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Hyphenations of Mass Spectrometry

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ABSTRACT:
Hyphenation is usually means coupling of two or more analytical techniques together in a direct fashion. As we know that chromatography produces nearly pure or pure fractions of chemical components in a mixture. And spectroscopic method provides the information for identifying the components using standards. This coupling combines the advantages of both the chromatographic and spectral methods.This combination has the power for both the quantitative and qualitative analysis of unknown compounds in complex natural product extracts. In order to get the structural information to identify the compounds in a crude sample, liquid chromatography usually a high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) is combined to spectroscopic detection techniques., for example- Fourier transform infrared (FTIR), photodiode array (PDA) UV-vis absorbance or fluorescence emission, mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR), which resulted in various new hyphenated techniques., e.g., CE-MS, GC-MS, LC-MS and LC-NMR. Here hyphenation in mass spectrometry is discussed. This includes the coupling of chromatography such as LC with MS, GC with MS, CE with MS and TLC with MS.
Key words: Hyphenations, LC-MS, GC-MS, TLC-MS, CE-MS, Interfaces

I. INTRODUCTION:

Mass spectrometry:

Analytical technique for determining the molecular mass of compounds by measuring the mass-to-charge ratio of ions in the gaseous phase is "mass spectrometry".

Principle:

In this process, by the ionization source the sample to be analyzed is ionized by using various methods like protonation or deprotonation. The ions formed in the gas phase are electrostatically channeled into a mass analyzer simultaneously where the ions are separated according to their mass and detected through signals recorded on mass spectra[18].

Instrumentation:

The primary components of the mass spectrometry instrumentation are**[Fig. 1]**

Sample inlet: at low pressure samples are steadily streamed into the ionization chamber through a pinhole called "molecular leak"

Ionizer: samples are bombarded with an electron beam in order to generate positively ions

Accelerator: positively charged sample ions pass through three slits, which have voltages in decreasing order. Acceleration ensures that all the ions have the same kinetic energy

Deflector: based on its charge and mass magnetic field deflects ions. If an ion is heavy or has two or more positive charges, then it is least deflected. If an ion is either light or has one positive charge, then it is deflected the most

Detector: the detector detects the ions reaching it through the mass analyzer. Detection is achieved based on the mass-to-charge ratio of ions[19].

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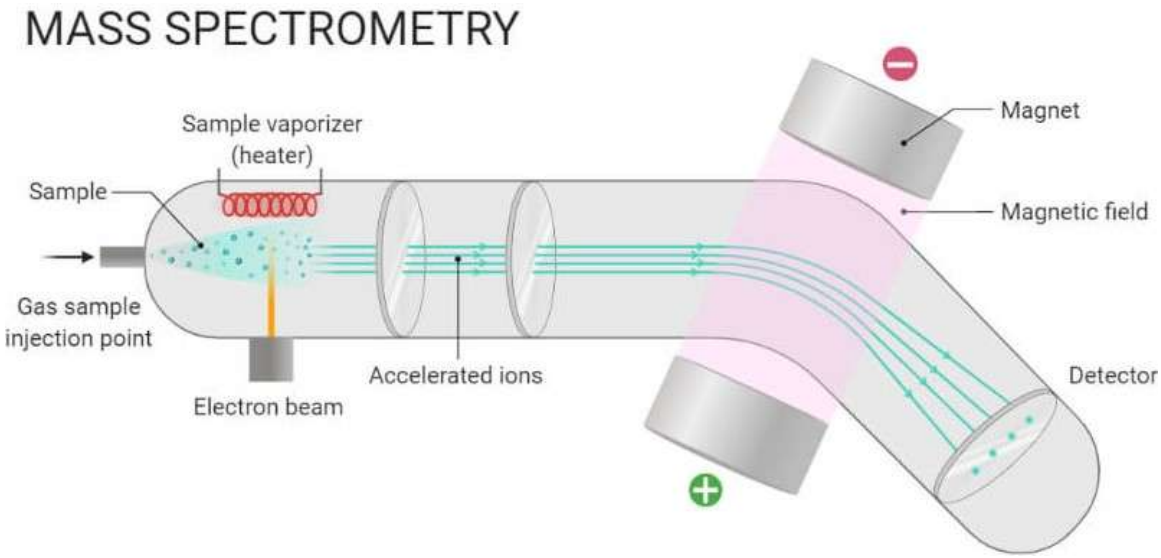

Fig. 1:Mass spectrometry instrumentation

Hyphenations of mass spectrometry:

Hyphenation is nothing but the combination of both chromatographic and spectral methods. As we know that chromatography produces nearly pure or pure fractions of chemical components in a mixture. And spectroscopic method provides the information for identifying the components using standards. This combination also combines the advantages of both methods.

In recent years in order to solve many complex analytical problems hyphenated techniques are being used. This combination has the power for both the quantitative and qualitative analysis of unknown compounds in complex natural product extracts. In order to get the structural information to identify the compounds in a crude sample, liquid chromatography usually a high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) is combined to spectroscopic detection techniques., for example- Fourier transform infrared (FTIR), photodiode array (PDA) UV-vis absorbance or fluorescence emission, mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR), which resulted in various new hyphenated techniques., e.g., CE-MS, GC-MS, LC-MS and LC-NMR. The most common and mostly used analytical separation for qualitative and quantitative analysis of compounds in natural product extracts is HPLC. LC-MS is mostly used than LC-NMR due to greater sensitivity[1].

