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Enhancing Pharmaceutical Analysis: Exploring Hyphenated Techniques"

Miss. Rutuja Ravindra Kokane [1] , Miss. Alfa Jain [2], Miss. Rutuja Haridas Mane[3], Miss. Rutuja Sanjay Kokane [4], Miss. Rucha Ravindra Gaikwad[5]
Institute of pharmaceutical science and research (for girls) (College code – 6914) Pune -Solapur Highway, Swami chincholi (Bhigwan), Tal. Daund, Dist – Pune 413130

ABSTRACT:

Hyphenated separation techniques refer to a mixture of two or more methods for separating and detecting chemicals from solution.The purpose of the pairing is to gain an information rich detection with a single analytical technique for both identification and quantification.The hyphenated technique is the combination or the coupling of the different analytical techniques. Mainly chromatographic techniques are combined with spectroscopic techniques. Hyphenated techniques in analytical chemistry involve the combination of two or more separation and/or detection methods to enhance analytical capabilities. This review explores the principles, applications, and advancements of hyphenated techniques, highlighting their synergy in providing comprehensive insights into complex sample analysis. The integration of chromatography with mass spectrometry, spectroscopy, or other methods leads to improved sensitivity, selectivity, and overall analytical performance. This review delves into the diverse applications across various scientific disciplines, showcasing the evolving landscape of hyphenated techniques and their pivotal role in modern analytical research.

KEY WORDS: Analytical techniques, spectroscopy, hyphenated techniques

INTRODUCTION :

The projects of drug development can help to develop hundreds of thousands of compounds that may analyze for their presence of impurities and structure and for qualitative and quantitative estimation of drug in analytical chemistry[1]. In analytical chemistry the analysis of drug is done to separation, estimation quantification of a compounds that are obtained from natural as well as artificial sources. The number of drugs is introduced into the market per year. They can be new drug entities or partial structural modification of the drug that exists already.[2,3]. For analysis of small chemical entities as well as larger molecules, life scientists increasing rely on methods like High performance liquid chromatography [HPLC] High performance thin layer liquid chromatography [HPTLC] Liquid chromatography – Mass spectroscopy [LC-MC] is an analytical technique that gives high resolution chromatographic separation with sensitive and specific mass spectroscopic detection. This also includes high performance liquid chromatography -Mass spectroscopy [HPLC-MS]. It is the most powerful technique for

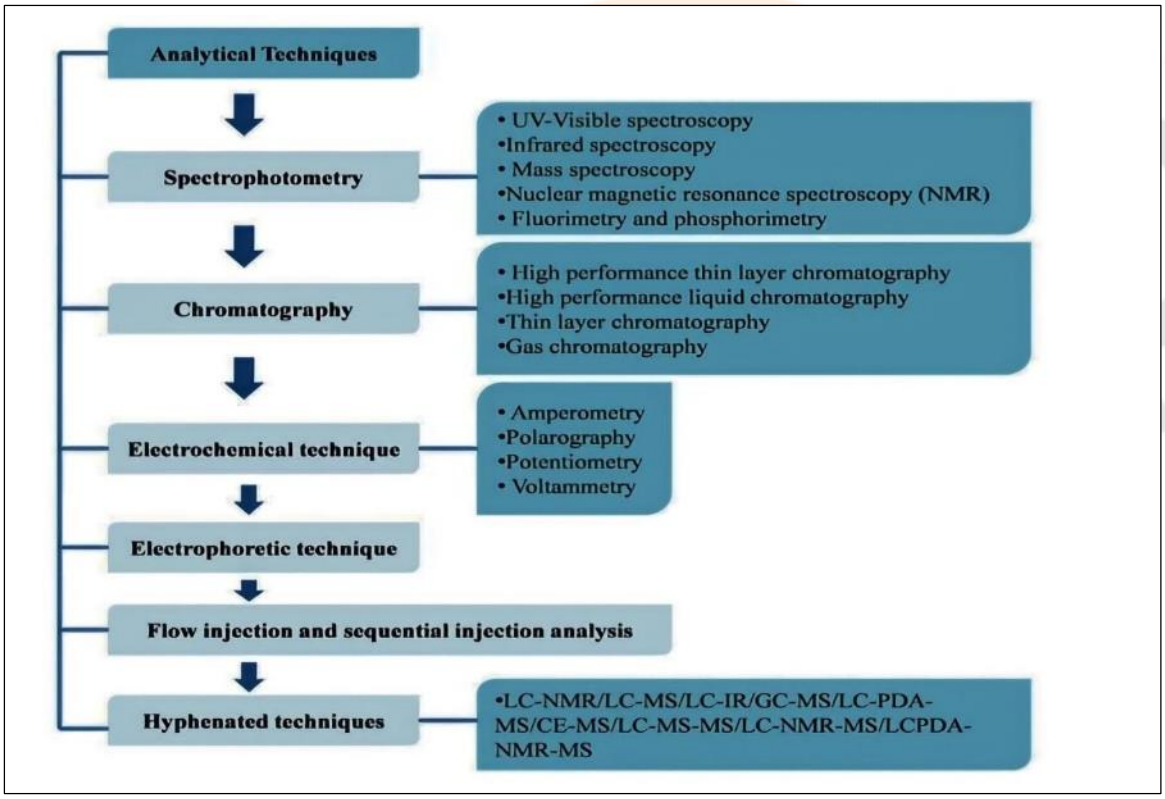
analysis. New analytical techniques are being used to improve drug discovery, development, and patient care. This technique also includes pharmacogenomics, which analyzes how an individual's genetic makeup affects their response to medications. There are also advanced analytical methods like HPLC and Mass spectroscopy which help in drug analysis and quality control. These techniques enable pharmacists to personalize treatments and ensure the safety and efficacy of the medications.[4]

