** HPTLC ensures that each batch of cosmetic products maintains consistent quality and composition.
- **Stability Testing:** It is used to monitor the stability of cosmetic formulations over time, 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 in delivering the desired cosmetic benefits.

2. Complex Mixtures:

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

• Safety and Compliance

1. Regulatory Compliance:

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

2. Contaminant Detection:

- **Heavy Metals:** HPTLC is used to detect heavy metals and other harmful contaminants 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 and optimizing their formulations.

2. Comparative Analysis:

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

• Environmental Analysis

1. Pollutant Monitoring:

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

2. Air Quality:

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

Biotechnology

• Genetic Engineering

• Gene Expression Analysis:

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

• Recombinant Protein Analysis:

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

• Microbial Biotechnology

- **Metabolite Profiling:**

- **Secondary Metabolites:** HPTLC is used to profile secondary metabolites produced by microorganisms, which are often used in pharmaceuticals and agriculture.
- **Fermentation Monitoring:** It helps monitor the fermentation process by analyzing the metabolites produced.
- **Strain Improvement:**
- **Mutant Screening:** HPTLC is employed to screen for beneficial mutations in microbial strains used for industrial applications.
- **Genetic Stability:** It helps in assessing the genetic stability of microbial strains over multiple generations.

- **Environmental Biotechnology**

1. **Bioremediation:**

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

2. **Waste Treatment:**

- **Effluent Analysis:** HPTLC is employed to analyze the composition of industrial effluents before and after treatment.
- **Toxicity Testing:** It helps in assessing the toxicity of treated waste to ensure environmental 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 the environment.

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 various stress conditions.

Medical Biotechnology

1. **Pharmacokinetics:**

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

2. **Diagnostic Applications:**

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

III. CONCLUSION

The pharma industry has observed a rise in the use of HPTLC alone or in combination with other techniques such as FTIR, MS for the determination of formulations and bulk drugs and flavonoids content in some leaf extracts of *Syzygium cumini* and phytochemical screening and HPTLC fingerprinting of extracts of *Thuja occidentalis* 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 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.

REFERENCES:

1. Attimarad M et al. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. *Pharm Methods*. 2011;2(2):71-75.
2. Joseph Sharma. Review of HPTLC in drug analysis: 1996-2009. *JAOAC Int*. 2010;93(3):754-764.
3. Srivastava M. An Overview of HPTLC: A Modern Analytical Technique with Excellent for Automation, Optimization, Hyphenation, and Multidimensional Applications. In: Srivastava M. (eds) *High-Performance Thin Layer Chromatography (HPTLC)*. Springer, Berlin, Heidelberg, New York. 3-24. 2011.
4. Sethi P. D., Sethi's HPTLC, High Performance Thin Layer Chromatography. Quantitative Analysis of Pharmaceutical Formulations. Volumes 1–3, CBS Publishers & Distributors, New Delhi, India. 2013.
5. Deval Rao G, A Text book of pharmaceutical analysis, Volume 2, Birla Publications, Shahdara, Delhi-110032:63-69. 2002.
6. Patel RB, Patel MR, Bhatt KK, Patel BG. Development and validation of an HPTLC method for determination of olanzapine in formulations. *JAOAC Int*. 2010;93:811–9.
7. Arup U, Ekman S, Lindblom L, Mattsson JE. High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist*. 1993;25:61–71.
8. Butler MS. Natural products to drugs: Natural product-derived compounds in clinical trials. *Nat Prod Rep*. 2008;25:475–516.
9. Gershell LJ, Atkins JH, 2003. A brief history of novel drug discovery technologies. *Nat Rev Drug Discov*. 2003;2:321-7.
10. Albert K, Krucker M, Glaser T, Schefer A, Lienau A, Zeeb D. Hyphenated techniques. *Anal Bioanal Chem*. 2002;372:25–6.
11. Sweedler JV. The continued evolution of hyphenated instruments. *Anal Bioanal Chem*. 2002;373:321–2.
12. Reich E, Schibli A. Stationary Phases for Planar Separations - Plates for Modern TLC. *LC GC*. 2015; 23:58–69.
13. CAMAG, Basic equipment for modern thin layer chromatography. Switzerland: Camag. Available : camag.com/downloads/free/brochures/CAMAG-basicequipment-08.pdf. 2010-2011.
14. Sudberg S, Sudberg EM, Terrazas J, Sudberg S, Patel K, Pineda J, et al. Fingerprint analysis and the application of HPTLC to the determination of identity and quality of botanicals, from an industry perspective. *J AOAC Int*. 2010;93:1367–75.
15. Arup U, Ekman S, Lindblom L, Mattsson JE. High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist*. 1993;25:61–71.
16. Patel PM, Patel KN, Patel NM, Goyal RK. Development of HPTLC method for estimation of charantin in herbal formulations. *Pharmacogn Mag*. 2010;2:224.
17. Wang J, Tang F, Yue Y, Guo X, Yao X. Development and validation of an HPTLC method for simultaneous

- quantitation of isoorientin, isovitexin, orientin, and vitexin in bamboo-leaf flavonoids. J AOAC Int.2010;93:1376–83.
18. Patel KG, Patel VG, Patel KV, Gandhi TR. Validated HPTLC Method for Quantitative Determination of Gallic Acid in Stem Bark of *Myrica esculenta* Buch.-Ham. Ex D. Don, Myricaceae. J AOAC Int.2010;93:1422–7.
 19. Chakraborty GS. Quantitative estimation of ascorbic acid by HPTLC in different varieties of amla. J Young Pharm, 2009;1:82.
 20. Jadhav VM, Kedar US, Gholve SB, Kadam VJ. Development and Validation of HPTLC Method for Determination of Glycyrrhizin in Herbal Extract and in Herbal Gel. Development. 2009;1:826–31.
 21. Patel RK, Kanani RJ, Patel VR, Patel MG. Development and Validation of HPTLC Method for Simultaneous Quantification of Vasicine and Piperine in *Vasavaleha*. Int J.2010; 2:14–7.
 22. Hong T, Jeong ML, Zahn M, Fay BA, Lee K, Hwangbo H, et al. Detection of the Potential Adulterant *Teucrium chamaedrys* in *Scutellaria baicalensis* Raw Material and Extract by High-Performance Thin-Layer Chromatography. JAOAC Int.2009;92:785–8.
 23. Pagi KB, Lahiri SK, Yadav GK, Shah MB. Development and Validation of HPTLC Method for Determination of Betulinic Acid in *Helicteres isora* root Extract Development. 2010;2:851–5.
 24. Gertrud E Morlock, Petar Ristivojevic, Elena S Chernetsova. Combined multivariate data analysis of high-performance thin-layer chromatography fingerprints and direct analysis in real time mass spectra for profiling of natural products like propolis. J Chromatogr A. 2014;1328:104–12.
 25. Morlock G, Schwack W. Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/MS and HPTLC-DART/MS. Anal Bioanal Chem. 2006;385:586–95.