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS):

Liquid chromatography-mass spectrometry (LC-MS) is a hyphenated technique that combines liquid chromatography with mass spectrometry. In chemical analysis the coupled chromatography-MS systems is popular as the combination itself enhances the capabilities of each technique. As the liquid chromatography separates the mixtures containing multiple components, and mass spectrometry provides structural information which is used to identify each separated component[2]. MS is not only sensitive but also provides selective detection. LC-MS system in addition to liquid chromatographic and mass spectrometric devices, It also contains an interface which transfers the separated components from LC column into the MS ion source. Because the LC and MS devices are fundamentally incompatible the interface is necessary. As the mobile phase in a LC system is a pressurized liquid and the MS analyzers mostly operate under high vacuum, hence interface is the simple part of the LC-MS system which transfers the maximum amount of analyte, removes a significant amount of used mobile phase in LC and preserves the chemical identity of the chromatography products[3].

INSTRUMENTATION:

LC unit basic componnets are -

Pump- It delivers the mobile phase with required flow rate

Autosampler-It injects the sample automatically

Column- It is the main component where separation takes place

Detector- It is used for the analysis of separated componnets

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Where as LC-MS instrumentation consists of**[Fig. 2]**

1) A LC unit
2) An interface between the LC and MS
3) An ion source that ionizes samples (e.g. API unit)
4) An ion guide (an electrostatic lens that efficiently introduces the generated ions into the MS)
5) A mass analyzer unit that separatesthe ions based on their mass to charge ratio (m/z)
6) A detector unit that detects the separated ions [6]

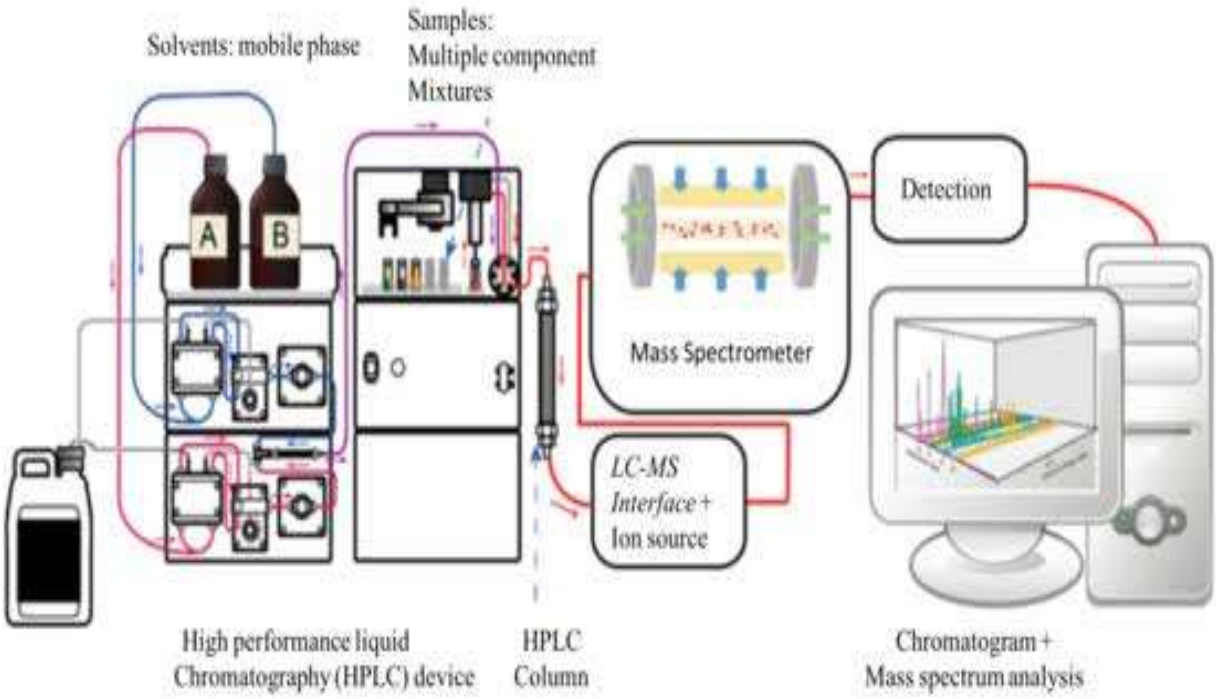

Fig. 2:Schematic diagram of LC-MS

Working Of Liquid Chromatography (Lc)& Mass Spectrometry:

LC:

Pump present in LC is used to deliver the mobile phase at a required flow rate. The autosampler injector injects the sample into the mobile phase flow, along with mobile phase the sample reaches the column. Separation of molecules takes place in the column based on the polarity of sample molecules by the principle of adsorption. The separated molecules moves towards the detector where it is detected.

MASS SPECTROMETTRY:

In mass spectrometry the molecules present in sample are bombarded with an electron beam. After bombardment the molecules gets ionized which causes the molecule to break into many charged fragments. According to their mass-to-charge ratios these ions are then separated by accelerating them and subjected to both magnetic and electric filed. Ions with same m/z ratios will undergo the same amount of deflection.They are detected and signals are recorded as mass spectra[4].

In case of LC-MS interfaces are used that transfers the components which are separated in the LC column into the MS ion source. Different interfaces used are-

Electrospray ionization (ESI)
Atmospheric pressure chemical ionization (APCI)
Atmospheric pressure photo-ionization (APPI)

Newer ionization techniques which facilitate the transition from a high-pressure environment to high vacuum conditions needed at the MS analyzer. Various drying and deposition techniques were used in the past but the most common of these was the off-line MALDI deposition.