In addition to pharmacogenomics, HPTLC, HPLC, and Mass spectroscopy there are other analytical techniques. One such is metabolomics, which involves studying the metabolites in the body to understand drug metabolism and identify potential biomarkers. Also, another technique called proteomics, which focuses on analyzing proteins to gain insights into disease mechanisms and drug targets. Also, bioinformatics plays a crucial role in analyzing large-scale biological data to identify patterns and make predictions. [6]

The necessity of method development is for at some point of manufacturing technique and development of drug, the principal purpose of analytical strategies is to get data regarding efficiency, impurity, bioavailability, stability, and effect of producing to verify that the assembly of drug product is steady.[7] The process of analytical technique starts with two main categories including qualitative and quantitative analysis. The qualitative analysis only the obtainable samples are estimated while in qualitative analysis the total number of elements in a compound should be identified. [8,9] By using these analytical techniques during the development of drugs there are many compounds generated by inventors and they can easily determine their structure, behavior, also helps to find the impurities in a compound. If all parameters are done of the targeted drug, then the bioassay of drugs will perform to find out that how it will be going to work, and functions analytically. [10,11]

Hyphenated techniques are essential to scientific analysis because they combine two or more analytical techniques to provide a thorough understanding of complex systems. This synergistic approach offers deeper insights into a variety of samples while enhancing the capabilities of individual techniques. The purpose of this introduction is to discuss the importance and uses of hyphenated techniques in developing analytical methodologies for a deeper examination of different phenomena. [12]

- **Analytical Techniques for Method Development**



- **SPECTROSCOPY:**

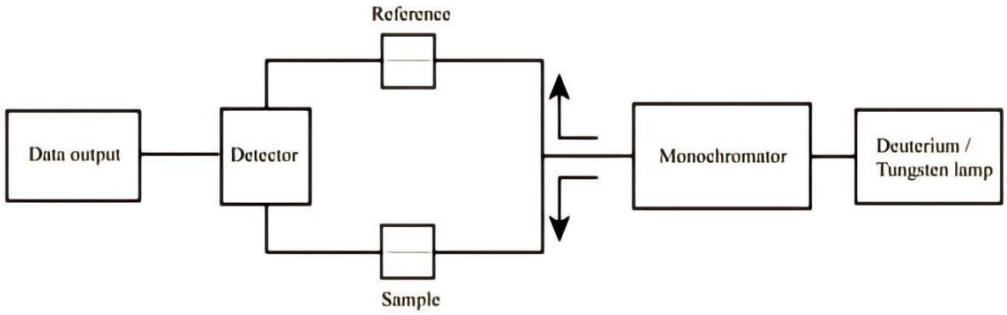
The most crucial approach for the method development process was the spectroscopic technique. This method is based on various chemical reactions and the natural absorption of UV rays as described in our pharmacopoeias. The wavelength function, characteristics transmission, and quantitative measurement are the three main pillars of spectroscopy.[13] This approach has a lot to offer in terms of labor cost or time savings. This method is also very accurate and precise. This technique was specifically used in pharmaceutical analysis to examine dose forms, and its use in the pharmaceutical industry has been steadily growing. Additionally, the following are some features of the colorimetric methods: [14]

UV – VISIBLE SPECTROSCOPY

The energy, radiation, or excitation of electrons is the basis of the UV visible spectroscopy technique. The UV-Visible technique uses light energy to excite electrons, and the absorbance ranges from 200 to 800 nm. This range is used to calculate the sample wavelength. Only in the presence of accessible conjugated pi-electron pairs does the absorption occur. [15]

The UV-Visible Principle The basis of spectroscopy is the way that chemical compounds absorb ultraviolet or visible light, producing unique spectra in the process. Spectroscopy relies on the way light and matter interact. A spectrum is created when the substance absorbs light and goes through excitation and de-excitation. [16]

Matter absorbs ultraviolet light, which excites the electrons within it. They transition as a result from an excited state, which has a comparatively big amount of energy connected with it, to a ground state, which has a relatively small amount of energy associated with it.



