Review Article on Hyphenated Techniques and Their Inter Face in Pharmaceutical Analysis

Dr.T. Yamini kumari[1], K. Keerthi[2], K. Girish[3], J. Saritha[4]
[1]Associate professor [1], Sri Vasavi institute of pharmaceutical sciences, pedatadepalli, Tadepalligudem, W.G. DIST (A.P)
[2,3,4]Student, Sri Vasavi institute of pharmaceutical sciences, pedatadepalli, Tadepalligudem, W.G. DIST (A.P)

Abstract—**The combination or coupling of the various analytical approaches is known as the hyphenated technique. Spectroscopic methods are generally paired with chromatographic methods. After that, an interphase will allow the mixture's separated components from the chromatographic process to enter the spectroscopic method. The separated components from gas chromatography are fed into the mass spectrometer (MS) for ionization, mass analysis, and the detection of mass-to-charge ratios of ions produced by each analysis. GC and MS can be connected using a jet/orifice separator, effusion separator, or membrane separator. The analytical flow cell was first built for continuous flow to NMR in LCNMR coupling. However, the use of the stopped-flow mode in LCMS has resulted from the necessity for a thorough structural evaluation of novel natural compounds. The use of LC-MS-MS is rapidly growing every day. When combined with biological screening, hyphenated techniques like HPLC coupled to UV and mass spectrometry (LC-UV-MS) have proven to be very helpful for a quick survey of natural compounds. These days, there are many distinct kinds of LC-MS systems with diverse interfaces that are sold commercially. Hyphenated procedures are used to describe separation and identification, and they provide improved sample analysis by incorporating specificity, accuracy, and precision.**

Index Terms—**Hyphenated Techniques, Interphase and Benefits**

I. INTRODUCTION

Double hyphenation methods:

A hyphenated approach is the combination of two different analytical methods with the help of a suitable interface. Hyphenated procedures include the combination of identification-identification, separation-identification, and separation-separation methods. To describe the hybridization of two or more instrumental analytical techniques in a single run, Hirschfeld first used the term "hyphenation" in 1980 (Hirschfeld, 1980). Producing a detection that is more information-rich for both identification and quantification than would be achievable with a single analytical method1 is obviously the aim of this connection. A few decades back, 101Hirschfeld first used the term "hyphenation" to refer to the online combination of one or more spectroscopic detection techniques with a separation approach. This approach, which is now called the hyphenated technique, was developed by combining spectroscopic separation and detection methods[1].

The use of three hyphens:

Chromatographic separation combined with detectors that provide high structural information is frequently necessary for the clear identification of known—and, more crucially, unknown—analytes in complicated mixtures. The two most used methods for identifying a compound's structure are mass spectrometry (MS) and nuclear magnetic resonance (NMR). MS and NMR both provide complementary evidence in studying unknowns and often are required to fully characterise them. While NMR just provides the organizational groups of structural moieties that an analyte´s atoms are involved in, MS can deliver the atomic formula of analyte. For example, NMR discerns between positional isomers and isobarics whereas the reverse applies for MS. Further, some functional groups that are 'NMR silent', such as nitro and sulphate groups, are 'visible' by MS but not by NMR. In such fields as natural product discovery, metabolomics, and drug metabolite identification, the synergistic nature of MS and NMR has facilitated their co-application for substances that cannot be characterized using either detector in isolation. challenging to couple LC-MS and NMR, requiring compromises in both technique development and hardware. For example, the more or less full MS analysis (plus fragmentation) can be completed in under one second using a nanogram of analyte. For the most basic 1D spectrum, however, NMR takes minutes to hours at the microgram level. This paper discusses the sacrifices that allow each technique to be employed at its maximum sensitivity and provides an overview of the various ways that LC-MS and NMR have been connected.

II. DEFINITION

Thomas Hirschfield first used the term "hyphenation" in 1980. Hyphenated approaches combine two or more approaches to address more intricate analytical issues. More precisely, it describes the fusion of spectroscopic detection technology with a separation approach.

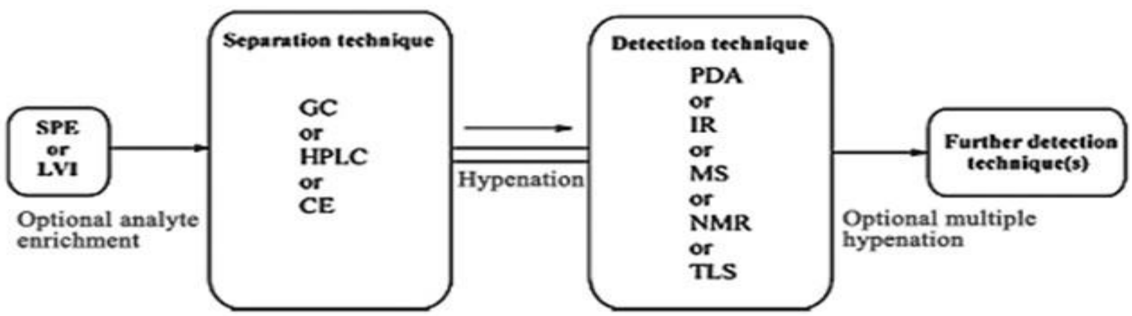

Fig 1: Hyphenated techniques flow chat

1. Hyphenated techniques:

Varieties of hyphenated methods

1. Double hyphenation methods.
2. The use of three hyphens.

The following are examples Double hyphenation:

LC-MS	GC-IR
LC-IR	GC-FTIR
LC-NMR	GC-MS
HPLC-DAD	

Table 1: Double hyphenation methods

The following are examples for Triple Hyphens:

APCI-MS-MS	LC-ESI-MS
LC-NMR-MS	SPE-LC-MS (2)
ESI-MS-MS	LC-UV-NMR-MS-ESI
LC-PDA-MS	LC-MS-TSPLC-UV-NMR-MS
LVI-GC-MS	LC-PDA-NMR-MS [2].
LC-DAD-API-MS	

Table 2: The use of three hyphens

Double hyphenation methods

1.GC-MS

While MS is able to categorize the molecule by providing molecular level structural data, it is unable to discriminate between volatile and semi-volatile compounds. Thus, the synthesis of these two methods occurred soon after GC's expansion. Both these methods have the sample in the vapour phase, making the samples quite compatible with one another. However, the two methods cannot work together because mass spectroscopy runs at a vacuum of 10-6 to l0-5 torr, whereas GC works at a high pressure, 760 torr, which contains a carrier gas. A sample is injected, vaporized, and separated in the GC column before being sent to the injection port of the GC.

system for GC-MS analysis. The movement of a sample the length of a column will differentiate the molecules in a mixture because of the differences in their chemical properties. The mass spectrometer does this by separating each molecule into ionized fragments and differentiating these pieces based on their mass-to-charge ratio.

GC-MS instrumentation generally provides an injection port at one end of a metal column, usually filled with a sand like substance to allow for the best possible separation), a detector MS at the other end of the column, and a carrier gas (such as argon, helium, nitrogen, or hydrogen) that pushes the sample down the column.

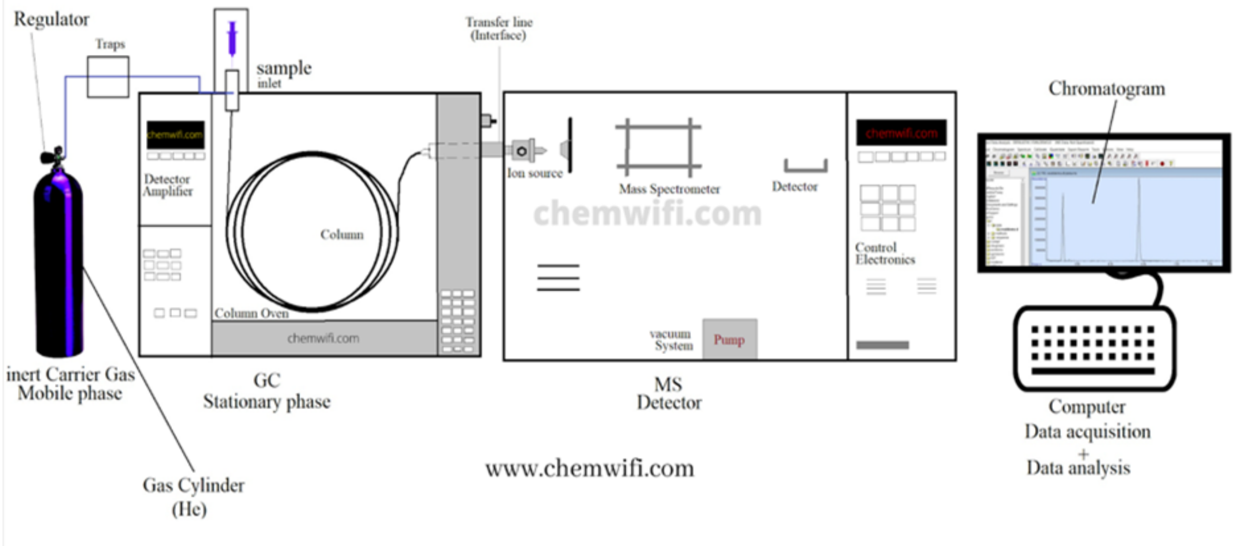

Fig 2: GC-MS Interface flow chat

GC-MS Interface

A gas chromatograph's vapor stream is under pressure, sometimes more than five times that of the atmosphere. In order to allow easier passage of the ions, mass spectrometer analysers are run under high vacuum. High vacuum is created using a fore pump and a turbo molecular pump. The turbo molecular pump can handle the gas flow from the GC under normal conditions, capillary Column: 1-2 ml/ min flow rate and internal diameter: 250-320 µ. In this case, the GC-MS interface consists of a hot metal tube. Before the sample and carrier gas enter the mass spectrometer, there is a separate pump that extracts the majority of the gas from the interface tube must separate part of the vapor flow for larger columns or at higher flow rates or Both. In the interface tube, the analyte molecules must be kept from condensing or breaking apart down.Typically, the interface temperature is set 10 °C higher than the oven's final temperature[3].

GC-MS Benefits

1. GC has a great resolution, and it is comparable to other methods.
2. This type has a great sensitivity when used with thermal detectors.
3. This technique also separates and analyzes samples rapidly, but with less reproducibility.
4. The accuracy and precision of this method are excellent.

2. LC-IR:

LC-IR or HPLC-IR is the hyphenated technique that was created by combining the LC coupling with the Infrared spectrometry (IR), or FTIR - detection concept IR or FTIR is a useful spectroscopic method for the identification of organic compounds, though HPLC is one among the most effective separation techniques being employed currently .This is because organic compound structures contain lots of structures within the mid-IR range .Moreover, infrared is far less sensitive than other detecting methods such as UV and MS. Recent developments in HPLC-IR have utilized two simple approaches that are based on interfaces. established in HPLC-IR or HPLC-FTIR. Because the 237 absorption bands of the hyphenated approachs mobile phase solvent are so large in the mid-IR a region in which the small signal produced by the sample constituents is often obscured, the coupling of HPLC with IR is challenging, and the advances within this hyphenated technique are very slow.

