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A REVIEW: MASS SPECTROMETRY HYPHENATIONS FOR THE COMMON SEPARATION TECHNIQUES, THEIR INSTRUMENTATION AND ITS DIVERSE APPLICATIONS.

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ABSTRACT
Many Traditional methods which involves single analytical techniques like HPLC, HPTLC etc. has become insufficient in order to carry out for analysis of large amount of compounds with accurate sensitivity as well as specificity. To overcome this type of difficulty in analysis hyphenated techniques in the year 1980 was introduced which mainly involved the coupling of spectroscopic technique and separation technique with the help of proper interface. In this review paper hyphenated MS techniques their instrumentation, Interfaces and applications were discussed for the commonly used separation techniques HPLC and HPTLC which have revolutionised in the field of analysis. Usage of HPLC-MS, HPTLC-MS is been increasing speeding day by day which has shown better analysis of sample.

KEYWORDS: Hyphenated technique, HPLC-MS, HPTLC-MS, Interfaces.

INTRODUCTION

In order to handle effective and increased in analysis of pharmaceutical drugs modern pharmaceutical techniques were included which is called as hyphenated techniques.[1] In the year 1980 Hirch Feld was the first person to introduce the term "Hyphenation" where there was on-line combination or coupling involving a spectroscopical technique and the separation technique with the help of proper interface connecting between those two techniques together. The main purpose involved in this type of coupling or combination is to increase the accuracy of analysis, faster analysis, increased reproducibilityand higher automation when compared to the traditional analytical approaches that includes High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) etc. which are single analytical techniques. The hyphenation technique on mass especially coupling of the chromatography technique along with mass spectrometry is been revolutionised in the field of analytical applications. When chromatography is coupled it is mainly involved in the identification of mixture components and spectroscocopical techniques provides information selectivity by usage of spectral library.[1-4]

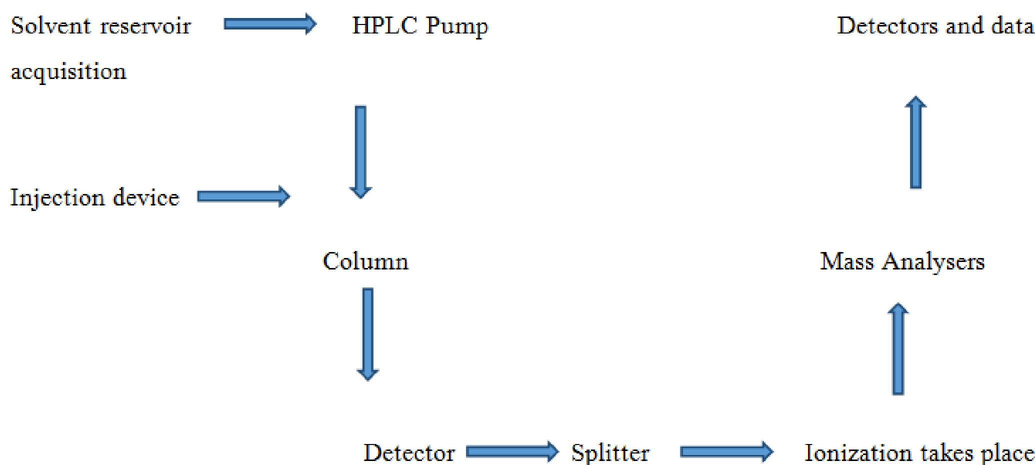
The different types of double hyphenated techniques discussed in this paper are

1. Liquid Chromatography and mass spectrometer
2. High Performance Thin layer chromatography and Mass spectrometer.

1. Liquid Chromatography and Mass Spectrometry (LC-MS)

It involves coupling of a liquid chromatography (most probably HPLC) with a mass spectrometry where HPLC involves the process of separation of the mixture of components and the mass spectrometry is involved in detecting those mixture of components which is been separated by HPLC. The sample is separated by using column then it is passed through the switching valve, these two techniques are combined.[2]

FLOW DIAGRAM OF LC-MS



Instrumentation of LC-MS

The LC-MS consist of following parts

a) Liquid chromatography

b) Mass spectrometry

Liquid chromatography consist of following parts

Table1: Parts of Liquid Chromatography (HPLC).

Parts	Description
Pump	Main role is to deliver the mobile phase from the solvent reservoir to the column compartment. The commonly used pumps are Constant pressure pumps Syringe type pumps Reciprocating type
Sample Injector	The role is to inject the sample along with the mobile phase into the column where the injection volume is in between 1 to 100µL The 2 types of injectors are available Automatic type Manual type Generally Automatic type pump is preferred because it is comfortable, user friendly more precise and accurate results.
Column	Mainly the column is been packed with silica combined with carbon chains. The different types of column which are commonly used are octadecyl columns, octyl columns, amino columns, phenyl columns and cyano columns. Always based on the components to be separated columns are chosen.
Detectors	Generally two types of detectors used Bulk property detectors- involved in detecting the physical property of the solute e.g. RI detectors, Conductivity detectors and dielectric constant Solute property detectors- involved in responding the property of the solute e.g. UV detectors, Fluoresce detectors, chemifluoresce detectors.