FTIR SPECTROSCOPY:

The absorption is led to a lower energy state by the infrared spectroscopy, which excites or causes some atoms and molecules to vibrate. This method advise the scientist identify the functional group and the original peaks related to the molecule, which would aid in the development of a new Approach [17]

The infrared region (IR) is the frequency range of the spectrum between 12,500 and 10 cm−1. It is separated into three areas. [18]

IR radiation range	Far-infrared	Middle-infrared	Near-infrared
Wavelength range (μm)	50–100	2.5–50	0.78–2.5
Wavelength number (cm^{-1})	200–10	4,000–200	12,500–4,000

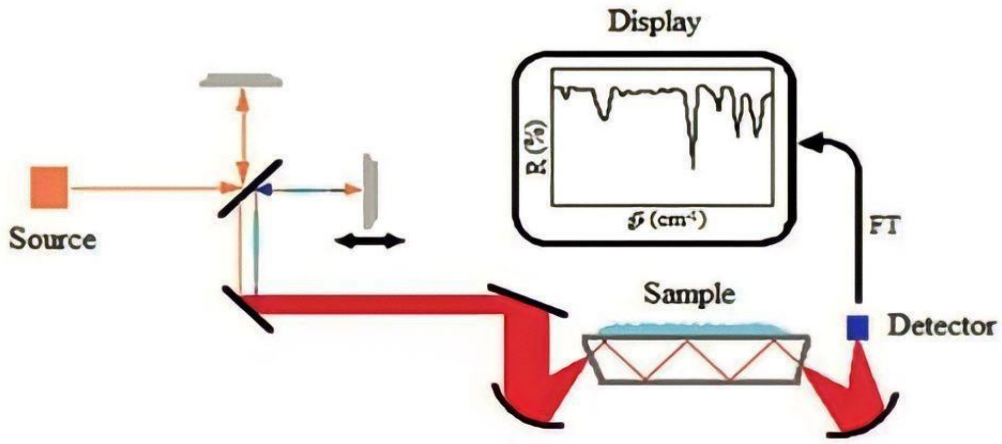


Fig. Schematic diagram of FTIR Spectroscopy

MASS SPECTROSCOPY :

A potent analytical tool with great sensitivity and specificity is mass spectrometry (MS). The MS system has developed over a century ago as a key instrument in analytical chemistry for studying and controlling ions in both chemical and biological species. [19]The mass-to-charge ratio (m/z) of analyte ions is measured in the electromagnetic fields of a mass spectrometer, and fragmentation information of the ions can help with tandem mass spectrometry (MS/MS) structure identification and confirmation.[20]The realm of clinical applications has expanded the use of MS in recent decades due to its excellent sensitivity and specificity. Since MS offers high-quality quantitative analysis even at low concentrations, it has been used more and more to handle clinical samples including complex matrices, such as tissues and biofluids. [21]

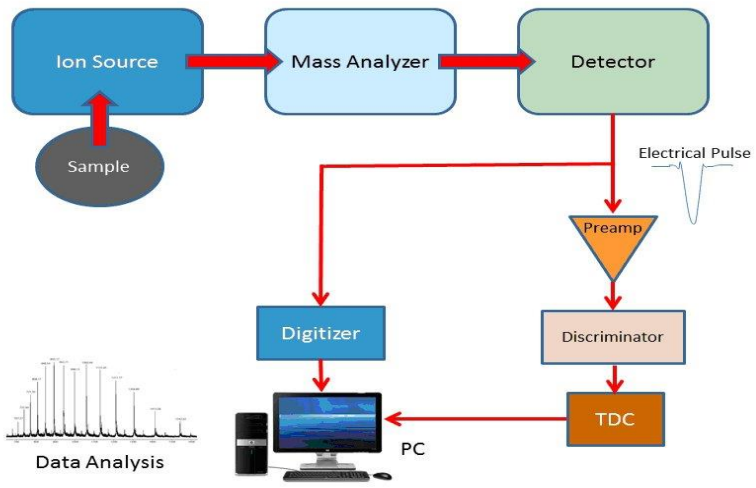


Fig. Schematic diagram of Mas Spectroscopy

NMR SPECTROSCOPY:

Scientists have developed a number of methods in recent years to address challenges with the analysis of novel drug molecules. The method of nuclear magnetic resonance spectroscopy was extensively employed in the creation of pharmaceuticals.[22]This method worked well for both drug identification and quantitative drug analysis, which allowed for the identification of individual molecules. Additionally, this method's procedure was useful in characterizing the chemical products, drugs used in pharmaceutical formulations, and biological fluids[23].