Since FT-IR is an absorbance system, the shape of the samples throughout the measurement procedure is important. When the diameter is reduced by a, four times the thickness and four times the optical density are deposited. factor of two for a fixed mass or volume of the analyte. Because the IR detector is limited by total light, this two-fold decrease in deposit diameter results in a four-fold increase in the signal-to-noise ratio. In order to develop a useful tool that provides complete mid-infrared spectra, the LC-IR hyphenation technique should be employed. The main components of an eluent are a KBr or KCl salt, usually employed to select sample components, and the volatile mobile phase. heating the medium before IR detection removes the solvents. The diffuse-reflectance infrared Fourier transform solution, DRIFT, and the buffer-memory technology are the Two interface types exist in the solvent-elimination solution.

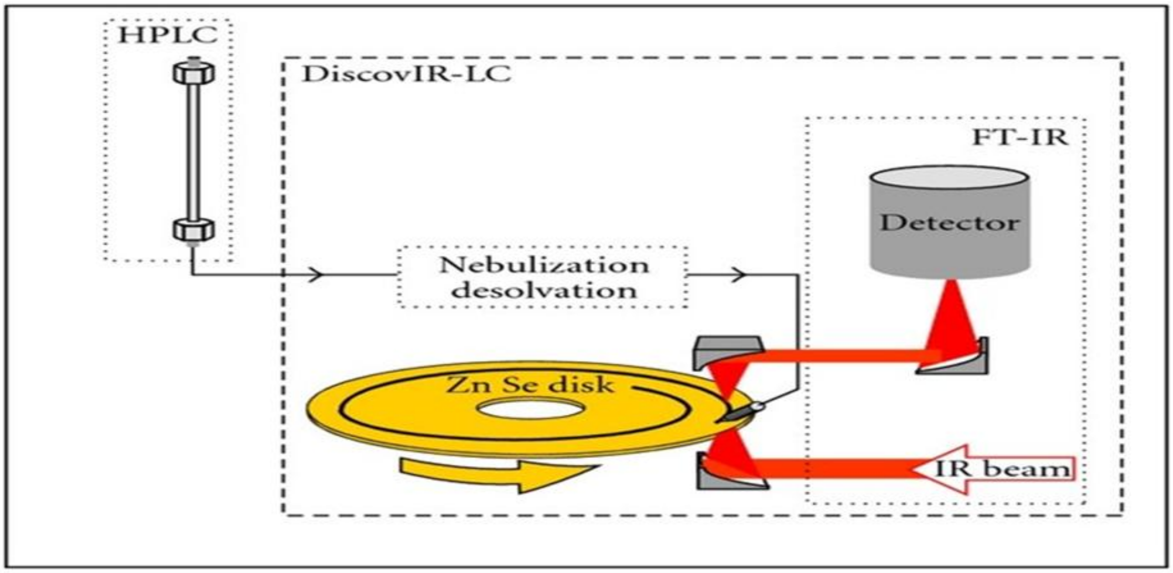

Fig 3: Schematic diagram of an LC-IR

LC-IR Benefits:

1. Remove the solvent without overloading the vacuum system with diluent gas or compromising the analytes' ability to withstand heat
2. Successfully transmit to the analyte spectrometer
3. Display the FT-IR in an analyte 89s thick deposition
4. Maintain the chromatography's resolution[4].

LC-IR Interface

In order to get a solvent-free, complete IR spectra of the analyte, the solvent-elimination interface efficiently removes the mobile phase solvent prior to the analyte reaching the IR spectrometer. Sensitivity and spectral quality are significantly enhanced by this. The general procedures are as follows:

Nebulization/Atomization: Usually, a nebulizing gas (such as nitrogen) is used to spray the LC effluent into a heated chamber.

Solvent Evaporation: The volatile mobile phase solvent is quickly evaporated by the heated chamber and gas.

Analyte Deposition: The now solvent-free analyte particles are moved and deposited onto a moving substrate, such as a rotating belt or disk (often composed of an IR-transparent substance like ZnSe).

IR Measurement: The solid-phase spectrum is obtained by passing the deposited analyte through the spectrometer's IR beam, which is usually a FTIR. composed of an IR-transparent substance like ZnSe)[5].