The signals from the detectors are stored in software and recorded in the form of peak were the peak area and retention time can be calculated

Interference

Various types of Interface are available. Certain characters should be exhibited by the interface they are

- Vaporisation of liquid and Nebulization
- Sample ionization
- Removal of excess amount of vapour present in the sample
- It should extract the molecular ions into the mass analyser

The most commonly employed interface are Electron Spray Ionization (ESI) and the Atmosphere Chemical Ionization (APCI)

Direct-Liquid Introduction Interface (DLI)

In the year 1980 Direct Liquid Introduction was first developed as interface. It involves the liquid solutions which are called as Direct Liquid Introduction which gets limited by the pumping capacity of the mass

spectrometer controlled up by orifice or the narrow capillary tube or the pinhole orifice of the diaphragm. DLI is mostly suitable for the RPLC (Reverse phase Liquid Chromatography) because of its good protonating action of the polar solvents like methanol or water.

This interface has produced good results to the molecules which are moderately difficult, but this method was not reliable because of frequently plugging in their restriction of flow so very small sample fraction (1% lesser) gets utilized from the LC system. Improved system is also suggested that will be replacing some of their drawbacks, but the benefits which are added was small but still the plugging problems were not solved. Slowly DLI interface were replaced and then thermo spray instrumentation came into existence.

Electron Spray Ionization Interface (ESI)
It was first developed by Fenn. Here around 3-5 Kilo volts potential is applied to the capillary tube which is made of stainless stay and then sample is passed to this tube as a result to the tip of the capillary tube charged droplets are formed then vaporisation takes place as well as the droplets are formed and then get evaporated and increased collision takes place as a result charged particles are converted to ions of gaseous state.

Advantage of ESI
- There is multiplication of charges where the charges are increased to 1-3 for the molecules above 5000 Daltons.
- M/Z ratio is always maintained and it always below Two thousand.

Application of ESI
It is most commonly employed with biological research and medical analysis to measure the molecular weight of sugars, Proteins, Polymers, Peptides, nucleotides etc.

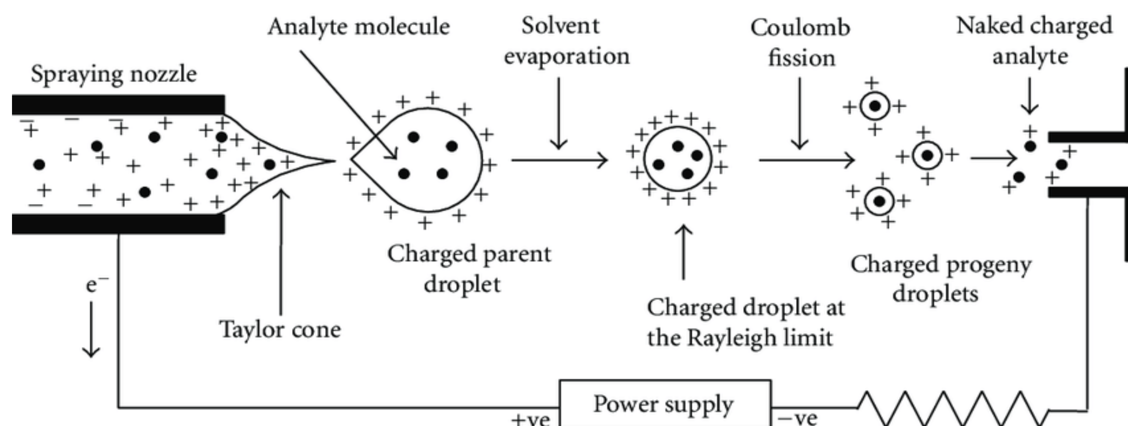


Figure 1: Electron Spray Ionization Interface.

Atmosphere Chemical Ionization Interface (APCI)
There are two major steps involved in APCI
a) Evaporation or desolvation of the analytes.
b) Charge transfer reaction to produce vapour phase ions

Mechanism
To the capillary tube sample is been nebulised and then to large chamber. Small droplets formation are formed in the large heating chamber at around 250 to 400 degree Celsius ionization of those sample molecule and then charge transfer takes place and then passed through mass analyser.

Application of ESI
It is commonly to analyse non polar analytes and less polar analytes which have modulate molecular weight of the sample molecules.

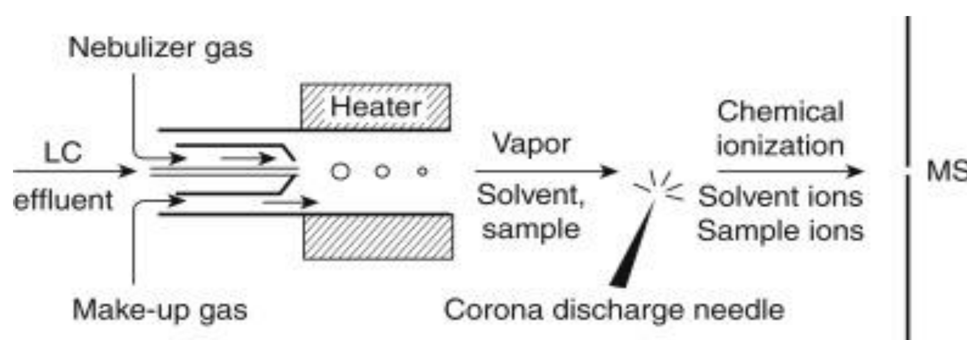


Figure2: Electron Spray Ionization Interface.