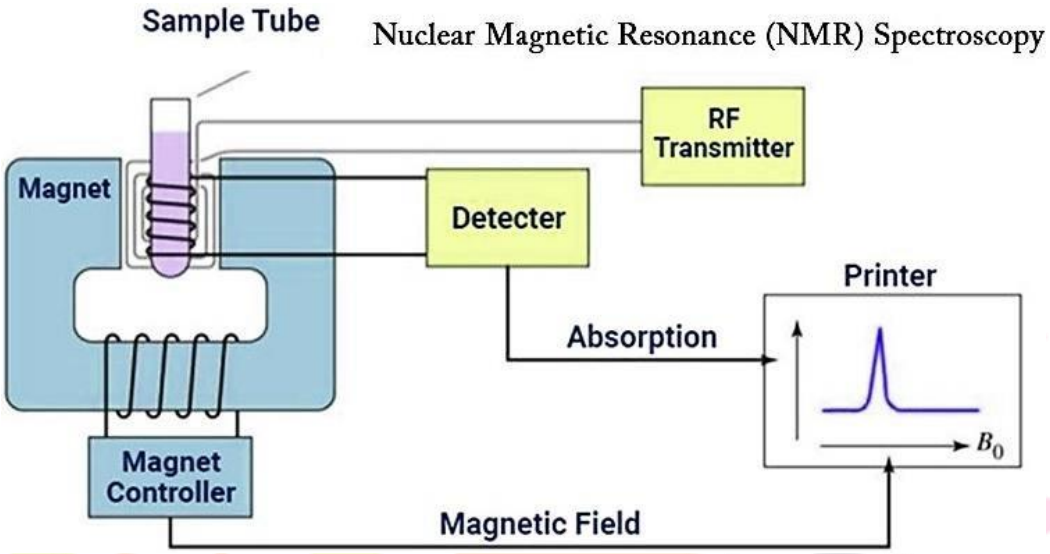


Fig. Schematic diagram of NMR Spectroscopy

FLUROMETRY AND PHOSPORIMETRY:

The use of fluorimetry and phosphorimetry in the pharmaceutical industry to analyze micro samples has been steadily increasing. The extremely sensitive system was examined using the fluorimetry technique without sacrificing the method's specificity or precision. Previous research has shown that the number of applications in fluorometry and phosphorimetry is constantly increasing. These methods, which were observed from previous years, are presented for the quantitative estimation of certain drugs that are available in the form of biological fluids.[24]

HYPHENATED TECHNIQUES:

Combining or coupling two distinct analytical techniques with the aid of an appropriate interface is known as a hyphenated technique. Spectroscopic techniques are primarily combined with chromatographic techniques.The pure or nearly pure fractions of chemical components in a mixture were separated by chromatography, and selective information for identification using standards or library spectra is produced by spectroscopy.A hyphenated technique will result from the combination of the separation technique and an online spectroscopic detection technology. Combining or coupling two distinct analytical techniques with the aid of an appropriate interface is known as a hyphenated technique. The combination of separation-separation, separation-identification, and identification-identification techniques are all included in the term "hyphenated techniques."[25]

