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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY(HPTLC)-MASS-SPECTROSCOPY(MS)

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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.

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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. Fromthe 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]

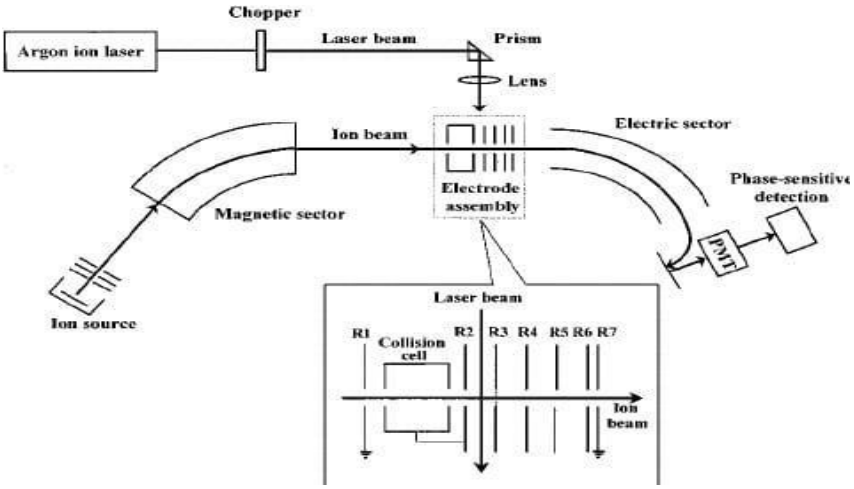
PRINCIPLE OF HPTLC :

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 moveaccording 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] ISSN: 2455-2631 August 2022 IJSDR | Volume 7 Issue 8 IJSDR2208094 International Journal of Scientific Development and Research (IJSDR) www.ijsdr.org 642 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]

SCHEMATIC DIAGRAM OF MASS SPECTROSCOPY:



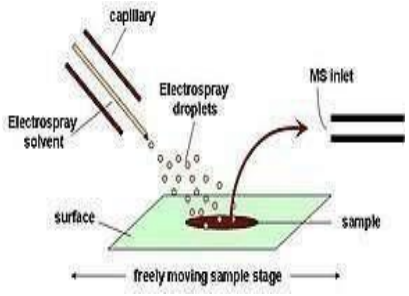
MASS SPECTROSOPY:

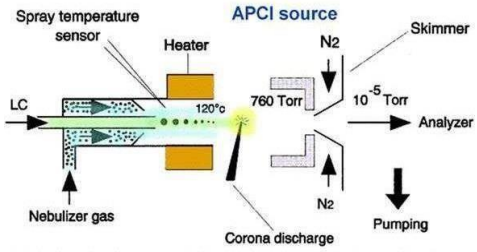
Types of Interfaces used in HPTL-MS

- Electron Spray Ionization
- Matrix Associated Laser Desorption Ionization Technique (MALDI)
- Atmospheric Pressure Chemical Ionization (APCI)

ELECTRON SPRAY IONIZATION:

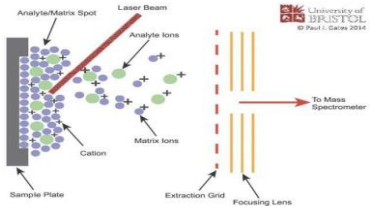
ESI provides the softest ionization method available, which means, it can beused for highly polar, least volatile or thermally unstable substances





APCI is an ionization technique using gas-phase ion-molecule reaction at atmospheric pressure.