Electrospray ionization (ESI):

Fenn and collaborators developed ESI interface for LC-MS systems in 1988. For analysis of moderately polar molecules (e.g., metabolites, peptides and xenobiotics) this interface is used.

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Through a metal capillary which is kept at 3 to 5kV the liquid eluate coming out of the LC column was pumped. At the top of the capillary the liquid is nebulized and a fine spray of charged droplets is formed. This capillary is usually perpendicularly located at the inlet of the MS system in order to avoid contamination. By the electric potential the heat is created which is used to rapidly evaporate the droplets in an atmosphere of dry nitrogen. As the charged ions flow through a series of small apertures with the aid of focusing voltages, ionized analytes are transferred later into the high vacuum chamber of the MS. Negatively and positively charged ions can be detected and it is possible to switch between the negative and positive modes of separation. Most of the ions produced in the ESI interfaced are multi charged[5]. The use of 1-3mm internal diameter microbore columns is recommended for LC-MS systems using electrospray ionization (ESI) interfaces because optimal operation is achieved with flow rates in the 50-200µl/min range[20].

Atmospheric pressure chemical ionization (APCI):

In the early 1973 the development of APCI interface for LC-MS started with Horning and collaborators. For analysis of small, neutral, relatively non-polar and thermally stable molecules like steroids, lipids and fat-soluble vitamins APCI interface can be used. By using ESI these compounds are not well ionized. APCI in addition can also handle mobile phase streams containing buffering agents. Through a capillary the liquid from the LC system is pumped and at the tip nebulization is also there where the corona discharge takes place. At the ion sourcefirstly the ionizing gas surrounding the interface and the mobile phase solvent are subjected to chemical ionization. These ions later react with the analyte and transfer their charge. Then the sample ions pass through small orifice skimmers by ion-focusing lenses. The ions are subjected to mass analysis inside the high vacuum region. This interface can be operated in both negative and positive charged modes and mainly singly charged ions are produced[5]. Flow rates between 500 and 2000µl/min also can be handled by APCI ion source and it can be directly connected to conventional 4.6mm internal diameter columns[7].

Atmospheric pressure photoionization (APPI):

In the early 2000s Bruins and Syage developed the APPI interface for LC-MS. The ions that cannot be ionized using ESI can be analyzed by using APPI[8]. APCI and APPI interfaces mostly similar but the difference is instead of corona discharge in APCI, in APPI the ionization occurs by using photons coming from a discharge lamp. By absorption of a photon and ejection of an electron singly charged analyte molecular ions are formed in the direct APPI mode. An easily ionizable compound is added to the mobile phase or the nebulizing gas to promote a reaction of charge-exchange between the dopant molecular ion and the analyte in dopant APPI mode.Later the ionized sample is transferred to the mass analyzer at high vacuums as it passes through small orifice skimmers[6].

Table-1: Advantages and disadvantages of LC-MS

ADVANTAGES	DISADVANTAGES
Suitable for analysis of relatively polar compounds with low, moderate or high molecular weights, depending on which ionization method is applied.	To operate and data analysis of the liquid chromatography and mass spectrometry (LC-MS) requires a skilled and trained person.
Derivatization not required. Thermo-stable compounds can be analyzed	Not all compounds in a plant extract will ionize under the same conditions: the technique is therefore more suited for targeted analysis
This can help in the manufacturing of pharmaceuticals to streamline their research or analysis processes and compliance with regulatory guidelines	Phosphate buffer is not compatible with the LC-MS analysis, which is the most commonly used buffer in HPLC method development
The LC-MS offers high selectivity, resolution, precise mass, and specificity as compared with other	LC-MS has another disadvantage is that the residual impurities being analysed

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chromatography techniques	should be ionized
This is an incomparable method to identify unknown components of a sample solution	This is a not a portable instrument: it requires special and more space

APPLICATIONS OF LC-MS:

1. It is used in determination of molecular weight
2. LC-MS is used in structural elucidation
3. For quantitative analysis of glucocorticoids and stimulants in biological fluids
4. Application of LC-MS in forensic sciences-toxins present in different material can be determined by using LC-MS. By using LC-MS any toxic metabolites in food or beverages can be determined.
5. e.g., identification of detergent added into orange juice can be determined by analyzing the juice and detergent sample
6. LC-MS is used in determination of different components present in the fertilizers and pesticides
7. For detection of phenyl urea herbicides and for detection of low level of carbaryl in food LC-MS is used
8. For determination of assay of drug substances, drug products, intermediates and their related compounds in pharmaceutical industry LC-MS is used
9. In the study of ADME properties of drugs LC-MS is used
10. LC-MS plays an important role in drug discovery and drug development process. A variety of LC-MS applications for the pharma industry have been developed for compound identification and quantification since the introduction of electrospray ionization[21]

Gas Chromatography-Mass Spectrometry (GC-MS):

Gas chromatography-mass spectrometry (GC-MS) is a hyphenated technique which combines both the features of gas chromatography and mass spectrometry in order to identify different substances in a test sample. Like LC-MS it allows analysis and detection even small amounts of a substance. 2 major building blocks are present in GC-MS, the gas chromatograph and the mass spectrometer. A capillary column is utilized by gas chromatograph,its properties regarding molecule separation depends not only on the column dimensions but also on the phase properties.As the sample travels the length of the column the separation of the molecules is promoted by the difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column. In the column the molecules get retained and then they elute from the column at different times and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratios (m/z). This hyphenation provides more advantages than the single component itself. Accurate identification of a particular molecule by gas chromatography or mass spectrometer alone is not possible[16].