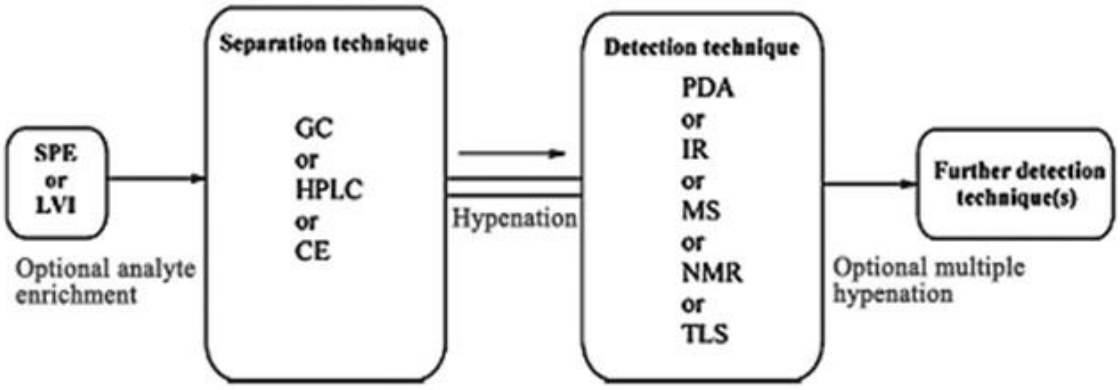


Fig. Schematic diagram of hyphenated techniques

LC- NMR

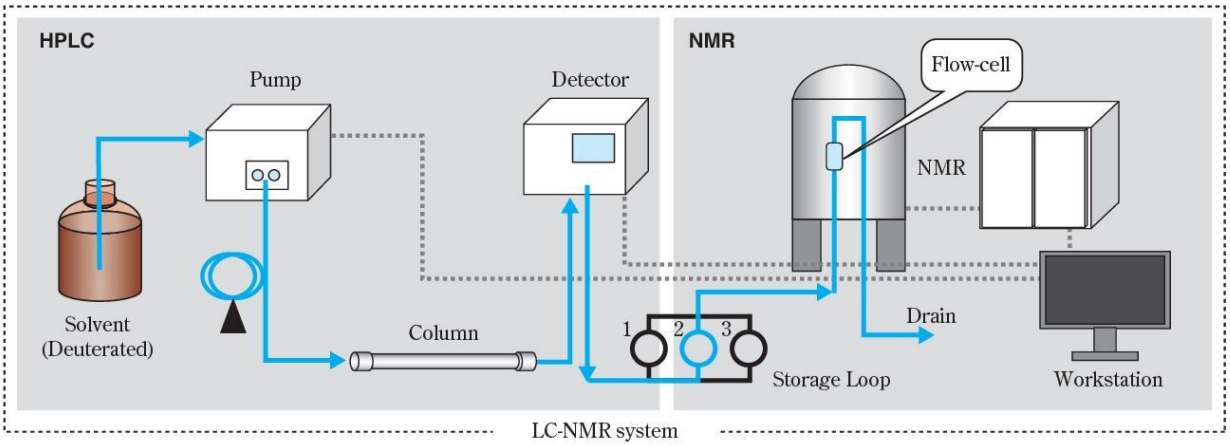
LC-NMR (Liquid Chromatography-Nuclear Magnetic Resonance) is a powerful analytical technique that Combines the separation capabilities of liquid chromatography with the structural information provided By NMR spectroscopy. The complete chemical characterization of natural goods is improved by the Remarkable technological developments and improvements in creative analytical hyphenated Approaches that have been made thus far. Studying intricate biological matrices, like unrefined plant Extracts, requires quick and effective characterization techniques. The initial identification of target Constituents is crucial for subsequent selective identification methods during the early stages of Separation.[26]

PRINCIPLE:

LC separates components in a mixture based on their chemical properties. NMR provides detailed Structural information about the separated compounds. It involves a HPLC separation followed by the Detection of separated components by UV or other methods and ultimately NMR analysis. LC-NMR is a Powerful analytical tool used to resolving complex mixtures.[27]

INSTRUMENTATION:

LC-NMR setups typically involve coupling a liquid chromatograph with a high-field NMR spectrometer.The eluent from the liquid chromatograph is directed into the NMR for real-time analysis. Hyphenated Techniques are analytical methods that combine or link spectrometers and chromatographs online. They Have gained popularity recently as high-throughput analytical methods that can simultaneously provide The spectra of different components' structural or compositional characteristics and separate mixtures. As a result, LC-NMR is a hyphenated method that combines the structurally informative spectroscopic .Detection method (NMR) with the separation technique (LC). Figure shows the general instrumentation Of an LC-NMR system. The isolation zone (column), interface zone, and detection zone—that is, the Probe used to record NMR spectra—are the three primary parts of an LC-NMR. Through a computer-Controlled data collecting system that automatically harmonizes the various procedures, the HPLC is Immediately connected to the NMR. A perceptive detector, like Drug Discovery: It aids in the identification and characterization of compounds in drug samples, Providing insights into the purity and structure of potential drug candidates.Metabolomics: LC-NMR is applied to study and identify metabolites in biological samples, contributing to Metabolomics research.Food and Beverage Analysis: Used for profiling and determining the composition of food and beverage Products.[27,28]



ADVANTAGES:

LC-NMR allows for the separation and identification of compounds in complex mixtures. Real-time Analysis enables on-the-fly structural elucidation during chromatographic runs.Challenges: Sensitivity can be a challenge, especially when dealing with low-concentration samples.Instrumentation costs and maintenance can be relatively high.[29]

Research and Development:

LC-NMR is a valuable tool in pharmaceutical R&D for compound identification and characterization. It Contributes to the understanding of the chemical composition of various samples in research fields such As environmental analysis and material science. Remember, the details may vary based on the specific LC-NMR setup and the application's requirements.[30

APPLICATIONS:

1. Natural Products Chemistry: LC-NMR is widely used for the analysis of complex mixtures like
2. Plant extracts, allowing researchers to identify and elucidate the structures of natural products.
3. In the middle of the 1990s, there was a report on the first practical application of LC-NMR for
4. Natural product analysis. Since then, a number of applications have been introduced to describe
5. Extracts from natural products.
6. Demonstrating products for drug degradation.
7. Impurities at moderate levels can be isolated and detected.
8. For environmental detection, this technique is utilized to track pesticides, herbicides and organic
9. Contaminants.[29,31]

LC-MS:

Because MS has such high sensitivity and selectivity, it has become a popular approach for structural identification of analytes found in complex mixtures when combined with liquid chromatography (LC-MS). For analytes with a high ionization efficiency, the MS detection limits are comfortably in the femtomole range. Because there is less competition for the amount of charge available at any given time when fewer charged analytes enter the mass spectrometer simultaneously, the LC separation significantly lowers the complexity of samples in MS, which in turn reduces ion suppression. MS gives information on molecular weight, and precise mass measurements typically allow for the deduction of a compound's elemental composition.[32]

Moreover, using fragmentation patterns, tandem mass spectrometry (MS/MS) provides structural information about compounds. Depending on the mass analyzer, spectral scan rates for MS fall between nanoseconds and microseconds, which makes it perfect for high-throughput analysis. [34]When MS and LC are combined, it is perfect for complex sample analysis. However, one drawback of MS is that, in the lack of reliable standards, it cannot definitively identify a compound's structure. When using LC-MS, definitive structural identification is typically achieved by comparing the retention time and MS/MS spectral pattern of the target analyte with those of a legitimate standard.[33]

The LC–IR hyphenation process must be carried out in order to create a useful instrument that generates complete mid-infrared spectra.

