

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY WITH MASS SPECTROMETRY: A REVIEW

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Abstract: HPTLC is a most versatile technique and is known for uniformity, purity profile, assay values and precision and accuracy of results. It can handle several samples of even divergent nature and composition. HPTLC is a modern analytical separation method with extensive versatility, although already much utilized, is still with great potential for future development in research and development. It is accepted as a time-saving and most economical machine practically with minimum trouble shootings. It speeds up analysis work which is usually not possible with other parallel chromatographic techniques available. The scope of hyphenation of HPTLC with other analytical techniques appears to hold considerable promise for the analysts who previously have had reservation towards the use of planar chromatography. Its hyphenation with mass/infra-red/laser spectroscopy, etc. opens a new dimension which makes it the most prestigious among the analytical chemists in the present perspective. HPLC-MS is more preferred method for separation and identification of compounds. But disadvantage of HPLC is that it requires more solvent as compared to HPTLC. This technique provides efficient, quick and simple method for identification and separation of Narcotic drugs and psychotropic substances. Therefore, taking advantage of less solvent requirement in HPTLC and also to enhance working hyphenation of both HPTLC and MS is done so as to provide wide scope for separation as well as identification of product within short period of time. The great advantage of the instrument is that exclusively questioned zones are transferred into the MS for identification and that within less than one-minute sensitive mass spectrometric information is available.

Keywords: HPTLC-MS, Technique, Analytical separation, applications.

INTRODUCTION

The science of analytical chemistry can be described in simplified terms as the process of obtaining knowledge of a sample by chemical analysis of some kind. The sample under investigation may consist of any solid, liquid or gaseous compound and the result of the analysis is data of some kind that is related to the initial question raised about the sample. From the data obtained in the analysis some knowledge about the sample can be extracted. This knowledge may be either qualitative or quantitative.^[1]

Examples of qualitative information are types of atoms, molecules, functional groups or some other qualitative measure, while the quantitative information provides numerical information such as the content of different compounds in the sample.^[2] Nowadays an analytical chemical analysis generally includes some sort of analytical instrument that performs the actual analysis, while the data processing and instrument control are taken care of by software run on a computer. Hence it is no exaggeration to say that analytical chemistry has become computerised. The shape of the data of analytical chemical analyses has, moreover, changed. From a single sample it is now possible after a very short period of analysis to obtain enormous amounts of data.^[3]

By means of techniques like ultraviolet-visible (UV-Vis) spectroscopy, fluorescence spectroscopy, infrared (IR) spectroscopy, near infrared (NIR) spectroscopy, Raman spectroscopy, mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR), High performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) large amounts of data on a sample can be collected in a short period of time.^[4]

Chemical analysis is an essential component in allowing a laboratory to ensure routine acceptable performance of analytical methods. Despite the considerable amount of important published work on this subject, diversity still prevails in the employed methodologies because validation of an analytical method depends on the specific purpose of that method.^[5] This can lead to difficulties in validation approaches and the interpretation of results. Aiming to assist in the planning of validation methods, we

discuss relevant approaches of various parameters in quantitative high-performance thin layer liquid chromatographic methods and validation fields in pharmaceutical analysis. Moreover, this article provides full review on HPTLC method development that should be useful as an introduction to analytical validation for practical applications in academic research or the industrial sector.^[6]

Principle of HPTLC

The HPTLC works on the same principles as TLC such as the principle of separation is adsorption. The mobile phase or solvent flows through the capillary action. The analytes move according to their affinities towards the stationary phase (adsorbent). The higher affinity component travels slower towards the stationary phase. A low-affinity component travels rapidly toward the stationary phase. On a chromatographic plate, then, the components are separated.^[7]

The specific intermolecular interactions between the molecules of a sample and the packing material define their time “on-column”. Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved.^[8]

Mass spectrometry

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios by using the one of a variety of techniques (e.g EI/CI/ESI/APCI/MALDI).^[9]

Mass spectrometry principle

Mass spectrometry (MS) is an analytical technique that separates ionized particles such as atoms, molecules, and clusters by using differences in the ratios of their charges to their respective masses (mass/charge; m/z), and can be used to determine the molecular weight of the particles.^[10]

HPTLC with TENDEM MASS SPECTROMETRY

THE direct coupling of TLC/HPTLC with mass spectrometry is of particular interest because of the latter’s high sensitivity, rapid analysis, and ability to aid structural characterization. TLC-MS is a versatile technique for separation as well as identification of pharmaceuticals and phytopharmaceuticals. Traditionally the separation was carried out by TLC/HPTLC then the separated materials was removed and then identified by mass spectrometry. This technique provides efficient quick and simple method identification and separation of narcotic drugs and psychotropic substances.^[11]