3. LC-NMR:

NMR is arguably the least sensitive spectroscopic method now in use, but it offers the most valuable insights into the structure of natural compounds. The new practical approach known as HPLC-NMR or LC-NMR, which has been widely used for more than 15 years, was made possible by technological advancements that allowed HPLC equipment to be directly parallel coupled to NMR. In the early 1980s, the first online HPLC-NMR experiment with superconducting magnets was published. However, it wasn't until the late 1990s that this hyphenated technique began to be used in analytical laboratories. The investigation of complex mixtures of various kinds, especially the analysis of natural products and drug-related metabolites in bio-fluids, is where LCNMR promises to be very useful. There are two ways to conduct LC-NMR experiments: continuous-flow and stop-flow. Systems featuring 1H, 13C, 2H, 19F, and 31P probes operating at 500, 600, and 800 MHz can be used to solve a variety of bioanalytical issues. Along with the NMR and HPLC equipment, the continuous-flow probe and a valve that is placed in front of the probe to record either continuous-flow or stopped-flow NMR spectra are the primary requirements for on-line LC-NMR. (17). For LC operation, a UV-Vis detector is also employed as the primary detector. For a typical HPLC-NMR coupling, magnetic field strengths greater than 9.4 T—that is, a 1H resonance frequency of 400 MHz—are advised. The analytical flow cell was first built for NMR acquisition in continuous flow. However, the use of the stopped-flow method has resulted from the requirement for complete structural assignment of unknown chemicals, particularly novel natural products. The LC unit typically consists of an auto-sampler, LC pump, column, and a non-NMR detector (such as UV, DAD, EC, refractive index, or radioactivity) in an LC-NMR system. An LC-NMR interface, which should have additional loops installed for the intermediate storing of certain LC peaks, receives the flow from this detector. The flow from the LC-NMR interface is then directed either to the waste container or to the flow-cell NMR. probe-head. The flow is directed to a fraction collector for recovery and additional study of the different fractions examined.by NMR after passing the probe-head. A splitter at the output from the LC-NMR interfaces can also be used to connect an MS to the system. Reversed-phase columns are employed, using a binary or tertiary solvent mixture with isocratic or gradient elution.in most of the LC-NMR work. Obtaining a sufficient NMR spectrum is severely hampered by the protons of the mobile phase solvents. The strong solvent signals and the weak material signals cannot be handled simultaneously by the NMR spectrometers receiver .Reversed-phase columns, using a binary or tertiary solvent mixture with isocratic or gradient elution, are utilized in the majority of LC-NMR activities. Obtaining a sufficient NMR spectrum is severely hampered by the protons of the mobile phase solvents. The strong solvent signals and the weak material signals cannot be handled simultaneously by the NMR spectrometer's receiver. One of the three main approaches—pre-saturation, soft-pulse multiple irradiations, or water suppression enhancement through T1 effects (WET) presaturation using a z-gradient—can be used to tackle this issue by suppressing the solvent signal[6].

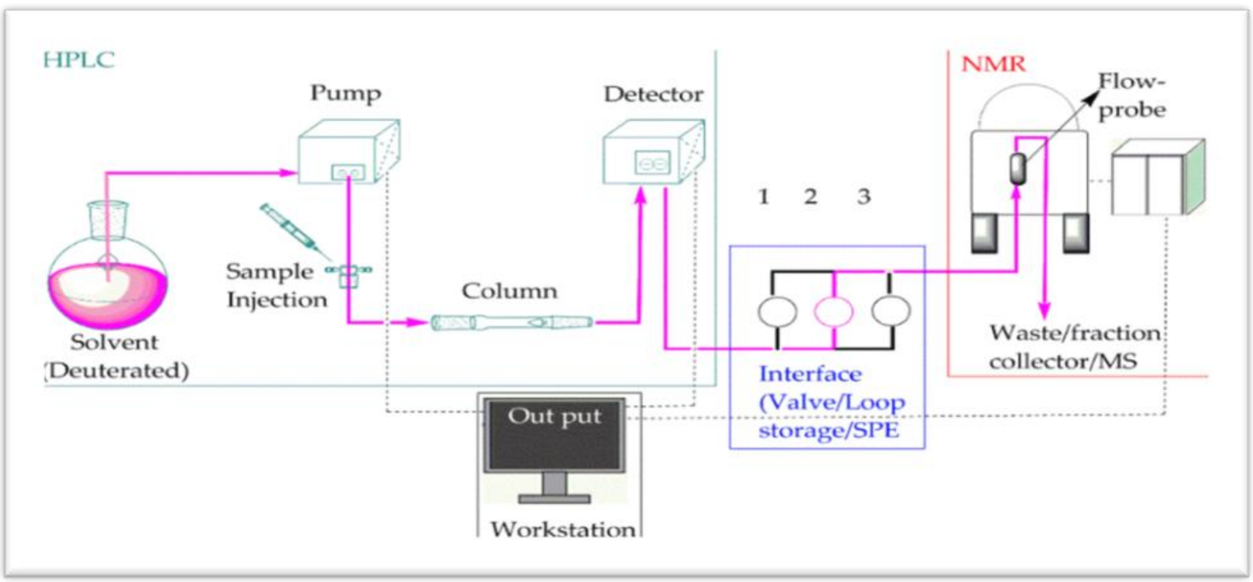

Fig 4: Schematic diagram of a LC-NMR

LC-NMR Benefits:

1. Analysis of natural products. Numerous applications have been developed since then to characterize
2. Natural product extracts.
3. Presenting drug degradation products.
4. It is possible to identify and detect impurities at moderate levels.
5. This method is used to monitor pesticides, herbicides, and organic contaminants for environmental detection.
6. Chemistry of Natural Products: LC-NMR is frequently used to analyze complicated mixtures, such as plant extracts, which enables scientists to recognize and clarify the structures of natural products[7].

4.LC-MS:

For decades, technical problems prevented the connection of HPLC separation to mass spectrometry for the analysis of non-volatile substances. It proved to be difficult to overcome the issues of solvent and flow rate incompatibility between HPLC and MS and to provide a suitable interface for the effective ionization of non-volatile and thermally instable chemicals (10). The instrument of choice for liquid chromatographers is rapidly evolving to be Liquid Chromatography/Mass Spectrometry (LC/MS). It is a potent analytical method that combines mass spectrometry's detection specificity with liquid chromatography's resolving strength. The components of the sample are separated using liquid chromatography (LC) before being introduced to the mass spectrometer (MS). Charged ions are produced and detected by the MS. The molecular weight, structure, identity, and quantity of particular sample components can all be determined using the LC/MS data. The combination of an LC with a mass spectrometer (MS) is called LC-MS or HPLC-MS. The separated sample that comes out from the column can be identified based on its mass spectral data. The two methods can be combined effectively with the use of a switching valve. A double three-way diverter connected in parallel to an auto-sampler, an LC system, and the mass spectrometer makes up the standard automated LC-MS system. Before the sample enters the MS, the diverter usually serves as an automated switching valve to direct Unwanted elute components from LC system to trash. This qualitative approach makes it possible to rebuild an unknown molecule from MS data. Soft ionization techniques, which exhibit primarily a molecular ion species with only a few fragment ions, are Usually applied in LC-MS. The information on the compounds structure that is gleaned from one LC-MS run, therefore, is not very good. In tandem mass spectrometry, the fragments are delivered through collision-induced dissociation of the has now been addressed by using molecular ions thus generated, for which purpose (11). Applications of LC-MS-MS are increasing rapidly. When coupled with biological screening, hyphenated techniques such as HPLC coupled to UV and mass LC-UV-MS has proven to be very helpful for a fast survey of natural compounds. Nowadays, many different types of LC-MS systems with a variety of interfaces are commercially available. The interfaces are designed to provide adequate liquid nebulization and vaporization, sample ionization, and solvent removal of vapor, ion extraction into the mass analyzer.