PRINCIPLE:

Into the GC inlet the sample solution is injected where it is vaporized and carried into the column by the carrier gas. The sample mixture which passes through the column gets separated by means of their relative interaction with the stationary phase and the carrier gas. The effluent from the column passes through a heated transfer line and enters the ion source where the effluents from the column are converted to ions and detected according to their mass to charge (m/z) ratio[14]

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INSTRUMENTATION:

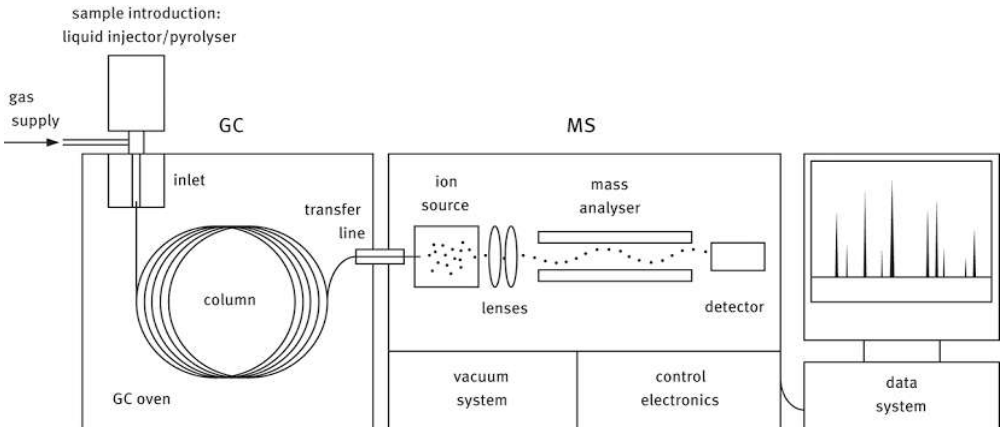

Fig. 3: Schematic diagram of GC-MS

With sample preparation, injection and separation on a GC column a GC-MS experiment begins. An interface is required to direct the molecules from GC column to the mass spectrometer since the operation of a mass spectrometer requires a high vacuum system. The molecules leaving the column enter an ionization chamber where they are bombarded with the stream of energetic electrons which ionizes and fragments some of the molecules in the most common type of instrument. Molecular ions (unfragmented) and ions are formed due to re-arrangement or fragmentation reactions. The ions are accelerated and rapidly sorted according to the mass to charge ratio by use of a magnetic or electric field in a mass analyzer. The mass analyzer can sort thousands of different ion masses (m/z) per second. The current of electrons generated is measured when the ions strike the detector for each m/z ion abundance is then counted by a detector. MS serves as the detector for GC which generates a chromatogram indicating the quantity of each compound as a function of retention time. The underlying dimension of data specific to MS is called a mass spectrum, which is a histogram of the abundance of each ion as a function of m/z and serves as the fingerprint to identify the compound represented by a peak on the chromatogram[14] **[Fig. 3]**

Interface-

An ideal interface should-

- Quantitatively transfer all analyte
- Reduce pressure/flow from chromatograph to level that MS can handle
- No interface meets all the requirements
- The major goal of the interface is to remove most of the carrier gas- the majority of the effluent

Different interfaces are-

- Classical or molecularjet interface
- Direct capillary infusion interface
- Permeation separator
- Open slit

Molecular jet separator:

In these separators, into an evacuated chamber the GC flow is introduced through a restricted capillary. A supersonic expanding jet of analyte and carrier molecules is formed at the capillary tip and its core area sampled into the mass spectrometer. In an expanding jet, in the core flow high molecular mass compounds are concentrated whereas the lighter and more diffusive carrier molecules are dispersed away, in part through collisions. Thus sampling of the core flow produces an enrichment of the analyte. The jet interface is very versatile, inert and efficient despite disadvantages of reduced efficiency with more volatile compounds and potential plugging problems at the capillary restrictor

Permeation interface:

This interface is made of a silicone-rubber membrane which acts as a barrier for (non-organic) carrier gases and transmits organic non-polar molecules. Despite being a very effective enrichment procedure, it also suffers from discrimination effects with more polar analytes and produces significant band broadening of their chromatographic peaks. Major problems regarding this approach- membrane selectivity was based on polarity and molecular weight, slow to respond, only a small amount of analyte actually permeates through membranes

Watson-Biemann effusion separator:

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The molecular effusion (or Watson Biemann) interface is based on the molecular filtering of the gas effluent by means of a porous glass frit. The column effluent passes through a fritted tube situated in a vacuum chamber. Small molecules traverse the microscopic pores in the walls and are evacuated whereas high molecular mass molecules are transferred to the ion source. The main drawbacks of this interface are the high dead volume added and its high surface. The three methods presented above are based on the enrichment of the analyte in the carrier gas by eliminating carrier molecules. In this way enough sample can be introduced into the ion source with total gas flows compatible with the pumping capacity of the system. Among the above the jet separator has been the most expensively used and is perhaps the most successful interface.

The simple alternative to reduced total gas flow is flow splitting. In this case no sample enrichment takes place and these procedures are most useful where sensitivity is not a critical factor. Flow splitting can be performed at the exit of the gas chromatograph allowing the diverted gas to be directed to a parallel detector, or at the interface itself such as in the open split interface. Later is based on a capillary restrictor that limits the flow entering the ion source to a manageable constant value. The GC column exit is situated close to the restrictor entrance in an open connector. The restrictor samples the effluent from the GC column exit and the excess column flow is removed from the connector by helium.