Atmosphere Pressure Photo Ionization Interface (APPI)

Here the molecules are ionized by the use of photons. It involves mainly 2 steps

- Excitation step
- Analyte Ionization

Similar to APCI, the eluents arrised from the LC into gaseous phase. Here photons are produced from the krypton lamp which causes the ionization as well as excitation of the molecules.

Application of APPI

It is mainly used in non-polar analytes were other techniques like ESI and APCI are difficult to perform for non-polar compound.

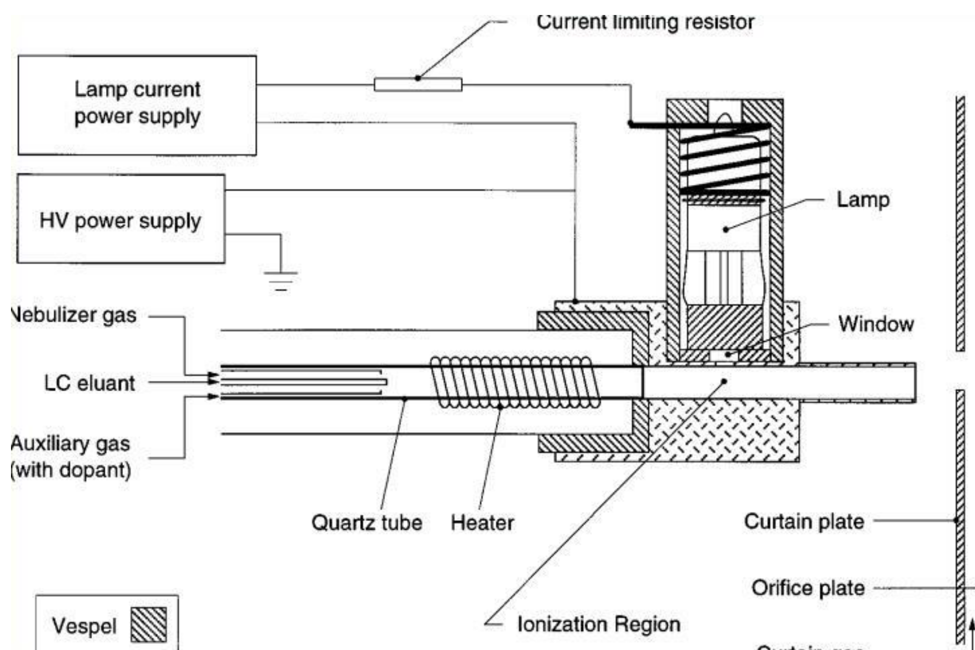


Figure 3: Atmosphere Pressure Photo Ionization Interface.

Moving belt

In the year 1977 Moving belt interface was first introduced. It act by separation of condensed liquid phase (LC) from the mass spectrometry which uses a belt in-order to transport the analytes gets deposited in the form of band and then it gets evaporated. Here the analytes gets cycled continuously and then transportation takes place from the atmosphere pressure into the vacuum of the ionization source through the pumped vacuum locks (2 in numbers). Mostly this type of Interface is used for volatile samples using EI/CI but less volatile compounds can also be analysed E.g. Nucleoside and nucleotide.

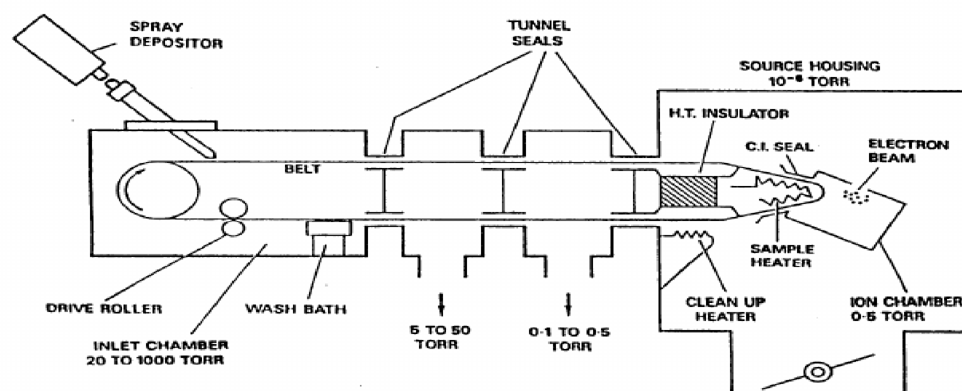


Figure 4: Moving belt Interface.

Fast Atom Bombardment Interface

This type of interface is the very sensitive interface. It has Argon or Xenon atoms which bombards with the sample molecule as a result ionization of the sample molecule takes place.

Application of Fast Atom bombardment

It is mainly involved for thermally and large unstable molecules. Proteins and surfactants ionization take place with this type of interface.