1. Eliminate the solvent without overflowing the vacuum system with diluent gas or thermally damaging the analyses.
2. Ensure that analyses are transmitted to the spectrometer efficiently.
3. In a thick deposit, present analyses to the FT-IR.
4. Maintain the resolution of the chromatography[35]

PRINCIPLE:

The Liquid Chromatography-Mass Spectrometry (LC-MS) Principle Utilizing an HPLC, the LC-MS technology first separates the constituent parts of a mixture before ionizing and separating the ions according to their mass/charge ratio.[36]

INSTRUMENTATION:

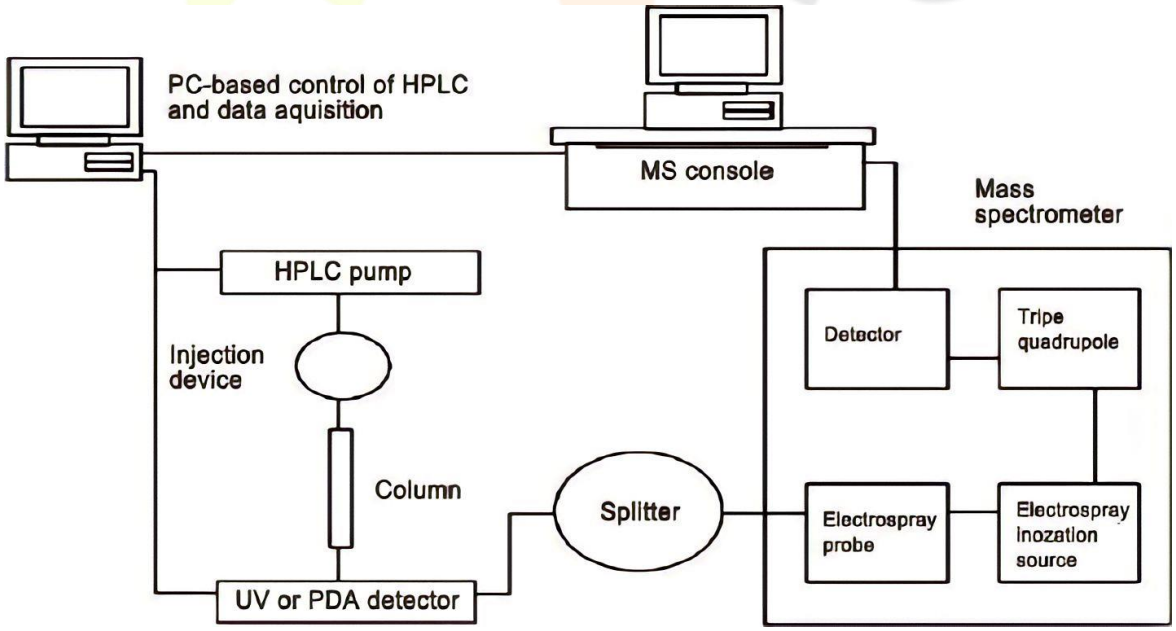


Fig. Liquid chromatography – Mass spectroscopy

A method of chemistry known as LC-MS combines mass spectroscopy with the physical separation of liquid chromatography, also known as HPLC. A double three-way diverter in line with an autosampler, an LC system, and a mass spectrometer comprise a standard automated LC-MS system. Before the sample enters the MS, the diverter typically functions as an automatic switching valve to direct undesirable portions of the eluting from the LC system to trash. Typically, LC-MS uses soft ionization techniques that primarily reveal molecular ion species with a small number of fragment ions. The identification of the molecule cannot be confirmed using the data from a single LC-MS run. However, tandem mass spectrometry (MS-MS), which delivers fragments by collision-induced dissociation of the generated molecular ions, has now solved the issue. The use of LC-MS-MS is growing daily at a rapid rate. When combined with biological screening, hybridized techniques like HPLC coupled to UV and mass spectrometry (LC-UV-MS) have proven to be incredibly beneficial for a quick survey of natural compounds.[37]

Many compounds of pharmaceutical interest can be analysed quickly and affordably using LC/MS methods due to their sensitivity, selectivity, and speed of analysis.These analytical features have kept getting better, making the instruments more dependable and easier to use. These advancements came at the right time and lined up with the pharmaceutical industry's previously mentioned changes. Analytical method that uses electric and magnetic fields to separate gaseous ions according to mass in order to identify chemical substances. A mass spectrograph, also known as a mass spectroscope, employs non-electric methods such as photography to detect the sorted ions, whereas a mass spectrometer uses electrical methods. [38]

APPLICATION:

1. Dissatisfaction with the expensive and inconsistent cross-reactivity of commercial immunoassays used in therapeutic drug testing has prompted the development of LC-MS assays as alternatives.