Open split interface:

It is somewhat similar to a jet separator. The MS pulls in about 1ml/min through the flow restrictor. If column flow is above that excess is vented. If flow is below that He from external source is pulled in. best for sources that have flows close to 1ml/min like capillary columns. Advantages include any gas producing source can be used, low cost and easy to use. Drawbacks include sample leaves column in split, Split changes as flow changes[15].

Table-2: Advantages and disadvantages of GC-MS

ADVANTAGES	DISADVANTAGES
GC-MS is fast and in expensive	Compounds with vapor pressure exceeding 10torr are analyzed by GC-MS
GC-MS is highly efficient and has high resolution	Determination of positional substituents on aromatic rings was difficult with GC-MS
Non-destructive sample recovery is possible	If MS feed is poor it results in background noise in the mass spectrum
Sample size is small	Certain isomeric compounds cannot be determined by mass spectrometer
Suitable for analysis of mixtures	Not suitable for non-volatile and thermo unstable compounds
Suitable for analysis of low molecular weight compounds	Requires derivatization depending on the molecules that are analyzed. Derivatization can mask the result

Applications of GC-MS:

1. GC-MS provides enhanced sample identification, higher sensitivity, an increased range of analyzable samples and faster results which enable a whole new range of applications for GC-MS in several areas
2. For the detection of several congenital diseases in screening tests GC-MS is used. It detects the oils present in ointments, creams and lotions. It detects minute amounts of chemicals present in the urine of patients with genetic metabolic disorders.
3. Environmental pollutants monitoring is major applications of GC-MS. It is widely used in the detection of dibenzofurans, herbicides, sulfur, phenols, dioxins, pesticides in air, soil, water
4. GC-MS is used in the synthesis and characterization of compounds and in pharmaceutical biotechnology
5. Fire debris analysis can be performed as per the American society for testing materials standards by using GC-MS. In forensic toxicology in order to identify poisons and steroids in biological specimens and also in anti-doping labs in order to detect performance enhancing drugs such as anabolic steroids GC-MSis widely used

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6. In public places explosive detection systems GC-MS technique is used for the analysis and detection of chemical warfare agents
7. For the analysis of body fluids in order to detect narcotics, barbiturates, drugs such as anesthetics, antihistamines, anti-epileptic drugs and anticonvulsants. It is also used in detecting metabolites and pollutants in serum and in fatty acid profiling in microbes
8. For the analysis of inorganic gases and aromatic solvents, detection of impurities and allergens in cosmetics GC-MS is used
9. It is also used in the synthesis of cellulose acetate, polyethylene, polyvinyl and synthetic fibers[22]

Table-3: Differences between LC-MS and GC-MS-

Aspect	LC-MS	GC-MS
Mobile phase	LC-MS uses solvent as mobile phase	GC-MS uses inert gases like helium as mobile phase
Used for	Samples which are thermally fragile and non-volatile	Samples which are thermally stable
Sample preparations for biological fluids	Protein precipitation, liquid-liquid extraction, solid-phase extraction	Liquid-liquid extraction, solid-phase extraction, derivatization (to make polar compoundsmorevolatile)[23]
Limits of detection and quantification	Picograms 10^{-12}g to femtograms 10^{-15}g	Nanogram 10^{-9} g to picograms 10^{-12} g
Forensic identification	Not preferrable	Mostly preferred technique as it tests for specific substances and not for general composition or identification
Cost	Costlier than GC-MS	Lesser costs than LC-MS
Operation process	Not Easier to operate	easy to operate

Capillary Electrophoresis-Mass Spectrometry (CE-MS):

Capillary electrophoresis is a separation method based on the differential rates of migration of charged species in an applied dc electric field[17]. The speed of movement is determined by their charge and size ratios. High voltage is generated over the capillary and due to this electric field the sample moves through the capillary at different speeds. The components move towards the opposite electrodes[13].Mass spectrometry is a process used to find out the mass of particles and also to find basic chemicals in a sample/molecule. It is a technique which is used to quantify known materials. To identify unknown compounds within a sample and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios and relative abundance [12]. A mass spectrum is displayed in the form of a plot of ion abundance Vs mass to charge ratio. Mass spectrometry is applicable across diverse fields including forensic toxicology, metabolomics, proteomics, biopharma and clinical research etc.

It is an analytical technique formed by the combination of the liquid separation process of capillary electrophoresis with mass spectrometry**[Fig.4]**. This hyphenation accelerates the sensitivity of CE analyses. MS in combination with CE, separates on the basis of an analytes charge to size ratio and then on basis of its mass to charge ratio. By this hyphenation both the separation and characterization molecules complete in single analysis. CE-MS has high resolving power, sensitivity, requires minimal volume and can analyze at high speed. Ions are typically formed by electrospray ionization, but they can also be formed by matrix assisted laser desorption and other ionization techniques. When a MS detector is linked to CE system for acquiring online MS data of the separated compound, the resulting combination is termed as CE-MS[24].

