

# High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery

## Abstract

Analysis of pharmaceutical and natural compounds and newer drugs is commonly used in all the stages of drug discovery and development process. High-performance thin layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on.

**Key words:** Drug discovery, HPTLC, natural products, planar chromatography.

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## INTRODUCTION

Science and technology have never been so promising nor have delivered so many opportunities to improve health and extend lives, but continued investments are being invested in both the public and private sector, in spite of the current economic climate.<sup>[1-3]</sup> Increasing pharmaceutical industry success rates and delivering more medicines are very challenging, but very few predictive scientific and analytical tools are available.<sup>[4]</sup>

Research on drugs involves production control of bulk drug and final product, toxicological analysis of side effects of the drug or its possible impurities, and determination of the fate of a drug and its metabolites in an organism by the monitoring of body fluids.<sup>[5]</sup> Common criteria for drug evaluation include the quality and therapeutic value of the bulk drug and pharmaceutical product, identification studies, purity, content, uniformity, chemical and physical stability, and biological availability.<sup>[6,7]</sup>

## PHARMACEUTICAL ANALYSIS AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY—AN OVERVIEW

Analysis of pharmaceutical compounds and newer drugs is commonly used in all the stages of drug discovery and development process. These analytical techniques provide more accurate and precised data, not only supporting drug discovery and development but also postmarket surveillance.<sup>[8]</sup> Pharmaceutical analysts work regularly to improve the reliability of existing techniques to cope up the demands for better chemical measurements. Modern pharmaceutical analysis is mainly dominated by costlier instrumental analysis. Hence, many analysts' focus is on developing newer applications, discoveries, and new methods of analysis to increase the specificity and sensitivity of a method.<sup>[9,10]</sup>

Analytical methods used in drug analysis are diversified and are still being improved to find better solutions to satisfy manufacturers and institutions

that test drug quality. Official documents dealing with the problem of QC of pharmaceutical products recommend diversified analytical techniques, with chromatographic methods playing a significant role in pharmaceutical analysis.<sup>[11]</sup>

Thin layer chromatography studies are among the key identity tests in most pharmacopoeial monographs. Pharmacopoeial standards are typically used by industry as a basis for meeting QC requirements and current good manufacturing practices (cGMPs). An extension of TLC is high-performance thin layer chromatography (HPTLC) is robust, simplest, rapid, and efficient tool in quantitative analysis of compounds. HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. Some of the enhancements such as the use of higher quality TLC plates with finer particle sizes in the stationary phase which allow better resolution.<sup>[12]</sup> The separation can be further improved by repeated development of the plate, using a multiple development device. As a consequence, HPTLC offers better resolution and lower Limit of Detection (LODs).

Visual detection is suitable for qualitative analysis, but a more specific detection method is needed for quantitative analysis and for obtaining structural information on separated compounds. UV, diode-array and fluorescence spectroscopy, mass spectrometry (MS), Fourier-transform infrared (FTIR), and Raman spectroscopy have all been applied for the *in situ* detection of analyte zones on a TLC plate. Van Berkel and coworkers have recently described couplings of TLC to atmospheric pressure chemical ionization<sup>[13]</sup> and electrospray ionization.<sup>[14-16]</sup> In both couplings, a special surface sampling probe is used for extracting the analyte on-line from the TLC plate to MS analysis.

The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography.<sup>[17]</sup>

One of the available chromatographic techniques is HPTLC, which is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, high-resolution sorbents with

selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization all make HPTLC an important alternative method to HPLC or gas chromatography. Specifically, HPTLC is one of the ideal TLC technique for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies are also the most appropriate TLC technique for conformity with GMPs.<sup>[18]</sup> Today the comprehensive use of TLC in pharmaceutical analysis is demonstrated by the great number of articles published in this field.<sup>[19-30]</sup>

HPTLC remains one of the most flexible, reliable, and cost-efficient separation technique ideally suited for the analysis of botanicals and herbal drugs. Used with standardized procedures, it guarantees reproducible results, a vital element in the routine identification of complex fingerprints of plant extracts and pharmaceutical products.<sup>[19]</sup> It has established itself as the method of choice for handling complex analytical tasks involving herbal drugs and botanicals. The unique combination of state-of-art instrumentation, standardized procedures, and solid theoretical foundations enables it to deliver reliable, cGMP-compliant results time after time.

High-throughput analysis using HPTLC is being aimed at the rapid analysis of large numbers of compounds. This field has been expedited by the requirement to provide analytical support for multiple drug targets emerging from the field of molecular biology, human genetics, and functional genomics. Further, drivers for development have been in the support for the analysis of large compound libraries arising from parallel and combinatorial chemistry, and economic pressure to reduce time-to-market for new drug candidates.<sup>[20]</sup>

## APPLICATIONS OF HPTLC

HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environmental analysis, and other areas. It is due to its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results as an image. Other advantages include simplicity, low costs, parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility of

multiple detection. Le Roux *et al*<sup>[31]</sup> evaluated a HPTLC technique for the determination of salbutamol serum levels in clinical trials and established as a suitable method for analyzing samples from the serum. Many lipids have also been analyzed and studied using HPTLC; 20 different lipid subclasses were separated using HPTLC with the reproducible and promising results. Many reports on studies related to clinical medicine have already been published in many journals. HPTLC is now strongly recommended in the analysis of drugs in serum and other tissues.<sup>[32]</sup>