The interfaces that are frequently utilized are

Direct liquid introduction	Continuous flow rapid bombardment of atoms
A moving wire or belt	Ionization of atmospheric pressure
A beam of particles	Ionization of chemicals at atmospheric pressure
Thermo spray	Ionization by electro spraying

Table 3: Interfaces of Lc-ms

LC-MS Benefits

For decades, technical problems prevented the connection of HPLC separation to mass spectrometry for the analysis of non-volatile substances. It proved to be difficult to overcome the issues of solvent and flow rate incompatibility between HPLC and MS and to provide a suitable interface for the effective ionization of non-volatile and thermally instable chemicals (10). The instrument of choice for liquid chromatographers is rapidly evolving to be Liquid Chromatography/Mass Spectrometry (LC/MS). It is a potent analytical method that combines mass spectrometry's detection specificity with liquid chromatography's resolving strength. The components of the sample are separated using liquid chromatography (LC) before being introduced to the mass spectrometer (MS). Charged ions are produced and detected by the MS. The molecular weight, structure, identity, and quantity of particular sample components can all be determined using the LC/MS data.

The combination of an LC with a mass spectrometer (MS) is known as LC-MS or HPLC-MS.

The separated sample emerging from the column can be identified based on its mass spectral data. The two methods can be combined effectively through the use of a switching valve. A double three-way diverter connected in parallel to an auto-sampler, the LC system, and the mass spectrometer makes up a standard automated LC-MS system. The diverter is usually used as an automatic switching valve before the sample enters the MS. Unwanted elute components from the LC system to trash. It is possible to rebuild an unknown molecule from MS Data is thanks to this qualitative approach. Soft ionization techniques, which typically exhibit mainly the molecular ion species with very few fragment ions, include Typically, employed in LC-MS. As a result, the information on the compounds structure gleaned from one single LC-MS run is not very good. Tandem mass spectrometry (MS-MS), which provides fragments through collision-induced dissociation of the This problem has now been addressed by the use of molecular ions generated, (11). Application of LC-MS-MS is expanding rapidly. Hyphenated techniques, such as HPLC coupled to UV and mass, when combined with biological screening, LC-UV-MS has proven to be very helpful for a quick survey of natural compounds. Nowadays, many different types of LC-MS systems, each with a variety of different interfaces, are available commercially. The interfaces are made to provide sufficient liquid nebulization and vaporization, sample ionization, solvent vapor removal, and ion extraction into the mass analyzer.

LC-MS benefits:

1. Dissatisfaction with the inconsistent cross-reactivity with metabolites and high -*cost 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 employing LC-MS for vitamin D assessments because commercial immunoassays react differently to different forms of vitamin D and its metabolites.
3. LC-MS analysis is pertinent to a number of steroid biochemistry topics. Numerous very sensitive LC-MS assays that can provide precise readings in these classes have been developed as a result of challenges with measuring low testosterone and dihydrotestosterone levels in women and children using conventional immunoassays[8].