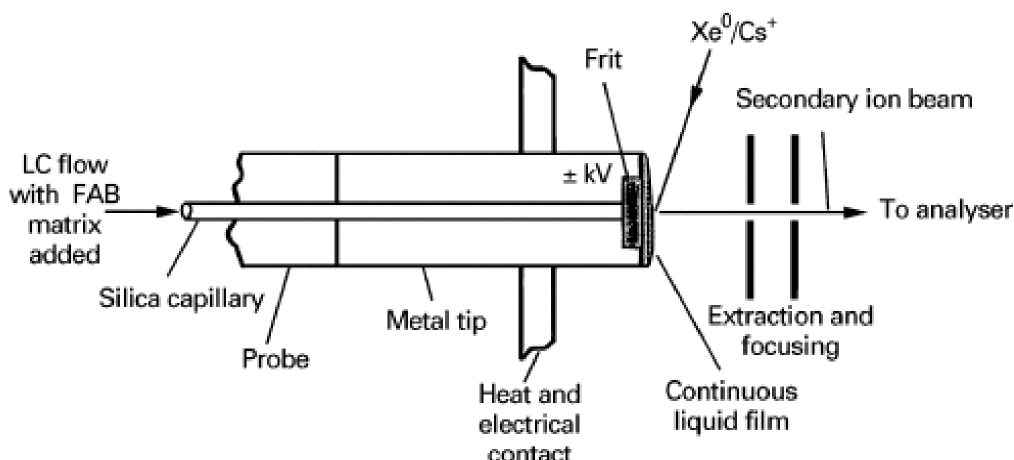


Figure 5: Fast Atom bombardment interface.

Particle Beam Ionization Interface

It was first developed by browner. Here the process involved in nebulization as well as evaporation is similar to APCI and ESI. In this method the eluent after HPLC is been passed through narrow tube. Along with liquid droplets Helium gas is been passed, then from nebulization it is passed into heating chamber similar to ESI and APCI mechanism.[5-8]

Mass Spectrometry

Here based on the m/z ratio measurement of the mass of the compound is been determined. In mass spectrometry important part is

a) Ionization sources
b) Mass analysers
c) Detectors

Ionization sources

Soft ionization is commonly employed for the molecular ions involved in LC-MS to produce few fragment of ions. But still with LC-MS structural characters of certain compounds are found to be studied hardly and this problem can be overcome by tandem mass spectrometry introduction (MS-MS) i.e. LC-MS-MS where the molecular ions are produced through collision induced dissociation.

The different types of Ionization methods commonly employed are

Table: 2 Ionization Techniques.

Types of Ionization Techniques	Description
Electron Impart Ionization	It is most commonly as well as highly developed ionization method. It involves neutral molecules placed inside the chamber and they are maintained at a pressure of 0.005 torr. An electron gun is located perpendicular to the incoming gas stream. Electrons are emitted from a growing filament are drawn off by pair of positively charged slits through which electron pass into the body of chamber. An electric field is maintained between these slits and then acceleration of ions takes place were the ionizing electrons are from the cathode are formed into the tight helical beam by small magnetic field on order of 100G which is confirmed in ionization region. Here the operating source is at 70V, which provides sufficient energy to ionize and fragmentation takes place.
Chemical Ionization	In chemical Ionization the sample molecules are not involved to the bombardment by the high energy electrons. Instead a reagent gas (Methane, Isobutane, Ammonia-commonly used) is involved in the ionization source and then ionized. In chemical ionization there are two types Positive Chemical Ionization Negative Chemical Ionization In Positive Chemical Ionization involves 2 steps

	Step-1: Reagent gas is ionized by electron by electron impart ionization method where the electron energy must be 200-500V to ensure penetration of the ionization electrons into active volume. Primary ionization is followed by second order processes in which primary ion reacts with additional reagent gas molecules to produce stabilized reagent ion plasma. Step-2: Second part of this reaction involves when the reagent ion encounters a sample molecule. Negative Chemical Ionization: It is typically counterpart for the positive chemical ionization where the negative ions are formed.
Field Ionization	When there is application of strong electric field it induces the emission of electrons. When a molecule is in between 2spaced electrodes in the presence of electric field, it experience electrostatic force similar to plates of charged condensed. If metal surface (anode) has proper geometry and is under high vacuum (10^{-6}) torr and this is sufficient to remove electron from a molecule without imparting much excess energy.
Field Desorption	It is similar to field ionization. It is dipped into or has deposited on solution of sample under study. Here emitter wire is heated with an electric current which is at high voltage condition used in the field ionization. Material gets evaporated into a source that may be chemical ionization plasma or electron impart plasma unit.
Matrix Assisted Laser Desorption Ionization	Compound is dissolved in the small organic molecules which is called as matrix. These molecules have strong absorption at the laser wavelength. This matrix is dried before analysis and any lipid solvent used in the preparation of solution is removed. Now, the result is solid solution of analyte and those molecules are embedded throughout the matrix so they are isolated from other. This step involves abblication of bulk portions of this solid solution by intense laser pulse over the short duration.

Analysers

After the ionization of sample then it is passed through analysers were separation of ion takes place in the analysers based on the (m/z) ratio. The different types of analysers that are commonly used are

Table 3: Types of Analysers.

