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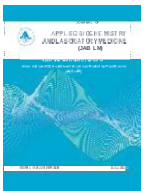

Newer developments

High performance thin layer chromatography (HPTLC)

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ABSTRACT

Both thin layer chromatography (TLC) and High-performance liquid chromatography (HPLC) are well known laboratory procedure for separating different analytes. Combination of both principles using recent software advances has led to development of a much more efficient high-performance thin layer chromatography (HPTLC) technique that is both more accurate and time efficient. The principles and techniques involved in this recently developed technique is discussed briefly in this review article.

INTRODUCTION

Thin layer chromatography (TLC) is the technology on board making sound advancement in everyday laboratory practices. The TLC machines that are a staple in most laboratory setups today, have paved a way to a newer methodology that is consistently rising in demand. The High-performance thin layer chromatography (HPTLC) is a form of thin layer chromatography. It takes into its stride the most recent advancement of software-based interpretation thus reducing human errors that may occur time and again. The sampling technique is accurate and along with it is a chromatogram that is standardised and can be duplicated without leaving room for error. HPTLC is a new method whose adaptation in modern laboratories will only further the advancement of the efficacy of laboratory practices of the day. A brief overview of the HPTLC method will be discussed in this article.

PHASES:

Stationary Phase:

It is the part of the column that interacts with the target molecule. HPTLC has a set of plates which have smooth layers due to a homogenous distribution of small particles. The HPTLC plates are only about 10x10 or 10x20cm. In the usual TLC, majority of the molecules are analysed using silica gel with a less polar mobile phase such as chloroform-methanol. Lipophilic chemically-modified silica gel phases with more polar aqueous mobile phases such as methanol-water are used for reversed-phase TLC. These plates improve the resolution and the sensitivity of detection. In addition to biochemical and clinical sciences, they are creeping into the pharmaceutical industry for various drug quantitative analysis too.

Mobile Phase:

This phase is the solvent that dissolves the target molecule. The mobile phase of the thin layer chromatography is based on the analyte's properties. The physical and chemical properties of the analyte and the stationary phase's composition are the two key factors for the choice of mobile phase. Chloroform, methylene chloride, diethyl ether are the proposed materials of the mobile phase, and these are used on an individual capacity or in combination with hexane in normal phase TLC. If the instrument uses reversed-phase TLC, tetrahydrofuran, acetonitrile or even methanol mixed with water can be used for strength adjustment.

For example, a reliable and quick way of estimation of cholesterol content was achieved by using the HPTLC method. The mobile phase consisted of chloroform mixed with methanol (9.5:0.5 v/v) while the stationary phase consisted of an aluminium-backed precoated silica gel.

PROCESS:

The entire process of HPTLC method consists of the following

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1. Prewashing

The plates for HPTLC have to be used in a way that there is no contamination by finger tips. The layers of the plate are pre washed with 20 ml methanol. This allows for quantitative analysis and reproduction of the result. The plates are cleaned and dried for about 20 minutes and at 120 degrees in a hot air oven. The plates are also equilibrated with the relative humidity and temperature of the inner atmosphere of the laboratory, away from fumes and dust.

2. Plate preparation

Precoated plates are usually made of aluminium or glass, with glass providing a superior quality of results. On the other hand, aluminium plates are cheaper and better suited for transport. Precoated layers are uniformly thick, resistant to scratches and are reproducible. Pre-coating is mostly done with high molecular weight polymer.

3. Sample application

The sample application process has to be precise and it is either by spot application or by spraying. The end result usually depends on the expertise of sample application and handling. Fixed volume spot application is the simplest method of application. For HPTLC, it is usually up to 1uL of volume per spot application. Larger volumes of samples can be dealt with by using spray on technique. The spray on techniques focuses the sample into narrow bands.

4. Chromatogram development

There is the presence of a gas phase along with mobile and stationary phases. The process of separation is influenced by this gas phase. The lower end of the plate has to be immersed and capillary action causes the movement upwards to the desired distance. The reproducibility of the chromatographic results depends on whether the parameters involved are always constant. Since there is a non-equilibrium relationship between the mobile, stationary and gas phases, the ultimate consequence of the chromatography cannot always be predicted. The contour of the chamber and the level of saturation always plays an important role as the mediator of the result. The developing chamber is dependent on many factors. The choice has to be economically viable. Horizontal Developing Chambers are economically viable, and give reproducible results.

There is an automated spraying device called the Derivatizer which sprays micro droplets onto the TLC plates. It is designed so that there is reproducible and homogenous spraying and application of reagents. There is a gradient of nozzles which can determine the properties of the spray according to the operator. The Derivatizer claims to be economical, environmentally friendly, with lower reagent consumption and user independent results.

The chromatogram thus obtained is evaluated under the UV light or white light. The results may be evaluated by the user or maybe by specific software. The chromatogram can be evaluated by the monochromatic light of densitometry or electronic image acquisition. To achieve high precision of evaluation, there are various requirements:

- The HPTLC plates used should be pre-made; this ensuring homogeneity and even distribution
- Sample application, if automatic and spray on method, removes individual human errors of measurement and application.
- The chamber used should be should be properly chosen so as to have reproducibility of the results.
- The evaluation software should be thoroughly calibrated keeping in mind fluorescence/adsorption properties of the substances used.
- The light, respective wavelengths and measurement parameters need to optimised.

Lastly, the electronic images need to saved and archived for comparison with other images, educational purposes and medicolegal documentation as well.

HOW IS IT DIFFERENT FROM HPLC?

HPLC is a type of reversed chromatography where the stationary phase is liquid and there is no conditioning phase. HPTLC, on the other hand, is a straight phase chromatography with a solid stationary phase and a gaseous conditioning phase. HPLC has a closed chromatography system and has a better resolution as compared to HPTLC. The most important advantage of HPTLC over HPLC is that it requires much less processing time and is capable of running up to 100 samples in parallel without requirement of high pressure or temperature. HPLC, however, can run just one sample at a time and is quite time-consuming. Post-chromatography derivatization is easier and valuable with HPTLC. Another important advantage of HPTLC is that it warrants less analytical skill on the part of the operator and is also less expensive as compared to HPLC.

APPLICATIONS:

HPTLC is being increasingly used in the pharmaceutical industry for the analysis of solutions for determining drug composition, quality control of manufactured drugs, impurity testing and also for the identification and characterization of herbal materials. In clinical chemistry, HPTLC can be used for the determination of lipid fractions, analysis of analytes for inborn errors of metabolism, quantification of metabolic products/enzymes in disorders of metabolic processes, doping tests and drug screening/drug level testing. HPTLC is also being used in the forensic investigations for dyestuff analysis, detection and identification of unknown poisons and forgery of documents.

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