Electron spray ionization



APCI

MALDI

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 isvaporized 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 thedetection 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 Rf 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]

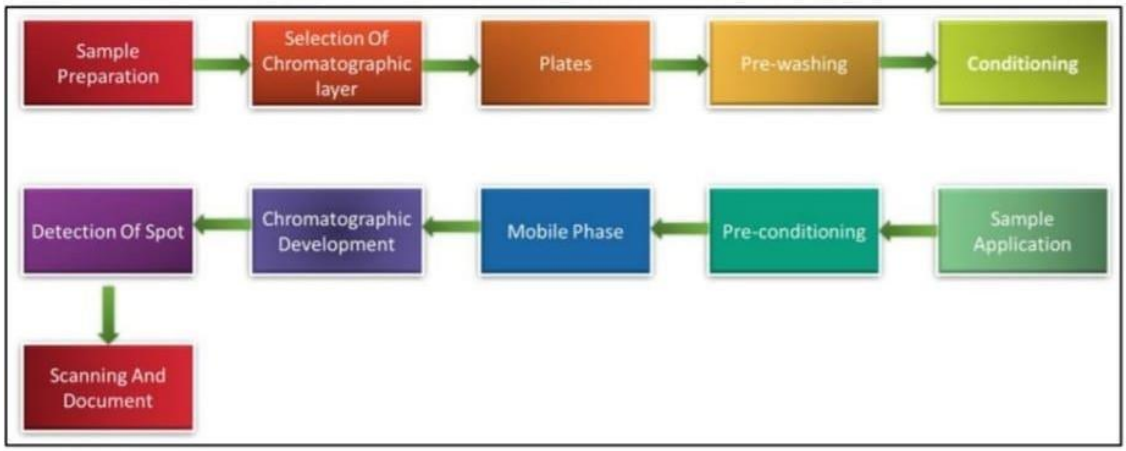


Fig 1. Steps involved in sample preparation.

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]

PLATE SIZE: 20 × 20 cm • 10 × 20 cm • 5 × 10 cm • 5 × 7.5 cm 17

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 solution1%.

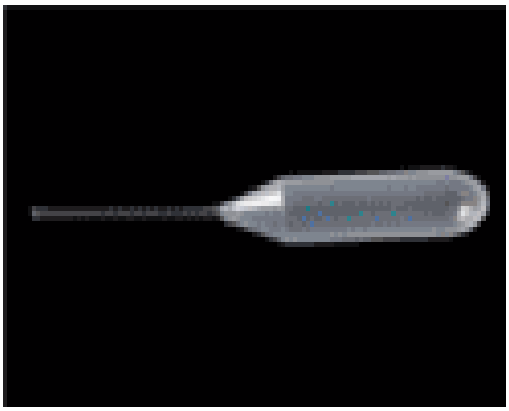
CONDITIONING:
The pre-washed plates are placed in oven at 120 for 15-20min. this process is known as conditioning.[21]

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



AUTOMATIC APPLICATORS:

CAMAG Nanomat: Samples applied in the form of spots. The volume is controlled by disposable platinum iridium of glass capillary which has volumeof 0.1-0.2µl

CAMAG Linomat: Automated sample application device. Sample is loaded inmicro syringe (Hamilton Syringe) 1ul capacity. Sample can apply either as spot or band by programming the instrument with parameters like spotting, volume,band length etc.

CAMAG automatic TLC sampler III: Applies sample as spot or bands automatically from the rack of sample vials.[2]

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. ISSN: 2455-2631 August 2022 IJSDR | Volume 7 Issue 8

IJSDR2208094 International Journal of Scientific Development and Research (IJSDR) www.ijsdr.org 644 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.

- 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

Twin trough chambers

Rectangular chambers **Automatic developing chambers**

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.

DRYING:

Drying of chromatogram should be done in vacuum desiccators with protection from heat andlight. 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 elutecan 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 triversa 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 functionprevents the elution path from becoming blocked.
- Easy handling ensures accurate and reproducible plate positioning
- low solventconsumption.[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].

CONCLUSION:

- ✓ This above short report a simple hptlc-ms method for the routine analysis of pharmaceutical product/formulation.
- ✓ Its play important role in the study of drug metabolism, discovery of new drug candident and the analysis,identification and characterization of impurities and degradant in drug substances.
- ✓ The hptlc-ms interface is versatile building block of multi-dimensional liquid chromatographic system.

