
Analytical Technique HPTLC For Herbal Analysis

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ABSTRACT

In herbal analysis, High-performance thin layer chromatography (HPTLC) is one of the useful analytical techniques for qualitative and quantitative analysis. It was developed to permit more precise auto spotting of the sample. HPTLC is one of the modern analytical technique by which quality of product can be authenticate in quality control. It is economic and reliable separation identify. It is most flexible, reliable and cost efficient separation technique, simultaneous processing of sample and standard –better analytical precision and accuracy no need internal standard. The advantage of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be powerful analytical tool for chromatographic information of complex mixtures of herbal product. This article updates the recent information about the HPTLC method development and validation for herbal product and its importance.

Keywords: HPTLC, Herbal product, Method development.

INTRODUCTION

HPTLC is very useful qualitative analysis method it combines the art of separation and identification with quickness at a low cost. It is moderate form of TLC. HPTLC Analytical technique is a powerful analytical tool which is equally suitable for qualitative and quantitative analytical work. It is playing an important role in today analytical world not as the level of HPLC but as complementary method. Herbal analysis done as per the WHO, according to there is three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal medicines are complex chemical mixtures obtained from a plant which is widely used in health-care in both developed and developing countries. The standard of herbal product authenticate for that quality assurance of herbal medicine is an important factor and basic requirement for herbal drug industry and other drug development organization. A great majority of drugs in modern medicine have been analysed and their

authenticity confirmed by chemical and instrumental analysis. Often the quality control and quality assurance of the drugs are confirmed by these tests [1-5].

One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae, is extracted with boiling water during the decoction process. This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug.

Unlike other methods, HPTLC produces visible chromatograms complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, so that reference and test samples can be compared for identification [6-7].

HPTLC is one of the sophisticated instrumental techniques based on the full capabilities of TLC. It is most flexible, reliable and cost efficient separation technique. The advantage of automation,

scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation with instrument for better quantification, and so on enable it to be powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples and food stuffs. The advantages of using TLC/HPTLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal products. It is worth noting that the new techniques of TLC are also being updated like forced-flow planar chromatography (FFPC), rotation planar chromatography (RPC), over pressured-layer chromatography (OPLC), and electro planar chromatography (EPC). A simple, but powerful preparative forced-flow technique was also reported, in this technique hydrostatic pressure is used to increase mobile-phase velocity [8-10].

Phyto-chemical standardisation also performed and generates data according to WHO. Phyto-chemical evolution for standardisation established the following [11-13].

Preliminary testing for the different functional group, Quantification of active constituent and important groups e.g. (total alkaloid, total tannins, total phenolic and total triterpenic acid), Establishment of fingerprint profile, Multiple marker-based fingerprint profiles.

The problems affect the quality of herbal product- Herbal products usually available in mixture of many constituents, not proper information of active constituents in most cases,

Commercially reference compound are not available for analytical evaluation, Plant materials are chemically and naturally variable, the source and quality of the raw material are variable.

Method of harvesting, drying, storage, transportation and processing are also affect.[11-13].

Phytochemical evolution done by HPTLC due to- Perform rapid analysis of herbal extract with minimum sample clean up requirement, gives qualitative and semi quantitative information of the resolved compound, Provide quantitative measurement of chemical constituents with the help of spectroscopy detector system.

In TLC fingerprinting the data that can be recorded using a high-performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (R_f) values, the colour of separated bands, their absorption spectra λ_{max} and shoulder inflection of all the resolved bands. All of these together with the profiles on derivatization with different reagent represent TLC the fingerprint of the sample. The information so generated has a potential in the application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug.

HPTLC have Important quality which makes different from other analytical technique these are like, Sample and standard can be analysed simultaneously with better analytical precision and accuracy no need internal standard, Lower analysis time and low cost affect make easy for the work to perform more than one scientist at a time, Routine analysis cost low –Simple sample preparation, no need to do filtration and degassing for the solvent and consumption of mobile phase per sample is very low, Always fresh stationary and mobile phase for each analysis –zero contamination, Scanning and quantification can be done by using scanner like UV/Visible/ Fluorescence and advanced type of densitometer.

PRACTICAL PROCEDURE IN HPTLC

Sample, Standard and Stationary phase, information of chemistry about the sample, standard and stationary phase is completed from the literature. Knowledge of functional group, pk-value and solubility information is helpful for the selection of stationary phase.