2. There is a lot of interest in using LC-MS for vitamin D measurements because commercial immunoassays respond differently to different forms of vitamin D and its metabolites.

1. LC-MS analysis is pertinent for a number of steroid biochemistry domains. Due to challenges in measuring low levels of testosterone and dihydrotestosterone seen in women and children, numerous highly sensitive LC-MS assays capable of delivering precise measurements in these classes have been developed.[37]

LC-IR

LC-IR, also known as HPLC-IR, is the hyphenated technique that resulted from the LC coupling and the infrared spectrometry (IR) or FTIR detection idea. While organic compounds have various structures in the mid-IR region, infrared (IR) or far-infrared (FTIR) spectroscopy is a valuable method for identifying organic compounds, even though HPLC is one of the most efficient separation procedures known today.

Furthermore, in comparison to other detection methods like UV and MS, infrared sensitivity is significantly lower. Recent advances in HPLC-IR technology have employed two straightforward strategies based on interfaces developed in HPLC-FTIR or HPLC-IR.

Combining HPLC and IR is challenging, and the hyphenated approach advances very slowly because the 237 absorption bands of the mobile phase solvent are so large in the mid-IR region that they frequently mask the tiny signal generated by the sample components. Since FT-IR is an absorbance system, sample geometry during the measurement procedure is important. For a given mass or volume of the analyte, decreasing the diameter by a factor of two results in a deposition that is four times thicker and four times more optically dense. Due to the IR detector's limitation by total light, this two-fold reduction in deposit diameter results in a four-fold increase in signal-to-noise ratio.

To create a useful tool that produces whole mid-infrared spectra, the LC-IR hyphenation technique must be applied.

Salts like KBr or KCl are typically employed to select sample components in the eluent, and heating the medium prior to IR detection eliminates volatile mobile phase solvents. The diffuse-reflectance infrared Fourier transform (DRIFT) solution and the buffer-memory approach are the two types of interfaces that exist in the solvent-elimination solution.[41]

INSTRUMENTATION :

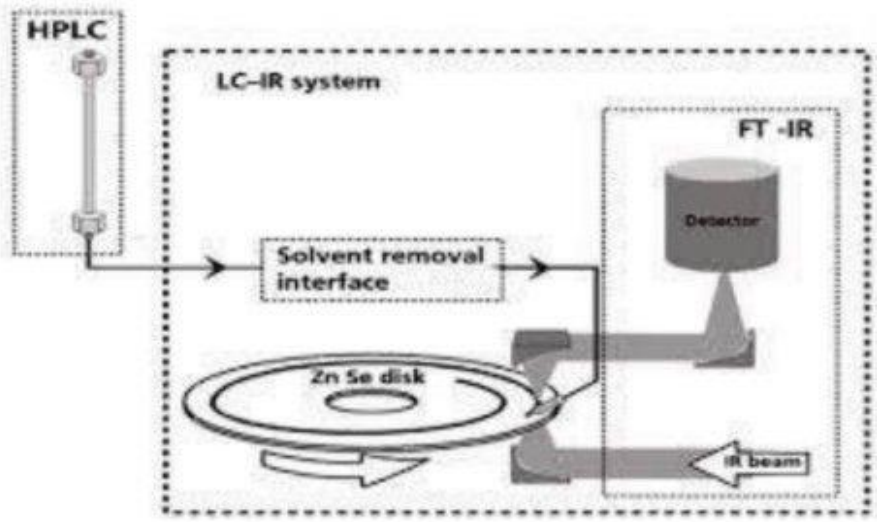


Fig.

Every component ought to be able to be identified in real time with this device without chromatographic resolution being compromised. The most widely used method for this is mass spectrometry (MS), however it has some drawbacks, especially when it comes to differentiating structural isomers like ortho-, meta-, and para-xylene, whose chemical-ionization mass spectra and electron-impact mass spectra are identical. A complementary technique to mass spectrometry is desired for such molecules. An alternative method for this purpose is Fourier transform infrared (FT-IR) spectrometry, which produces unique spectra for the majority of structural isomers.[40,41]

APPLICATION :

1. Eliminate the solvent without compromising the analytes' thermal stability or overloading the vacuum system with diluent gas.
2. Ensure that the analytes are successfully transmitted to the spectrometer.
3. Display the FT-IR in an analyte dense deposition.
4. Maintain the chromatography's resolution.