Types of Analysers	Description
Quadrupole mass analyser	It consist of 4 rods which are in cylindrical in type and positive and negative current flows through these rods. The flow acceleration is been applied to the ions entering into the quadrupole. The scan speed is 4000 m/z.
Time of flight analyser	It is also commonly used mass analyser, here the ions are subjected to the accelerating voltage. When the charge is single the time taken to reach the detector is been directly proportional to their masses. Lighter ions will move faster and involved in striking the detector at first.
Ion trap analyser	It consist of three dimension ion trap where at the ends two capped electrodes are present where the quadrupole field is being applied. On one side of the electrode contains a small aperture and smaller in size in which the ions are introduced into it and also contains Helium gas bath in order to stabilize the ion in trap were collision of ion takes place with the helium and it causes increased motion on the ions get escaped from the trap based on m/z ratio and then mass spectrum is recorded.
Fourier transfer Ion cyclotron Resonance	It is also called as FT-ICR. They are the important group of mass analysers were the mechanism involved is from the ionization source ions have been produced and then separation takes place through m/z ratio. Acceleration of the ions involves two fields Electric field Magnetic field As a result both of these fields the ions are excited as well as generation of time dependent current. Ion trapping takes according to M/Z ratio.

Detectors

It involves production of current and that current which is produced is being directly proportional to the ions that are separate and recording of those current (signal) in the form of data is carried out.

Table 4: Detectors in Mass Spectrometry.

Detector	Description
Electron Multiplier	For current less than 10^{-5}A, Electron Multiplier is necessary. The ion beam strikes the conversion dynode. It is a metal plate that convert imping ions to electrons. Either positive or negative ions are accelerated by the constant high voltage of conversion dynode and they are converted into electrons of positively charged ions when they strike the plate. The current created and then multiplied in either of two configuration. A discrete dynode multiplier has 15 to 18 individual dynode coated with a metal oxide that has high secondary electron emission properties.
Channel Electron Multiplier Array	A channel plate is composed of regularly (Usually hexagonal) close packed array of channel in a flat plate of semiconducting material. The pore diameter in the range of 10-25 µm. The plate is about 1 mm thick. Each pore or channel is coated with secondary electron emissive material, thus channel constitutes an independent electron multiplier.
Faraday Cup Collector	It is simple and effective means of monitoring ion current in the focal plane of Mass spectrometer. It consist of a cup with suitable suppressor electrodes (To supress secondary ion emission) and guard electrode.
Array detector	Here the collector of ions are placed in place. Ions get separated based on the M/Z ratio after that values get recorded
Point Ion collector detector	It involves ion collectors to be in fixed position were the electric current is produced when the arrival of ions are recorded.
Photographic Plate	Here simultaneous focusing of ions in a plate and so the mass spectrum can be recorded on a photographic plate. It can give greater resolution than electrical detector because the plate is a time integrating device, it can provide highest sensitivity of any detector. Spectra from extremely small samples with low vapour pressure and with short life can be detected.

Application of LC-MS[5]

In forensic science

It is used in trace analysis toxicity determination especially in food or beverages, drug analysis and toxic metabolites can be determined through this LC-MS hyphenation technique. E.g. If any detergent is added into juice can be determined by analysing detergent as well as the juice in the same chromatographic conditions were the spectra is studied.

Compound Identification and their characterization

Carotenoids

They are thermally not stable compound so analysis of carotenoids is not possible with Gas chromatography (GC). LC-MS is the most accurate technique to determine the carotenoid content. Up-to-date it involves 5 LC-MS techniques involved

A) Atmosphere Pressure Chemical Ionization (APCI)
B) Particle beam ionization
C) Fast Atom Bombardment
D) Electrospray
E) Moving belt

Among this type of interface commonly used is APCI which is easier to use and rapid analysis is possible when compared to other interface used in LC-MS

Product degradation

LCMS technique most suitable for identification, characterization as well as separation of degradation

products which has undergone various oxidative, photolytic, hydrolytic and thermal stress which indicates unstable hydrogen, change in the fragment pattern and their mass change if the product has been degraded structural characterization is also possible with LC-MS. It is very useful in understanding of degradation pathway.

Qualitative and Quantitative analysis
For Biological samples
For the biological sample analysis generally LC-MS-MS instrument is preferred where the biogenic amines, pharmacokinetics can be quantified.

Qualitative and quantitative analysis of complex lipid mixtures is also carried with LC-MS

LC-MS Doping test
For example determination in urine for 4-methyl-2-hexaneamine is carried with doping agent with the positive mode of LC-MS. Standard used is 4-methyl-2-hexaneamine and the suspected compound is analogue which is seen in the dietary supplement were two peaks are generated with Retention time is 3.43 minutes and 3.78 minutes which is identical to the unknown compound.

In Pesticidal and Agrochemical industries
The components which is present in the pestici9des as well as fertilizers can be determined with LC-MS.

2-D Hyphenated technology
LCMS is used in various bio analytical and various analytical techniques so it has become one of the powerful (2D) technology were two techniques are also combined in determination of lipids, carbohydrates, peptides, proteomics etc. and the method is also very sensitive and rapid determination.