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INSTRUMENTATION:

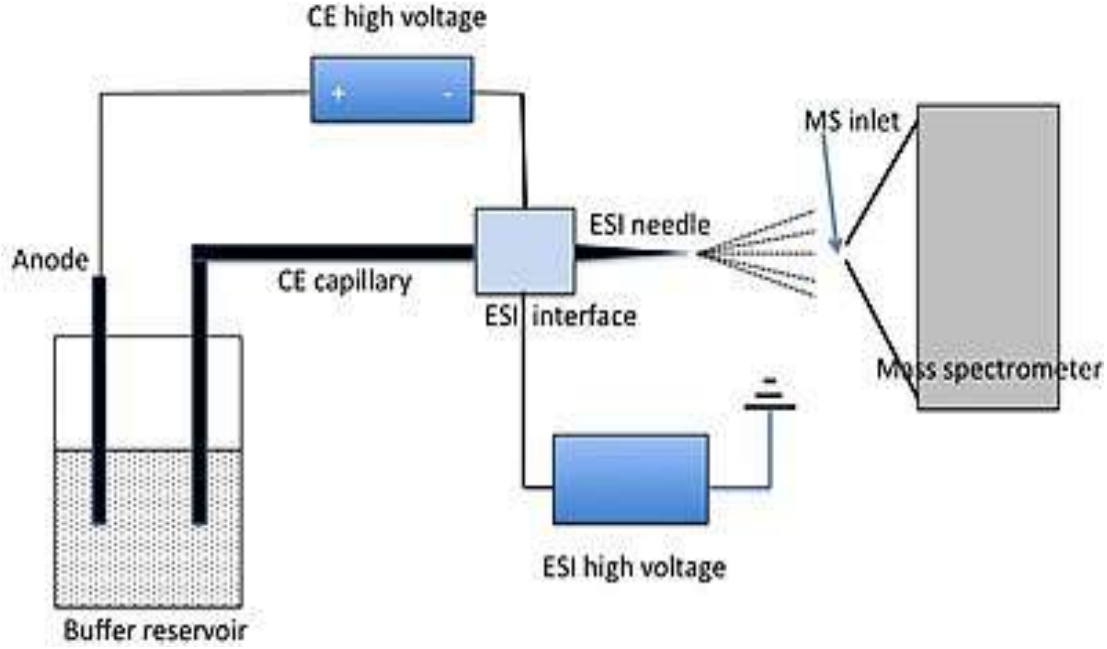

Fig. 4:Schematic diagram of CE-ESI-MS system

CAPILLARY ELECTROPHORESIS-
Injector
Capillary
Background electrolyte vessel
High voltage supply
Electrode
Detector

MASS SPECTROMETRY-
MS interface
Analyzer
Detector

INJECTOR-

Sample is injected into capillary tube. The most common sample introduction is electrokinetic and hydrodynamic type of injection. With pressure injection, the sample introduction end of the capillary is also placed momentary into a small cup containing the sample, and pressure difference is then used to drive the sample solution into the capillary. The pressure difference can end by pressurizing the sample or by elevation the sample end. Hydrostatic injections do not discriminate due to ion mobility, but cannot be used in gel filled capillaries

CAPILLARY-

A buffer filled fused silica capillary which is 40 to 100 cm long and 10 to 100µm internal diameter extends between 2 buffer reservoirs that also holds platinum electrode. The sample is introduced at one end and detection at another end. The detector should be placed at right side i.e. near the cathode electrode. The sample is injected through the hole created on the capillary tube i.e. 0.5 mm detector window. If a provision is absent, then the capillary tube is heated with 96-98% of conc.H_2SO_4 or conc. KOH at 130ºc[22.]

BGE (background electrolyte) VESSEL-

The selection of buffer for electroosmotic flow and electrophoretic mobility is based upon analyte behavior, pH constants. The selected buffer should be of good quality and should be prepared under optimized concentration. Phosphate buffer, ethanoate buffer and borate buffers are the most commonly used buffers in capillary electrophoresis. Buffer additives like certain organic compounds, surfactants, organic & inorganic salts also are used.

HIGH VOLTAGE SUPPLY-

High potentials can be applied in CE with current technology, up to 30kV can be applied for extremely fast and efficient separation. When the elements are migrating at completely different rates along the length, though separated by the electrophoretic migration, the complete sample is drawn towards cathode by electro endosmosis. Cooling systems are maintained to reduce the temperature or reduce heat from the system [23]

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ELECTRODES-

An electrode is an electrical conductor that contacts with the nonmetallic parts of a circuit, such as an electrolyte, semiconductor. It is referred to as either an anode or a cathode in an electrochemical cell. The electrode at which electrons leave the cell and oxidation occurs is anode and cathode as the electrode at which electrons enter the cell and reduction occurs. Each electrode may become either the anode or the cathode depending upon the direction of current through the cell. An electrode that functions as the anode of one cell and the cathode of another cell is called as bipolar electrode[25]

DETECTORS-

Separation by capillary electrophoresis can be detected by several detection devices. Different detector configurations are possible. As the amount of each analyte passing through the detector is very minute, shooting the source lamp along a short section of the capillary increases the path length and if absorbance is being used, decreases the detection limit.

The most commonly used are:

1. UV Absorption
2. Fluorescence
3. Conductivity
4. Potential gradient detector
5. Diode array detector
6. Refractive index detector
7. Atomic absorption[8]

MS INTERFACE-

The major problem faced when coupling CE to MS arises due to insufficient understanding of fundamental processes when two techniques are interfaced. The separation and detection of the analyte can be improved with a better interface. Mostly used ionization technique is ESI.