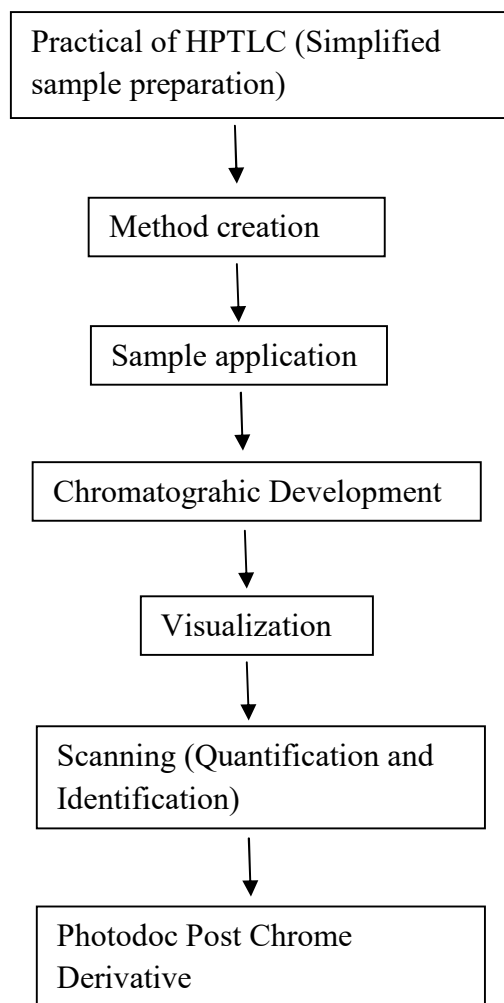


Figure 1: Schematic Diagram Of HPTLC Method For Separation

Preparation and Dilution of Sample, Standard and Conditioning of HPTLC Plate

For the development of chromatogram, it is necessary to develop LOD which can achieve by

low ratio of signals to noise in straight base line conditioning of plate of can be done at temperature 100-120°C around 20-30 mins. Proper selection of mobile phase and TLC chamber saturation help to get develop precise and authentic chromatogram.

Application Of Sample And Standard

Sample application play very important role for the separation of peaks in chromatogram. For this analyst need to select low quantity of sample usually concentration range is 0.1 -1 µg/µl. Over loading of sample gives poor separation.

Chromatographic Development

Development of chromatogram needs precaution and skill to handle the HPTLC. Removing of mobile phase and prevent from lab atmospheric contamination drying the HPTLC plate in vacuum desiccator. Also automatic developing chamber is use to develop better chromatogram.

Detection and Visualization Of Spots

In this step selection of detecting and quantification is depended upon the chemical nature of analyte. TLC and HPTLC are the only technique where the samples are visible throughout the chromatographic procedure. Under UV light condition different analytes detection is done and it is first choice - non-destructive. Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length) · Spots of non-fluorescent compounds can be seen - fluorescent stationary phase is used - silica gel GF · Non UV absorbing compounds like ethambutol, dicylomineetc - dipping the plates in 0.1% iodine solution · When individual component does not respond to UV - derivatisation required for detection. After development of chromatogram the sample and reference compound on same plate are quantified by using standard calibration method by using a standard instrument. Calibration of single and multiple levels with linear and non-linear

regression are possible. The preferred choice of method of calibration in HPTLC is external method.

Documentation Of Chromatogram

E - Merck introduced plates with imprinted identification code - supplier name. Item number, batch number and individual plate number - Avoid manipulation of data at any stage - coding automatically get recorded during photo documentation [15-17].

VALIDATION OF ANALYTICAL METHOD

All validation parameters such as precision, accuracy, LOD, LOQ, Ruggedness,

Robustness can be performed as according to WHO guidelines.

DISCUSSION

Routine exercise of applying modern technologies to quality control of the products that have been in constant use for centuries. Development of HPTLC in modern era which is play very important role on quality control of herbal medicines. Of course, the proposal of the use of chromatographic fingerprints of herbal medicines for quality control of herbal medicines is definitely a progress for this HPTLC is best option. However, using the chemical fingerprints for the purpose of quality control of herbal medicines can only address to the problem of comparing the integrated sameness and/or difference and controlling their stability of the available herbal products.

CONCLUSION

By HPTLC study the possible work on contamination study in herbal products, such as excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, and other also. The research field of quality control of herbal medicines is really an interdisciplinary research. It require crossover of chemistry,

pharmacology, medicine and even statistics to provide a platform for the quality control of traditional herbal medicines and further to discover the novel therapeutics composed of multiple chemical compounds.

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