Quality control tool-LCMS
LC-MS method is used for the topiramate determination (Narcotic drug) in the pharmaceutical application. The condition used for determination is

- Column- C18 column
- Elution Technique- Isocratic elution technique
- Mobile phase- Acetonitrile: Water (85:15)
- Flow rate- 0.5 ml/min
- Interface- ESI interface
- Analyser- Quadrupole analyser
- M/Z =338.1
- RSD=2.05%
- Recovery=99.53%
- Concentration range-0.25 to 10µg/ml

Steroid hormones
The level of detection of testosterone as well as dihydrotestosterone levels for children's and women's is difficult to detect as well as to analyse with the conventional immunoassay method, but it has prompted with reliable measurement in these groups by usage of sensitive LC-MS assay.

Chemical Screening
By combination of HPLC along with the mass spectrometry is used as chemical screening strategy has been recently developed of hyphenation techniques were the separation of the metabolites along with the structural characters at the same time information is obtained.[5,9-12]

2. High Performance Thin Later Chromatography and Mass spectrometer (HPTLC-MS)
HPTLC-MS is one of the versatile tool involved in separation as well as identification for the wide range of components in the pharmaceuticals and phytopharmaceuticals within short period of time. The TLC-MS coupling is mainly used for structural identification of unknown compound present in the drug substance and the coupling is with TLC-MS interfaces.[13] A special surface sampling probe is required for on- line extraction of analytes from HPTLC plates into Mass spectrometer.[14]

The HPTLC-MS consist of following instrumentation parts

A) HPTLC
B) Mass – Spectrometry
C) Coupling techniques
D) Interfaces

HPTLC
HPTLC is one of the analytical technique which is based on TLC but it has increased resolutions for the qualitative analysis and components to be separated. It is offering better resolution as well as lower limit of detection when compared to TLC [14] .HPTLC is mainly involved for separation of the crude form of drugs, synthetic drugs as well as semi-synthetic drugs. The HPTLC plates is made of glass or aluminium sheets of foil which consist of various adsorbents like silica gel, aluminium oxide, cellulose that act as stationary phase.

With the auto-sampler the sample is been kept on the bottom of the HPTLC plate and then mobile phase (solvent mixture) is prepared and is drawn up by capillary action via plate which flows against the gravity. Separation of the compound takes place which is based on the relative affinity between the mobile phase and stationary phase for the compounds.

Here the higher affinity compounds towards the stationary phase will be travelling slowly and the compound which has more affinity towards the mobile phase will be eluted rapidly. Generally silica is used as stationary phase which is polar in nature and if a mixture of components needed to be separated the more polar compounds exhibits strong interaction with the silica gel (Stationary phase) as a result the eluting time is more and elutes at last. Less polar compounds are eluted rapidly, if the solvent mixture for mobile phase is changed to more

polar it is capable of solute dispelling from the silica binding places and from the TLC plate as a result it moves higher to the plate. Compound separation is based on completion between mobile phase and solute to bind on stationary phase. After the separation spots are visualized and then their RF value is calculated and then by suitable detection technique their characters or nature of the compound is been studied.[13, 15]

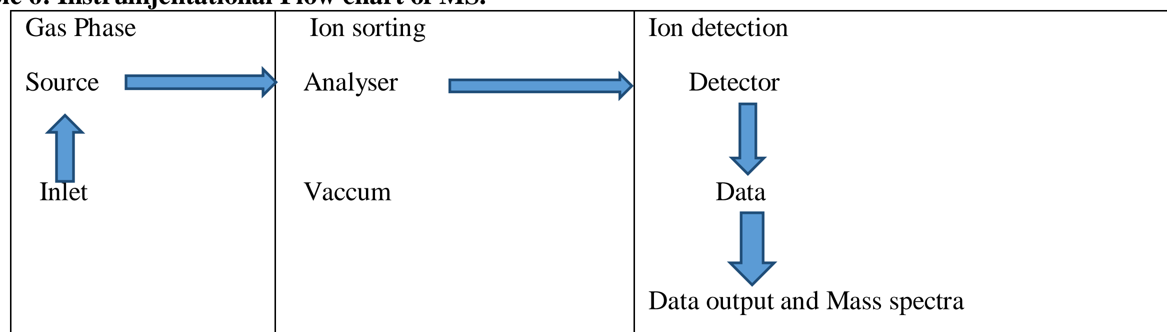
Mass spectrometry

Major components involved is Mass spectrometry are

Table 5: Major components of Mass spectrometry.

Main components	Description
Ion Source	It is involved in production of gaseous ions
Analysers	According to mass - charge ratio resolvation of ions takes place according to their characteristic mass components
Detectors	It is involved in detection of ions according to their relative abundance.

Table 6: Instrumjentational Flow chart of MS.



Vacuum system

A high vacuum is required in order to operate at a very low pressure, as a result in mass analyser the collision of ions with the other molecules is been reduced. If there is any collision of ions it can interfere the results in the mass spectrum. Generally the pressure maintenance is 10-4 to 10-7 torr. There are 2 stages in producing this vacuum

- Mechanical pump is the first stage where the vacuum is reduced to 10-3 torr.
- Diffusion pumps are there in second stage which consist of orturbo molecular pump which produces high vacuum.

Ion source

It involves loading of the sample into the mass spectrometer in liquid form or in dried form and then vaporisation and ionization of those molecules takes place with the help of ion sources e.g. for commonly used ion source in HPTLC-MS is Electron spray Ionization (ESI) and Matrix Adsorbed Laser Desorption Ionization (MALDI) etc. After the ions are produced, then it gets accelerated due to presence of electric as well as magnetic fields present in the mass spectrum.