The three setups commonly used in CE-ESI-MS coupling are:

- ✓ Coaxial sheath liquid
- ✓ Liquid junction
- ✓ Sheath-less or Nano-spray

ELECTROSPRAY IONISATION (ESI):

1. The first CE-MS interface had cathode end of CE capillary terminated within a stainless-steel capillary.
2. At that point electrical contact was made completing the circuit and initiates the electrospray. Few drawbacks are there in this interface system like mismatch in the flow rates of two systems.
3. Since then, the interface system has been improved to have a continuous flow rate and good electrical contact. At present, three types of interface systems exists for CE-ESI-MS, which are discussed briefly.

Sheath Less Interface-

CE capillary is coupled directly to an ESI source with a sheath less interface system.The electrical contact for ESI is realized by using capillary coated with conductive metal.The system has high sensitivity, low flow rates and minimum background as sheath liquid is not used. However, these interface designs all have changes including low mechanical robustness, poor reproducibility.The latest sheath less interface design features porous ESI emitter through chemical etching. Robust interfacing with mass spectrometry was provided by this design and shows the reproducibility challenges associated with previous designs[9].

Sheath Flow Interface-

When the CE separation liquid is mixed with sheath liquid flowing coaxially in a metal capillary tubing the electrical connection is established with the sheath flow interface.1:1 mixture of water-methanol with 0.1% CH_3COOHor HCOOH is commonly used sheath liquid.The system is more reliable and has a wide selection range of separation electrolytes.Due to sheath liquid, there might be chances for decrease in sensitivity[10]

Liquid Junction Interface-

This technique uses a stain-less steel tee to mix separation electrolyte from CE capillary with makeup liquid. Through opposite sides of the tee the CE capillary and ESI needle are inserted and a narrow gap is maintained. The electrical contact is established by makeup liquid surrounding the junction between 2 capillaries. This system is easy to operate. However, the sensitivity is reduced and the mixing of 2 liquids could degrade separation. Pressurized liquid junction is one of the type of liquid junction interfaces, where pressure is applied to reservoir with makeup liquid. In this method dilution is less than in traditional liquid junction interface due to low flow rates. Resolution increases due to defocusing of the CE effluent which is prevented by additional pressure.

Continuous Flow Fast Atom Bombardment-

Using continuous flow interface CE can be coupled to fast atom bombardment ionization.

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The interface should match the flow rate between the two systems. The Continuous flow fast atom bombardment requires a relatively high flow rate but capillary-electrophoresis needs low flow rate for better separation. A makeup flow can be used using a sheath flow or liquid junction.

$$Xe + e^- \longrightarrow Xe^+ + 2e^-$$

Formed radical ion react with Xe which is already present in the chamber

$$Xe + Xe^+ \longrightarrow Xe + Xe^+$$

Neutral atoms which are accelerated hits the matrix-sample mixture and ionize the sample
Electric field is used to remove free radical cations

Coupling of CE with MALDI-MS-

Off line coupling of Capillary Electrophoresis (CE) to MALDI, on the MALDI target plate the CE effluent is sprayed, dried and analyzed by MS. A moving target with continuous contact to CE capillary end is required for online coupling. After the moving target entered into MS it is desorbed and ionized. It is a desorption technique.Musyimi et al. developed a new technique where rotating ball was used to transfer CE to MS.The sample from CE is mixed matrix coming through another capillary.As the ball rotates the sample is dried before it reaches ionization region.As makeup fluid is not used, this technique has high sensitivity.

ADVANTAGES:

The main advantage of using CE-MS: It is automated

1. It provides spontaneous and faster separation with high resolution
2. Small volumes of sample is required for analysis which is quite robust and inexpensive capillary is used
3. It is compatible with most buffers and analytes
4. CE-MS enables sequence analysis via MS/MS with platform-independent sample separation which facilitates the independent entry of different sequencing platforms for peptide sequencing of CE-MS defined biomarkers from highly complex mixtures

DISADVANTAGES:

1. For analysis of high molecular weight proteins (greater than 20 kDa) CE-MS is not suitable as they tend to precipitate in the acidic buffers that are generally used for CE-MS analysis

It is a desorption ionization technique**.** Sample-matrix mixture is formed by mixing sample and a matrix. Gases like Xe or Ar becomes radical by entering the chamber

2. Small samples can only be loaded onto the capillary which leads to lower selectivity compared with LC[26]

APPLICATIONS-

1. CE-MS can separate analytes even in minute concentrations at high speed with high efficiency has made it applicable in all fields of science
2. CE-MS can be used for routine clinical checkup
3. CE-MS is a proteomic technology widely used for proteomic analysis of urine[11]
4. CE-MS is a useful tool for discovery of potential biomarkers. For early prostate cancer diagnosis and monitoring
5. CE-MS has been used for bioanalytical, pharmaceuticals, environmental and forensic application
6. Major application CE-MS has been used for biological studies, mostly for protein and peptide analysis
7. For synthetic in-vitro glycolysis studies- A synthetic in-vitro glycolysis was reconstructed from 10 purified E. coli enzymes to obtain a better understanding of the regulation of sequential enzymatic reactions
8. Characteristics of Monoclonal Antibodies- Monoclonal antibodies are highly heterogenous proteins, thereby requiring a battery of sophisticated analytical techniques. For their complete characterization it has become a routine tool for analysis of recombinant protein therapeutics in the biotechnology industry
9. Analysis of O-Glycopeptides by Acetone enrichment
10. Identification of anthraquinone coloring matters in natural red dyes

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11. Development for profiling metabolites of steroid hormone metabolism[9]

TLC-MS:

Thin layer chromatography:

A technique used to isolate non-volatile mixtures is thin layer chromatography. The experiment is conducted on adsorbent material coated sheet of aluminum foil, plastic or glass. The adsorbent material is usually made of aluminum oxide, cellulose or silica gel. After completion of separation each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f= distance travelled by sample/distance travelled by solvent

Principle of TLC- TLC depends on the separation principle. Separation depends on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. On completion of separation the individual components from the mixture appear as spots at respective levels on the plates.