## HPTLC IN PHARMACEUTICAL PRODUCTS

HPTLC is also used in analyzing the purity and efficacy of many pharmaceutical preparations and dosage forms. Puranik *et al* developed and validated a simple, rapid, and accurate chromatographic methods (HPLC and HPTLC) for simultaneous determination of ofloxacin and ornidazole in solid dosage form. The amount of ofloxacin and ornidazole estimated as percentage of label claimed was found to be 100.23 and 99.61% with mean percent recoveries 100.47 and 99.32%, respectively. Both these methods were found to be simple, precise, accurate, selective, and rapid and could be successfully applied for the determination of pure laboratory prepared mixtures and tablets.<sup>[33]</sup>

A relatively fast, simple, and accurate method has been established for analysis of celecoxib, etoricoxib, and valdecoxib in pharmaceutical preparations. Małgorzata Starek *et al* reported that the procedure can be readily used for selective analysis of drugs, and repeatable results are obtained without interference from auxiliary substances.<sup>[34]</sup> Similarly, HPTLC method was successfully used to analyze fixed-dose tablets samples of lamivudine, stavudine, and nevirapine.<sup>[35]</sup> Two simple, accurate, and precise HPTLC methods have been established for the determination of mexiletine hydrochloride, an antiarrhythmic agent, in Mexicord capsules. The established methods are in accordance in terms of linearity, accuracy, precision, sensitivity, and specificity.<sup>[36]</sup>

Patel *et al* developed a simple and rapid HPTLC method and validated for quantitative determination of olanzapine on silica gel 60F254 layers using methanol-ethyl acetate (8.0 + 2.0, v/v) as the mobile phase. The developed method was found to be simplest among existing analytical methods.<sup>[37]</sup> A sensitive, simple, selective, precise, and accurate

HPTLC method of analysis for paracetamol, diclofenac potassium, and famotidine both as a bulk drug and in tablet formulation was developed and validated.<sup>[38]</sup>

A novel HPTLC method has been developed and validated for quantitative determination of omeprazole in capsule dosage form. The method was validated according to the International Conference on Harmonization guidelines for accuracy, precision, linearity, specificity, and robustness. The method proposed can be used for QC and stability testing of different dosage forms such as tablets and capsules, as well as for bulk drug analysis of omeprazole.<sup>[39]</sup>

A new, simple HPTLC method for determination of etoricoxib and thiocolchicoside in combined tablet dosage form has been developed and validated. The pharmaceutical dosage form used in this study was Nucoxia-MR tablets. The method was validated with respect to linearity, accuracy, precision, and robustness in accordance with the International Conference on Harmonization guidelines. The method has been successfully applied to the analysis of drugs in the pharmaceutical formulation.<sup>[40]</sup>

## HPTLC IN NATURAL PRODUCTS

The HPTLC technique is rapid, comparatively simple, robust, and extremely versatile. HPTLC not only confirm but also establish its identity. It is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability. A simple and reproducible method using HPTLC was successfully performed for the quantitative analysis of above diterpenoids in the root bark of *Photinia integrifolia*. In which Diterpenoids 1 $\beta$ ,3 $\alpha$ ,8 $\beta$ -trihydroxy-pimara-15-ene (A), 6 $\alpha$ ,11,12,16-tetrahydroxy-7-oxo-abieta-8,11,13-triene (B) and 2 $\alpha$ ,19-dihydroxy-pimara-7,15-diene (C) were used as chemical markers for the standardization of *Photinia integrifolia* plant extracts.<sup>[41]</sup>

A simple HPTLC method has been developed for the simultaneous determination of isoorientin, isovitexin, orientin, and vitexin, both pure and in commercial samples of bamboo-leaf flavonoids. It was found that HPTLC is a simple, precise, specific, and accurate and can be used for manufacturing QC of bamboo-leaf flavonoids or for governmental regulatory purposes.<sup>[42]</sup>

Many such reports<sup>[43-49]</sup> present the evidence of utilization of HPLTC in fingerprinting analysis of drugs of natural origin, and hence, the increasing

acceptance of natural products is well suited to provide the core scaffolds for future drugs; there will be further developments in the use of novel analytical techniques in natural products drug discovery campaigns.

## HPTLC IN OTHER FIELDS

In recent years, HPTLC is a globally accepted practical solution to characterize small molecules in quality assessment throughout the developing world. HPTLC is used for purity control of chemicals, pesticides, steroids, and water analysis.<sup>[50]</sup> HPTLC is also widely used for analysis of vitamins, water-soluble food dyes, pesticides in fruits, vegetables, and other food stuffs.<sup>[51]</sup> Beate *et al.*<sup>[52]</sup> reported the analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS. HPTLC is useful in detecting chemicals of forensic concern,<sup>[53,54]</sup> including abuse drugs, poisons, adulterations, chemical weapons, and illicit drugs.

## CONCLUSIONS

Applications of HPTLC for 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 made HPTLC a power analytical tool in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, Bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future.

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