The use of high-performance thin layer chromatography in combination with high resolution time of flight mass spectrometry for the detection, identification and imaging resulted in increase in its analytical importance. It has been successfully hyphenated with HPLC, MS, FTIR and Raman spectroscopy to give far more analytical data on separated compounds.^[12]

Dr. Luftmann developed HPTLC-MS hyphenation. The technique is divided into elution based and desorption based. Elution based; analyte on the plate is first scraped, extracted, purified and concentrated, then transferred in the liquid phase to mass spectrometer ion source for further analysis. Desorption based; analyte is vaporized from the silica, and transferred to the mass spectrometer in the gas phase. But these days substance of interest is eluted directly from the HPTLC plates and transferred online to the mass spectrometer.^[13]

Key feature

- HPTLC-MS is a cost-effective because the chromatographic run is decoupled with the detection step.
- Rapid and contamination- free elution of selected zones.
- Online transfer into the mass spectrometer.
- Advantages of HPTLC include that the technique is simple to learn, operate, several analysis works could be done on same time, is a fast and economic technique.
- Thin layer chromatography/high performance thin layer chromatography can be used interchangeably for methods developed in the twenty-first century.^[14]

HPTLC-MS Principle

- The versatile instrument is used to isolate unknown compounds form a HPTLC/TLC plate and transfer them into a mass spectrometer for identification or structure elucidation.
- TLC/MS interface can be brought together to any brand of LC coupled mass spectrometer.
- Plug and play installation by two HPLC fittings at a given HPLC-MS system.
- Semi-automation instrument involving automatic piston movement for pressure seal the HPTLC/TLC zone on both glass plates and aluminium foils take out directly from the plate using a suitable solvent delivered by the HPLC/HPTLC pump online transfer into the mass spectrometer.
- Automatic cleaning of the piston between the extraction.^[15]

INSTRUMENTATION

It consists of;

- Double three-way diverter in line with an autosampler
- Lc system
- Mass spectrometer
- The diverter generally operates as an automatic switching valve.^[16]

SAMPLLE PREPRATION AND APPLICATION

A good solvent system is one that moves all components of the mixture off the baseline but does not put anything on the solvent front. (Fig 1)

The peaks of interest should be resolved between R_f 0.15 and 0.85. Pharmaceutical preparation with sufficiently high concentration of analyte is simply dissolved in a suitable solvent that will completely solubilize the analyte and leave excipients undissolved to yield a test solution that can be directly applied on HPTLC plate.

Solvent used for dissolving the sample can be ethanol, methanol, chloroform N-hexane etc. ^[17]

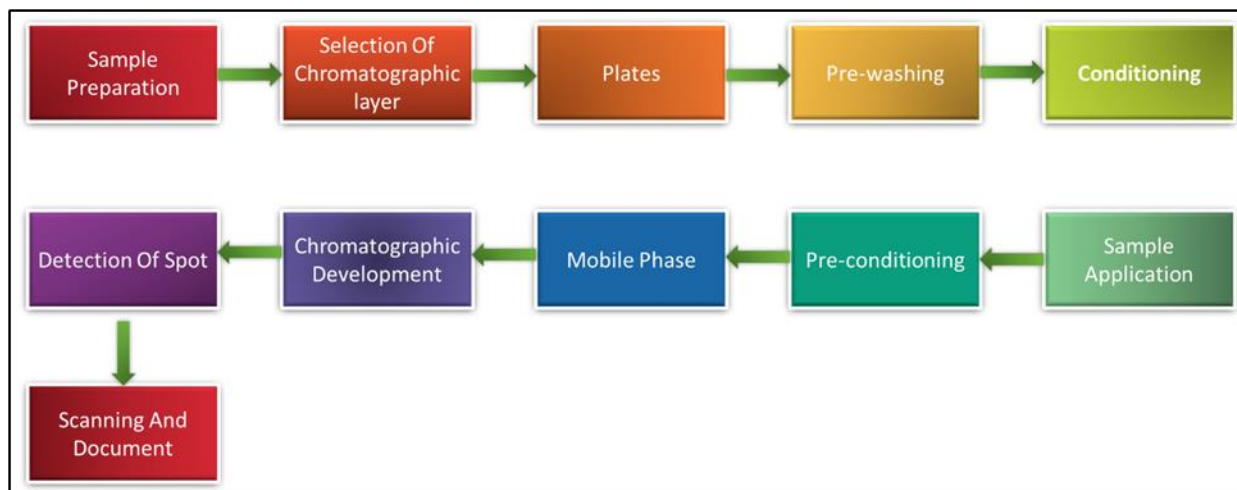


Fig 1. Steps involved in sample preparation.

HPTLC-MS Plates

The techniques for coupling TLC with mass spectrometry can be divided into elution-based, or desorption based. Both approaches are offline, and are performed after the separation is completed and the plate dried. Sample transfer to the mass spectrometer is fast and typically takes less than one minute.^[18]

Elution-based TLC-MS; The analyte on the silica plate is dissolved in a solvent and transferred to the mass spectrometer in the liquid phase.