Mass Analysers

After the acceleration of ions from the ion source it then enters into the mass analysers were separation of ions takes place according to the m/z ratio. The operating characters for each of the mass analysers differ from the other type of mass analysers. Analysers can be pulsed one or continuous one. In case of continuous type of analysers after separation of ions according to m/z, then it is passed to the detector where certain m/z is selected, then ions at other m/z ratio will be lost which reduces S/N for the continuous analysers. The m/z for an ion can be setted in case of Single Ion Monitoring (SIM) which enhance the S/N but any other information about that ion will be lost.

Pulsed type of Mass analysers are also used but they are less common but the advantage is that it can able to produce the entire mass spectra. E.g. for Pulsed type of mass analysers used in HPTLC-MS is time of flight, Quadrupole ion trap analysers and cyclotron resonance. Commonly used mass analyser is quadrupole in HPTLC-MS where the m/z range is 1000 and it requires modest vacuum condition which act by high transmission in its efficiency.

Detectors

Detection of ions in HPTLC-MS is based on the either on charge or momentum. In case if the signal is large then faraday cup can be used to collect the ions and then current is measured. Previously photographic plates were used. The detector should be very sensitive, where even single ions should be detected. Based on its dynamic range of detection, geometry again as well as detection speed, detector must be selected.

Data system

This is the final part of the mass spectrometer. This part has continuously for past 20 years has undergone revolutionary changes. From photographic plates, strips chart recorders it is changed to data system where there is hundreds of spectra is obtained within a minute.[13]

Coupling techniques of TLC-MS

TLC can be directly coupled with the mass spectrometry by involving 2 techniques.

a) Elution based technique
b) Desorption based technique

Elution based technique

In this type of technique TLC-MS interface is utilized that enables the analyte dissolution from the silica plate with the help of solvent and then the analyte gets transferred into the liquid phase of the mass spectrometer.

Desorption based technique

In this type of technique involves the analyte vaporization from the TLC plate surface and then analyte is transferred into gas phase of mass spectrometer. E.g. for this type of vaporization technique involves MALDI, Ion bombardment, DART, Gas beam

MALDI: Laser energy is involved by absorption of matrix where there is involvement of minimu7m fragmentation to create ions from the large molecules. This technique is commonly employed for the larger organic molecules and biomolecules.[16]

DART: It is called as direct analysis in Real time. It involves instantaneous ionization takes place in atmosphere pressure.[17]

Both of these coupling techniques is performed after the separation process is completed by the TLC plates are allowed to dried. In both of these techniques transfer of the analytes into the mass spectrometer is rapid and fast.[16]

INTERFACE

One of the versatile instrument which is involved in the extraction of the HPTLC/TLC plates into the mass spectrometer in order to determine structural characters of that particular compound which gets separated in HPTLC or TLC plates.

The HPTLC which consisted an auto sampler called as Linomat V is connected to the nitrogen cylinder and then scanner of TLC plates is attached with PC-CATS software. The HPTLC-MS examination is carried with the help of TLC-MS interface were the eluting agent is acetonitrile and its flow rate is 1ml/min. The material which gets eluted is then automatically gets transferred into single-quadrupole mass spectrometer and then their spectrum is obtained. Now a day's universal and most suitable TLC-MS interface is available where there is semi- automatic extraction of the zones of interest and it can direct into HPLC-MS system. The interface is connected simply to any of the LC coupled mass spectrometer and possess certain characters are

- It should be compatible to all HPLC-MS system
- It should exhibit semi-automatic performance
- It must be compatible to HPTLC or TLC layers
- It should produce reproducible results
- There should not be scrapping in the plates.[13,15]

Applications of HPTLC/TLC –MS Hyphenated technique

Identification of the substance present in the mixture

Example- Acetyl salicylic acid, paracetamol, caffeine

The mass spectrum for the standard mixtures involving caffeine, paracetamol and the acetyl salicylic acid is obtained and their identification of their zone on hRF15 as well as their mass spectra is been recorded. The background spectra for the plate at the same plate is also gets recorded and it is subtracted from the substance spectrum so the mass spectrum throws only the system peak.

Separation and Identification of Q10 complex co-enzyme

Q10 co-enzyme which is called as ubiquinone (Vitamin like substance). They are present naturally in the human cells and they act as antioxidants and protects the skin from free oxygen radicals. These Q10 agents are also added into various cosmetic products is carried out with HPTLC and then their identification is carried with MS.[13]

Detection of Sibutramine in Slimming food products

HPTLC-MS also confirms the detection and their fraudulent addition of sibutramine over the slimming products (Coffee or dietary food supplements). It involves MS confirmation after the marking zones based on their RF values for the reference substance obtained from densitometry and detector is operated in ESI mode and data's are recorded.[18]

HPTLC-MS act as one of the Neoteric hyphenation technique for forensic identification as well as separation of drugs

HPTLC-MS is also used in identification and separation of Narcotic drugs and psychotropic substance. The drug involved in the study is

Table 7: Narcotic and Psychotropic drugs under study for HPTLC-MS.