GC-MS

It is a separation technique applicable to compounds which can be volatilized in a gas stream. This is typically done by injection of 0.1-1 µL of sample into an injection port heated to 250ºC. These two techniques highly compatible with each other, the sample is in the vapour phase in both the techniques. (11) But the incompatibility between these techniques is GC operates at high pressure (760 torrs) and in this the carrier gas is present, whereas in the case of mass spectroscopy it operates at a vacuum 10-6 to l0-5 torrs.[41]

PRINCIPLE :

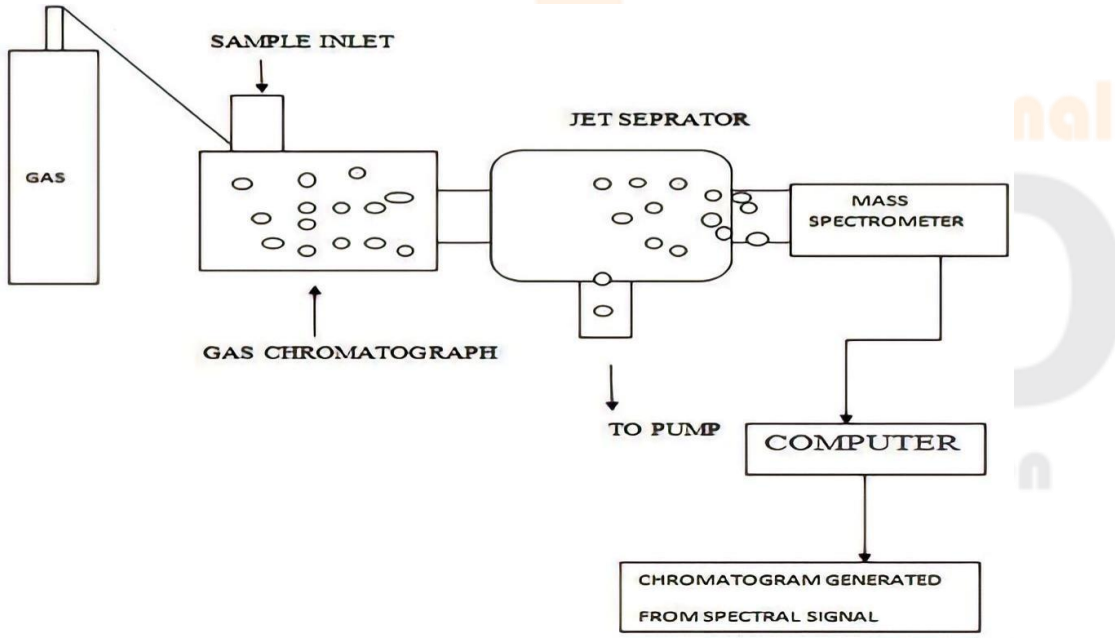
The basis for gas chromatography/mass spectrometry (GC/MS) is the principle that heat causes a mixture to separate into its constituent substances. Prior to the analyte molecules being eluted into the MS for detection, the sample mixture is separated by the GC-MS instrument.[42]

INSTRUMENTATION :

Vaporized analytes undergo separation in the GC column when they are passed through it with the aid of heated carrier gas ; this carrier is also referred to as the mobile phase (helium). The chemicals separate as a result of interactions between the analyte, mobile phase, and stationary phase. The parameters of the column (length, diameter, and film thickness), the type of carrier gas, the gradient temperature of the column, and the characteristics of the stationary phase all affect the analyte's separation. As the sample moves through the column, the mixture's constituent parts separate due to variations in boiling points and other chemical characteristics. Because of their varied adsorption or variations in the partition between the mobile phases, the components have varying elution and retention times. After that, an interphase will allow the mixture's separated components to enter the MS. Ionisation, mass analysis, and the determination of the mass-to-charge ratios of the ions by each analysis by the mass spectrometer come next. GC and MS can be connected via an interface, such as a membrane separator, jet/orifice separator, or effusion separator. Ionization is a process that separates a molecule into its positive and negative modes in addition to ionizing it. [42,43]

APPLICATION :

- Gas chromatography having the highest resolution power to compare other techniques.



- This technique having higher sensitivity when used with heated detectors.[41]

CONCLUSION :

In many domains, the hyphenated technique is a useful strategy that combines the advantages of multiple approaches to improve overall performance. Because of its adaptability, effectiveness, and capacity to offer thorough insights, it holds great promise for use in upcoming studies and applications. Its full potential and widespread adoption across disciplines, however, depend on ongoing advancements and standardized protocols. Hyphenation implies both isolation and labeling, which facilitates sample examination. These days, hyphenated techniques are more common than traditional spectroscopy or chromatography methods. Chromatographic methods like GC, LC, and so on are used for separation, while spectroscopic methods like NMR, MS, and IR are employed for identification. Hyphenated techniques including LC-MS, GC-MS, LC-NMR, CE-MS, and have been developed to address a variety of complicated analytical problems in a variety of sectors. Therefore, it can be concluded that hyphenated techniques are far more effective and beneficial than standard single techniques.

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