REFERENCES:

1. McPolin O. An introduction to HPLC for pharmaceutical analysis. Lulu. com; 2009 Mar 1.
2. Virus ED, Sobolevsky TG, Rodchenkov GM. Introduction of HPLC/orbitrap mass spectrometry as screening method for doping control. Journal of mass spectrometry. 2008 Jul;43(7):949-57.
3. Kirbiš A, Marinšek J, Flajs VC. Introduction of the HPLC method for the determination of quinolone residues in various muscle tissues. Biomedical Chromatography. 2005

May;19(4):259-65.
4. Ahuja S, Dong M, editors. Handbook of pharmaceutical analysis by HPLC. Elsevier; 2005 Feb 9.
5. Srivastava M, editor. High-performance thin-layer chromatography (HPTLC). Springer Science & Business Media; 2010 Nov 15.
6. Zlatkis A, Kaiser RE. HPTLC-high performance thin-layer chromatography. Elsevier; 2011 Aug 26.
7. Sonia K, Lakshmi KS. HPTLC method development and validation: An overview. Journalof Pharmaceutical Sciences and Research. 2017 May 1;9(5):652.
8. Shewiyo DH, Kaale EA, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of pharmaceutical and biomedical analysis.2012 Jul 1;66:11-23.
9. Ansari MJ, Ahmad S, Kohli K, Ali J, Khar RK. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. Journal of pharmaceutical and biomedical analysis. 2005 Sep 1;39(1-2):132-8.
10. Zlatkis A, Kaiser RE. HPTLC-high performance thin-layer chromatography. Elsevier; 2011 Aug 26.
11. Rawat S, Gupta A. Development of novel HPTLC method for estimation of Qurcetine in Ocimum sanctum. Asian J. Pharm. Tech. 2011 Oct;1(4):149-51.
12. Domon B, Aebersold R. Mass spectrometry and protein analysis. science. 2006 Apr 14;312(5771):212-7.
13. Glish GL, Vachet RW. The basics of mass spectrometry in the twenty-first century. Nature reviews drug discovery. 2003 Feb;2(2):140-50.
14. Biemann K. Mass spectrometry. Annual review of biochemistry. 1963 Jul;32(1):755-80.
15. Zubarev RA, Makarov A. Orbitrap mass spectrometry.
16. Gross JH. Mass spectrometry: a textbook. Springer Science & Business Media; 2006 Sep 21.
17. De Hoffmann E, Stroobant V. Mass spectrometry: principles and applications. John Wiley& Sons; 2007 Oct 29.
18. Yamaguchi K. Cold-spray ionization mass spectrometry: principle and applications. Journal of mass spectrometry. 2003 May;38(5):473-90.
19. Milman BL. General principles of identification by mass spectrometry. TrAC Trends in Analytical Chemistry. 2015 Jun 1;69:24-33.
20. Watson JT, Sparkman OD. Introduction to mass spectrometry: instrumentation, applications, and strategies for data interpretation. John Wiley & Sons; 2007 Nov 12.
21. Dass C. Fundamentals of contemporary mass spectrometry. John Wiley & Sons; 2007 May11.
22. Luftmann H, Aranda M, Morlock GE. Automated interface for hyphenation of planar chromatography with mass spectrometry. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2007 Dec 15;21(23):3772-6.
23. Klöppel A, Grasse W, Brümmer F, Morlock G. HPTLC coupled with bioluminescence and mass spectrometry for bioactivity-based analysis of secondary metabolites in marine sponges. JPC-Journal of Planar Chromatography-Modern TLC. 2008 Dec 1;21(6):431-6.
24. Van Berkel GJ, Sanchez AD, Quirke JM. Thin-layer chromatography and electrospray mass spectrometry coupled using a surface sampling probe. Analytical chemistry. 2002 Dec 15;74(24):6216-23.
25. Sherma J, Rabel F. Review of advances in planar chromatography-mass spectrometry published in the period 2015–2019. Journal of Liquid Chromatography & Related Technologies. 2020 Jul 20;43(11-12):394-412.
26. Cebolla VL, Jarne C, Vela J, Garriga R, Membrado L, Galbán J. Scanning densitometry and mass spectrometry for HPTLC analysis of lipids: The last 10 years. Journal of Liquid Chromatography & Related Technologies. 2021 Feb 25;44(3-4):148-70.