Basic Drugs	Acidic Drugs
Methadone, Cocaine, Ketamine, Caffeine, Codeine, Diazepam, Heroin, Caffeine, Heroin, Carbamazepine, Narcotine, Tebaine, Ephedrine, Morphine, and Methamphetamine	Phenobarbital and Acetaminophen

The study involved in development of TLC plates and with the various solvent system those plates are developed and then their conformation of their mass spectrum for those individual drugs is done based on their m/z values.[19]

The detection, identification and imaging of plant ecdysteroids (which is known as insect moulding hormones) that is present in various plant extracts is studied with HPTLC-MS.[20]

Forced degradation studies

Various degradation studies is carried by HPTLC-MS where there is degradation product separation as well as isolation is carried by HPTLC plate and product identification by MS-MS e.g. Empagliflozin and their forced degradation studies is also carried by performing at various stress conditions.[15]

CONCLUSION

In the summary it is stated that with the help of appropriate interference the common separation techniques like HPLC as well as HPTLC is been coupled with the mass spectrometry. This type of coupling technique with the mass spectrometry has become an indispensable tool in the area of analysis. Over the last two decades this type of hyphenation technique gets significantly get broad banded in their diverse applications. In this review article the detailed Instrumentation and applications of LC-MS and HPTLC/TLC -MS has been discussed.

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REFERENCE

1. Article R. Joshi et al, 2012; 3(11): 4184–91.
2. Patel K, Patel J, Patel M, Rajput G, Patel H. Introduction to hyphenated techniques and their applications in pharmacy. Pharm Methods, 2010; 1(1): 2.
3. Nagajyothi S, Swetha Y, Neeharika J, Suresh P V, Ramarao N. Hyphenated Techniques- A Comprehensive Review, 2017; 2: 63–71.
4. Tanna S, Ogwu J, Lawson G. Hyphenated mass spectrometry techniques for assessing medication adherence: Advantages, challenges, clinical applications and future perspectives. Clin Chem Lab Med, 2020; 58(5): 643–63.
5. Pratima NA. Liquid Chromatography-Mass Spectrometry and Its Applications: A Brief Review. Arch Org Inorg Chem Sci, 2018; 1(1): 26–34.
6. Lee H. Pharmaceutical applications of liquid chromatography coupled with mass spectrometry (LC/MS). J Liq Chromatogr Relat Technol, 2005; 28(7–8): 1161–202.
7. (PDF) History of LC-MS development and interfacing [Internet]. [cited 2021 Feb 14]. Available from:https://www.researchgate.net/publication/291339319_History_of_LC-MS_development_and_interfacing#pf5
8. Lemi F. Interfaces for LC – MS Accelerating electrode. Guid to LC-MS, 2001; 21–7.
9. Koba M, Marszall M, Sroka W, Tarczykowska M, Bucinski A. Application of HPTLC and LC-MS Methods for Determination of Topiramate in Pharmaceutical Formulations. Curr Pharm Anal, 2012; 8(1): 44–8.
10. Kumar PR, Dinesh SR, Rini R. Lcms- A Review and a Recent Update. J Pharm Pharm Sci, 2016; 5(5): 377–91.
11. Pitt JJ. J.J.Pitt 2009 - Clin Biochem Rev, 2009; 30: 19–34.
12. Hostettmann K, Wolfender J-L, Terreaux C. Modern Screening Techniques for Plant Extracts. Pharm Biol, 2001; 39: 18–32.
13. Sambherao AB, Bhairav BB. Tlc- Ms Hyphenated Technique in Analytical, 2017; 6(10): 1392–410.
14. Attimarad M, Mueen Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods [Internet] [cited 2021 Feb 6], 2011; 2(2): 71–5. Available from: /pmc/articles/PMC3658041/?report=abstract
15. Bhole RP, Jagtap SR, Chadar KB, Zambare YB. Review on hyphenation in HPTLC-MS. Res J Pharm Technol, 2020; 13(2): 1028–34.
16. 50 Years of TLC-MS | Sigma-Aldrich [Internet]. [cited 2021 Feb 6]. Available from: https://www.sigmaaldrich.com/technical-documents/articles/analytix-reporter/50-years-of-tlc-ms.html
17. TLC and HPTLC MS-Grade Plates | Sigma-Aldrich [Internet]. [cited 2021 Feb 7]. Available from:https://www.sigmaaldrich.com/technical-documents/articles/analytical-applications/tlc/tlc-and-hptlc-ms-grade-plates.html
18. Chamber AD. Detection of sibutramine in slimming products by HPTLC and confirmation by HPTLC-MS, 2016; 3–5. Available from: www.camag.com
19. Verma KL, Kumar M, Singh AP. HPTLC-MS as a Neoteric Hyphenated Technique for Separation and Forensic Identification of Drugs. J Anal Sci Methods Instrum, 2018; 08(01): 1–15.
20. Claude E, Tower M, Lafont R, Wilson ID, Plumb RS. High Performance Thin-Layer Chromatography of Plant Ecdysteroids Coupled with Desorption Electrospray Ionisation–Ion Mobility–Time of Flight High Resolution Mass Spectrometry (HPTLC/DESI/IM/ToFMS). Chromatographia[Internet], 2020; 83(8): 1029–35. Availablefrom:https://doi.org/10.1007/s10337-020-03917-9