TLC-MS combines the speed and ease of thin layer chromatography with powerful on-line identification by mass spectrometry. Enabled by the recent development of special interfaces and MS-grade plates for TLC and HPTLC, the coupling technique allows the fast trace analysis with extremely low detection limits. This makes TLC-MS ideal for analytical use in a wide range of industries, such as pharmaceuticals, cosmetics, food and beverages.

TLC-MS Techniques:

TLC-MS coupling can be performed using an elution-based, or desorption-based technique. Both methods are offline and are commenced after the separation is completed and the plate dried. Sample transfer to the mass spectrometer is fast and typically takes less than one minute**[Fig.5]**

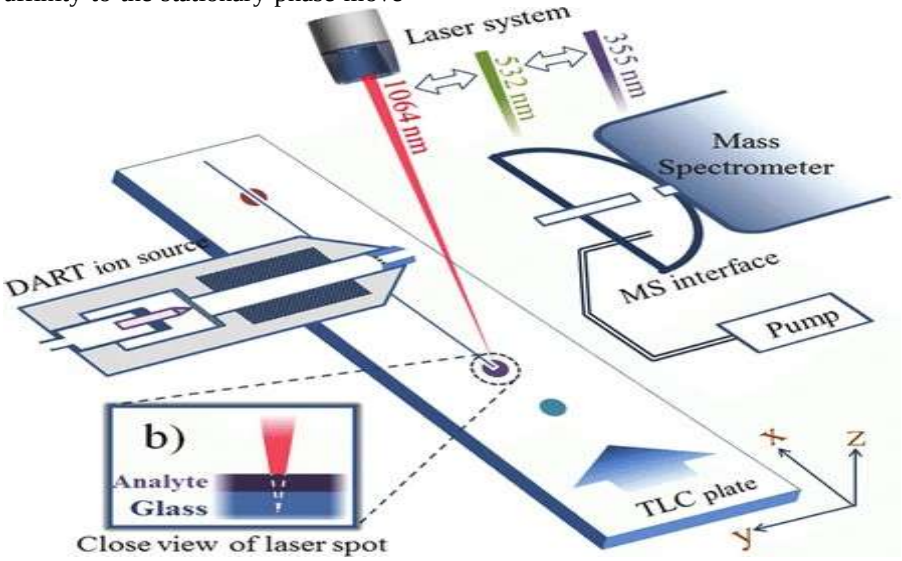

Fig. 5: Schematic diagram of TLC-MS

Elution based TLC-MS (indirect MS analysis): the analyte on the TLC plate is first scraped, extracted, purified, concentrated and then transferred in the liquid phase to the mass spectrometers ion source for further analysis

Desorption based TLC-MS (Direct MS analysis): the analyte is vaporized from the silica layer, and transferred to the mass spectrometer in the gas phase. Vaporization techniques include gas beam, ion bombardment and MALDI (matrix assisted laser desorption/ionization). Working conditions-desorption based TLC-MS is conventionally performed under vacuum, however analysis under atmospheric pressure is also possible through the use of ambient mass spectrometry

Camag TLC-MS interface2:

Contamination free and rapid elution ofselected zones. Online transfer to the mass spectrometer. Plug and play installation. Compatible with any LC-MS system. Confirmation of known substances within a minute. Highly effective back washing function prevents the elution path from becoming blocked. Easy handling ensures accurate and

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reproducible plate positioning. Low solvent consumption.

Other developing TLC-MS techniques-

TLC-MALDI-MS:

Bruker daltonics introduced an adapter that allows you 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 separations. However the data evaluation software enables to 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 a different color.

TLC-DART-MS and TLC-DESI-MS:

Like MALDI, direct analysis in real time (DART) and desorption electro spray ionization (DESI) are general surface analysis techniques and therefore also lend themselves to TLC analysis. The mode of operation at the surface of the plate is somewhat similar, but in DART-MS a gas stream is focused on the TLC plate: in DESI the stream hitting the surface is a solvent mixture. The AccuTOF-DART 4G is a powerful problemsolving tool for a wide range of applications

Advantages:

a) Excellent efficiency and resolution- silica gel matrix of MS-grade plates has considerably fewer impurities than standard plates, mass spectra are obtained quickly by direct sample access on TLC plates at room temperature
b) High sensitivity with low background-MS-grade plates are much cleaner than standard TLC or HPTLC plates, thus ensuring superior sensitivity with extremely lower background signals, MS-grade plates are packed in aluminum foil to prevent contamination
c) Direct coupling of TLC plate with MS without any pretreatment of the separated chromatographic bands
d) With MS there is possibility of identification of individual bands
e) There is possibility of coupling TLC plate with a variety of MS types[27]

II. CONCLUSION-

The technique which was developed from the coupling of a separation technique and on-line spectroscopic detection technology is known as hyphenated technique. In this current article, introduction, instrumentation, working and applications of various hyphenated techniques, e.g., LC-MS, GC-MS, CE-MS, TLC-MS are discussed.

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Conflicts of interest:

The authors declare no potential conflicts of interest

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