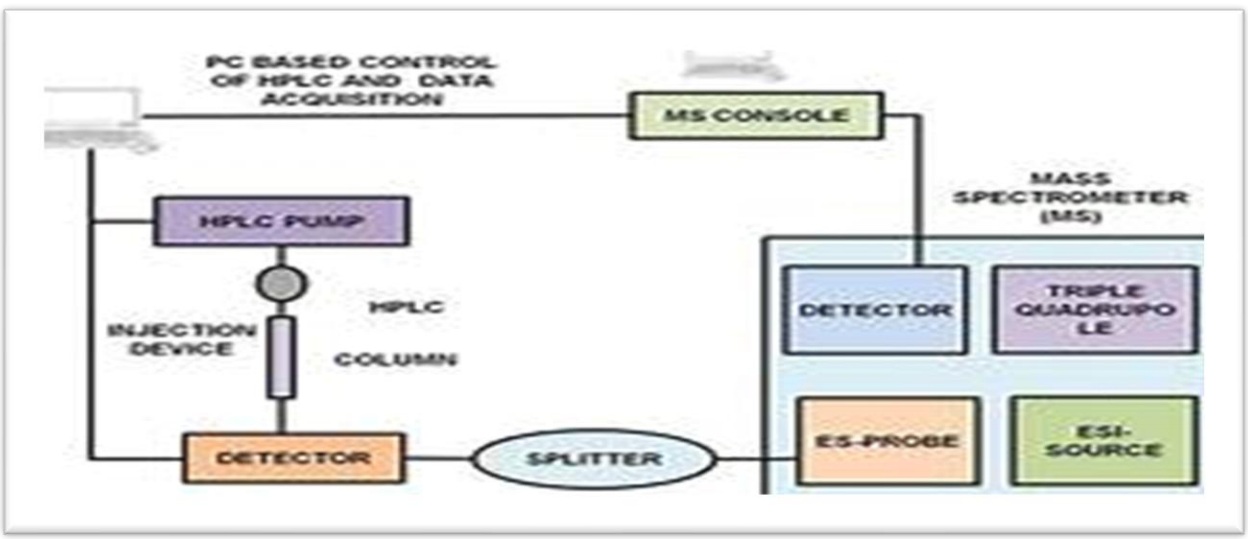

Fig 5: Schematic diagram of a Lc-ms

5. GC -FTIR

Gas chromatography with Fourier transforms infrared spectroscopy, or GC-FTIR, is a hybrid technology where samples are separated by the GC stage and identified by the IR stage. A capillary GC column is commonly used for improved chromatographic efficiency, and an interferometric (Fourier transform or FT) spectrometer is usually included in the IR stage. Quantitative results are possible, even though GC-FTIR is usually employed to offer qualitative information about different kinds of analytes. Modern GC-FTIR equipment can separate complex mixtures of volatile substances and provide molecular characterisation of the constituent parts. Their functional groups can be determined via IR spectroscopy. Software for searches and spectrum libraries are available. Fractions may be identified automatically. This type of equipment produces reliable, quick results without requiring extensive sample preparation. It has been applied in several fields in the past. The combination of gas chromatography and FTIR is a powerful technique for detecting the components of complex mixtures, including natural products, pesticide residues, environmental pollutants, and drug metabolites. The conventional light pipe interface is the most often used method of connecting a gas chromatograph to an FT-IR[9].

GC-FTIR Interface

A Discovers solid phase FTIR detector (Spectra-Analysis Instrument Inc., Marlborough, MA, USA) was attached to the transfer line that emerged from the GC oven. The restrictor was placed directly above a Zn Se disk that was cleaned every day with acetone and cooled to −50°C using liquid nitrogen. A cryogenically cooled Mercury-Cadmium-Telluride (MCT) detector was part of the Discov IR FTIR apparatus. With a resolution of 4 cm−1, a scan rate of 2 Hz, and a disc rotation speed of 3 mm/min, solid phase infrared spectra of the GC-eluted chemicals were captured in real time from 100 µm × 100 µm spots in the 650–4,000 cm−1 range[10].

Benefits:

1.Environmental: GC/IR has been used extensively to analyze typical environmental contaminants both qualitatively and quantitatively.

2. Flavour and fragrance: Taste and aroma chemical identification is the primary application of GC/IR.

3. Pheromones: The IR spectra confirmed the hydroxyl group of the pheromonal molecule, and the secondary alcohols had an OH stretching frequency of 3668 cm-1. An additional stretching frequency (3605 cm-1) revealed intramolecular hydrogen bonding, most likely as a result of the molecule interacting with a carbon-carbon unsaturation. Intramolecular hydrogen bonding is present in this system.

4. High Temperature Coal Tar Compositional Studies: Coal tar, a byproduct of cooking, is an essential raw resource for industrial and environmental uses.

5.Analysis of perfumes: Perfumes, whether synthetic or natural, are intricate blends of various ingredients. Perfume analysis also makes use of GC-FTIR in addition to GC-MS.

6. A quantitative analysis of caffeine.

7. Odour Essential Oil Analysis.

8. Spirit analysis.

9. Examination of unknown illicit substances[11].

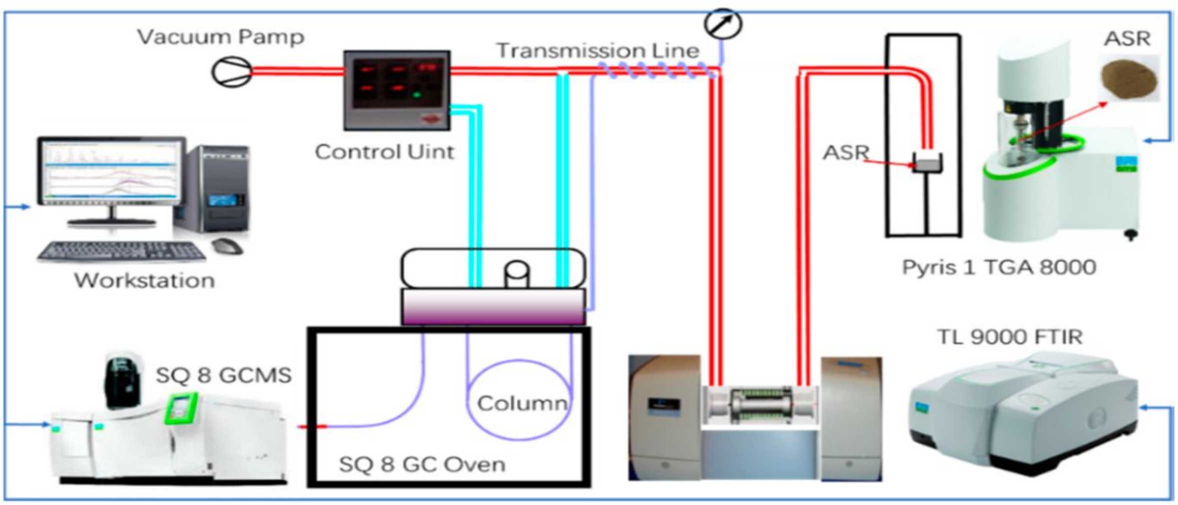

Fig 6: Schematic diagram of a GC FTIR

III. TRIPLE HYPHENATED TECHNIQUE

6. LC-MS AND NMR:

An overview of LC-MS and NMR is given in the section that follows. The analytical benefits are discussed, along with some of their contrasting characteristics and the structural information obtained from each detector.