Desorption-based TLC-MS; the analyte is vaporized from the silica, and transferred to the mass spectrometer in the gas phase. Vaporization techniques include gas beam, ion bombardment, and MALDI.^[19]

Features and benefits

- Enhanced sensitivity
- Extremely low background signal
- Trace analysis in nanogram range
- Flexible choice of mobile phases^[20]

PRE-WASHING

Plates need to be washed to be remove water vapours or volatile impurities. The plates are cleaned by the methanol, chloroform; methanol, ammonia solution 1%.

CONDITIONING

The pre-washed plates are placed in oven at 120 for 15-20min. this process is known as conditioning.^[21]

SAMPLE APPLICATION

The selection of sample application technique and device to be used depends on;

- Sample volume
- No. of sample to be applies

The sample should be completely transferred to the layer. Micro syringes are preferred if automatic application devices are not available. Volume recommended for HPTLC-0.5-5microliter. Sample spotting should not be excess or not low. Problem from overloading can be overcome by applying the sample as band.

Sampling application used for spotting are;

- Capillary tube
- Micro bulb pipette
- Micro syringe
- Automatic sample applicator

PRE – CONDITIONING

For low polarity mobile phase there is no need of chamber saturation. However saturation is needed for highly polar mobile phase. Time required for the saturation depends on the mobile phase.

If plates are introduced into the unsaturated chamber, during the course of development, the solvent evaporates from the plate mainly at the solvent front and it results in increased Rf values.

MOBILE PHASE

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte.

General mobile phase systems that are used based on their diverse selectivity properties are diethyl ether, methylene chloride and chloroform combined individually or together with hexane as the strength-adjusting solvent for normal-phase TLC. Methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC.

MOBILE PHASE

- Volume smaller than 1ml are measured with a suitable micropipette.
- Volume upto 20ml are measured with a graduated volumetric pipette of suitable size.
- Volume larger than 20ml are measured with a graduated cylinder of appropriate size.
- To minimize volume errors, developing solvents are prepared in a volume that is sufficient for one working day.^[22]

CHROMATOGRAPHIC DEVELOPMENT

Ascending, descending, horizontal. Plates are spotted with sample and air dried and placed in the developing chambers. After the development plate is removed from chamber and mobile phase is removed under fume cup-board to avoid contamination of laboratory atmosphere.

The plates should be always laid horizontally because when mobile phase evaporates the separated components will migrate evenly to the surface where it can be easily detected.

DEVELOPMENT OF CHAMBERS

- Automatic developing chambers
- Rectangular chambers
- Twin trough chambers

DRYING

Drying of chromatogram should be done in vacuum desiccators with protection from heat and light.

If hand dryer is used there may be chances of getting contamination of plates, evaporation of essential volatile oils if any present in the spot or compounds sensitive to oxygen may get destroyed due to the rise in temperature.

HPTLC-MS interface

HPTLC-MS coupling allows for verification of the chemical structure of analytes by mass spectrometry. Analytes can be directly eluted with the MS-Interface from the plate and the elute can be injected into an MS or collected for further analysis offline.

- LESA
- TLC-MALDI-MS
- TLC-DART-MS
- Elution based HPTLC MS^[23]

LIQUID EXTRACTION SURFACE ANALYSIS TLC MS

LEAST technology was originally developed to investigate tissue slices, but it can analyse almost any surface with its nano-robotic ESI source and that includes TLC plates.

The traversa nanomate automatically works its way across the plate taking a fresh pipette tip to analyse each "zone" which practically eliminates carry over. The robot picks up a pipette tip, draws extraction, solvent from a reservoir, moves to the zone of interest, allows a small droplet of solvent to mix with the sample spot for a present time, and draws up the mixture before nano spray injection into any high end MS system^[24]

TLC MALDI-MS

Broker daltonics introduced an adapter that allows us to directly insert your TLC plate into a MALDI instrument.

The fully automated measurement process allows an entire plate to be scanned and produces a visual representation of separation. However the data evaluation software enables so called MALDI chromatograms that plot molecular mass against TLC position, producing a two dimensional view, analytes that overlap on the TLC plate are separated by mass and shown in different colours.

ELUTION BASED TLC-MS

- Rapid and contamination free elution of selected zones.
- Plug and play installation.
- Compatible with any LC-MS system
- Confirmation of known substances within a minute highly effective backwashing function prevents the elution path from becoming blocked.
- Easy handling ensures accurate and reproducible plate positioning low solvent consumption.[25]

APPLICATIONS OF HPTLC-MS

- TLC-MS of protein and peptides.
- TLC-MALDI-MS of small molecules.
- TLC-MS of dirty samples.
- UV filters in sun screen.

- Paracetamol in different formulation.
- Caffeine in energy drinks ^[26]

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