LC-MS:

Because of its excellent sensitivity and selectivity, MS in conjunction with liquid chromatography (LC-MS) has emerged as the preferred technique for structural identification of analytes present in complex mixtures since the development of electrospray ionization. For analytes with high ionization efficiency, the limits of detection of MS are easily within 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 at once, the LC separation significantly lowers the complexity of samples in MS, which in turn lowers ion suppression. Molecular weight information is provided by MS, and the elemental makeup of substances can typically be inferred from precise mass measurements. Additionally, tandem mass spectrometry (MS/MS) uses the fragmentation patterns of substances to provide structural information.MS is perfect for high-throughput analysis because its spectral scan speeds range from nanoseconds to microseconds, depending on the mass analyzer. It is perfect for complex sample analysis when MS and LC are combined. However, one drawback of MS is that, in the absence of reliable standards, it cannot definitively identify a compound's structure. Comparing the retention time and MS/MS spectrum pattern of the target analyte with those of an authentic standard is often the basis for definitive structural identification by LC-MS.

NMR (and LC-NMR)

When more precise structural characterisation is required, NMR (particularly LC-NMR) is typically the preferred analytical technique. The interaction of magnetically active nuclei with an external magnetic field provides the basis for the NMR phenomena. NMR may theoretically detect any magnetically active nucleus; however, because most nuclei have poor sensitivity, 1H, 13C, 19F, and 31P are the most commonly examined nuclei in NMR. Compared to nuclei like 13C, which is found at 1.1% abundance, some nuclei, such as 1H and 19F, are found at ~100% abundance and are detected with greater sensitivity. Although it is identified indirectly through attached 1H, 15N is also often researched.

(i) The chemical shift, which depends on how sheltered a nucleus is by surrounding electrons, provides structural information
(ii) Splitting patterns that reveal the number of nearby nuclei
(iii) Multidimensional tests that show atomic interconnectedness. Moreover, NMR is inherently quantitative because, unlike in MS, the NMR signal is independent of matrix effects. Moreover, since the NMR analysis is non-destructive, a sample can be recovered and subjected to additional Analysis using different methods. The fact that NMR measurements are consistent and repeatable across many NMR devices (independent of Another advantage of the use of NMR over MS is the enhancement in resolution with respect to vendor or field intensity. Which is not the case in MS analysis, where data depends on the type of mass instrument and software used, as well.as the ionization. However, NMR requires a relatively higher material concentration for analysis compared to MS[12].

Interfacing LC, NMR and MS

Because of proton-deuterium exchange, groups like carboxylic acid, phenol, and am in o groups are NMR-silent in all solvents. No protons are present in nitro groups and sulfate conjugates that are readily detectable by mass spectrometry even if they are not directly visible in proton NMR spectra (it is rarely possible to solve the structure of a novel molecule by NMR alone).typical functional MS). On the other hand, molecular weight, fragmentation, and molecular formulas obtained from MS data may not be adequate to clearly identify the molecular structure of an unknown drug. Even in the most challenging situations, LC-MS can discriminate between tightly eluting isobars and isomers. It is possible to collect HPLC fraction s for off-line analysis in NMR tubes. However, parallel on -line NMR and MS detection efficiently provides complementary data and minimizes ambiguities between LC–MS and LC–NMR systems. Indeed, the characterization of some reactive and volatile metabolites was impossible by LC–MS and only feasible by LC–NMR. Interfacing LC with NMR and MS was difficult until recently. However, many of the challenges associated with placing the LC and MS in close proximity to the NMR magnet have recently been solved. A bench-top ion trap MS may now be positioned less than one meter from the centre of a 500 MHz magnet without any performance loss, thanks to the newest shielded magnets. Besides, this small distance reduces LC peak broadening, which improves NMR sensitivity. In practice, a post-column splitter is employed to perform a simple hyphenation of LC to NMR and MS. Through a 1-2 m capillary, this delivers 90–95% of the flow to the NMR and the remaining portion to the MS. The BNMI - Bruker NMR-Mass Spectrometry Interface-is the name given to this powerful valve-switch interface. Substitute To provide a suitable make-up flow for the best ionization in the MS, this splitter and double dilutor are controlled by a computer. Moreover, it allows its exchange of protons and deuterium to simplify MS spectra that would otherwise be Acquired in LC-NMR-MS. This BNMI is important in the LC–NMR–MS loop storage method, in which a portion of the loop contents can be stored in a delay loop upon transfer to the NMR. The resulting dilutor then gradually fuses the analytes into the MS during NMR acquisition. These integrated LC-NMR-MS systems are highly flexible and can be used when required. In fact, regular use of the LC-MS in longer running NMR experiments is possible. Other researchers have utilized the higher sensitivity of MS to target major analytes, which require longer NMR acquisition times for high-quality NMR data. Expert optimization of chromatographic separation conditions using a set of predefined ground rules greatly improves the routine production of LC-NMR-MS data. Analytical scale reversed-phase HPLC typically employs columns with dimensions of 2–4.6 mm in diameter × 50–250 mm in length and flow rates of 0.2–1.5 m l m in −1. Effective solvent suppression techniques are known, and mobile-phase combinations using deuterated water and non-deuterated organic modifiers (acetonitrile/D2O or methanol/D2O) are frequently used. In order to minimize solvent artifacts in the NMR spectra, a completely deuterated organic solvent may sometimes be used. However, when the NMR signals of two or more compounds overlap, one-dimensional 1H NMR is rarely able to properly characterize co-eluting analytes unless there is a considerable dynamic range variation in the concentration of the co-eluting analytes. Of course, coeluting components can be deconvoluted via online MS detection, but only if they are neither isomers nor isobars. It is possible to utilize two-dimensional H–H NMR spectroscopy to find correlations between the resonances in the co Eluting analytes if sufficient material is provided. Although optimization of chromatographic resolution is often the logical approach, this can be very time-consuming and counterproductive. Combination of the NMR designations with the MS data often provides unambiguous structural elucidation[13].

LC–NMR–MS hardware and software control

As seen in Figure I, the hardware is changed from LC–NM R–M S to LC–SPE–NM R–M S with little reconfiguration. A quaternary LC pump for both deuterated solvents - for LC–NM R–M S - and for nondeuterated solvents - for LC SPE–NM R–M S, auto sample, column and oven, UV or other analogue detector, NM R–M S interface Common hardware includes the (BNM I), and peak selection units, which are shown in white. The yellow-coloured BPSU-36 represents the automatic loop storage mode. A make-up flow pump dilutes HPLC peaks with water mobile phase post-column for effective trapping on 96 well plate SPE cartridges. The solid phase extraction (SPE) units for online concentration are shown in blue. Although quadrupole and time-of-flight instruments are also possible, the usual mass spectrometers employed are. The peaks are extracted from the NM R flow probe via a fraction collector. While the NMR apparatus is controlled by one computer, the LC and MS collection and data processing are managed by another. shows the acquisition window of the LC control softwares during an automated loop storage experiment. Hardware control is indicated by the blue windows

(a) Real-time diode array UV data
(b) are obtained. According to the legend
(c) and different mass spectral profiles
(d) shows the detection of HPLC peaks. Peaks stored in loops for a subsequent NMR analysis are indicated by the vertical lines. Unexpected peaks that appear for a sample can also be manually recorded. The main window is expanded in (f), where decisions about the subsequent eluting peaks are made possible by a time delay. Lastly, the log of hyphenated events is represented by (g).
(e) which defines UV and M S peaks, the main acquisition window[14].

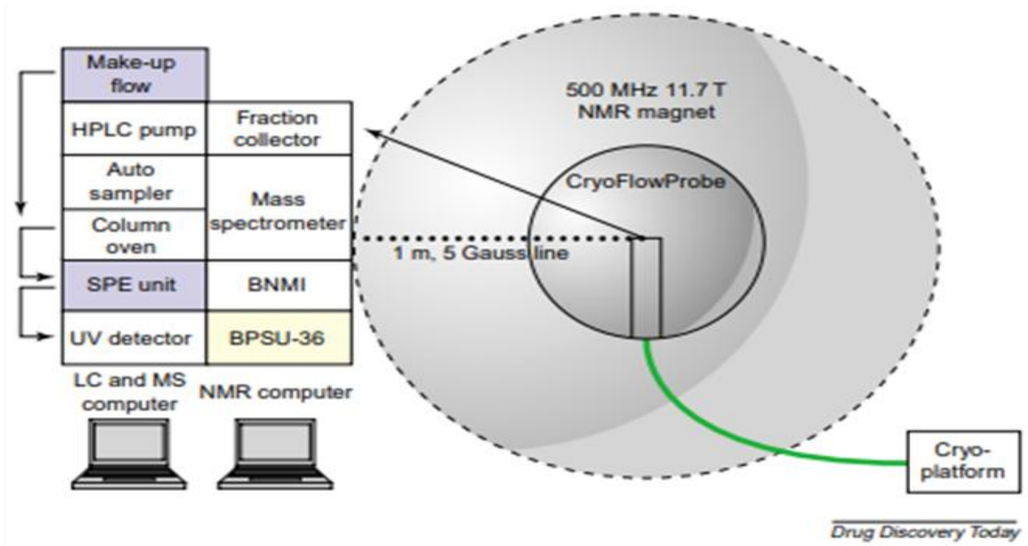

Fig 7: Schematic diagram of a LC–NMR–M S hardware control.

REFERENCES

[1] Pardeshi J, Darade R, Sarukh V, Garad M, Sonawane K. A Review on Hyphenated Techniques and Their Applications in Analysis of Pharmaceutical. Int J Novel Trends Innov (IJNTI). 2025;3(1): a71-a82.

[2] Patel PB, Dave TH. A Review on Big Data in Business Analytics. Int J Adv Res Dev (IJARND). 2017;2(4):25-8.

[3] National Institute of Standards and Technology. Guide to Operational Technology (OT) Security. Gaithersburg (MD): U.S. Department of Commerce; 2023. NIST Special Publication 800-82 Revision 3.

[4] Editorial Board. Overview of World Journal of Pharmaceutical Research. World J Pharm Res. 2020;9(14): i-iii.

[5] Somsen, G. W., Gooijer, C., & Brinkman, U. A. (1999). Liquid chromatography-Fourier-transform infrared spectrometry. Journal of Chromatography A, 856(1-2), 213-242.

[6] Jayanthi A, Nappinnai M, Poongothai S, Ganesan A, Hemapriya M. A Review on Ethosome: A Novel Carrier for Transdermal Drug Delivery. IOSR J Pharm Biol Sci. 2016;11(6, Ver. II):65-74.

[7] Pardeshi J, Darade R, Sarukh V, Garad M, Sonawane K. A Review on Hyphenated Techniques and Their Applications in Analysis of Pharmaceutical. Int J Novel Trends Innov (IJNTI). 2025;3(1): a71-a82.

[8] Ahmad S, Al-Suwayeh M, Rizwan M, Al-Obaid M, Husain S. A Review on Ethosomes as Novel Vesicular Carriers for Enhanced Transdermal Drug Delivery. Int J Pharm Sci Drug Res. 2014;6(2):107-13.

[9] Pallavi. Review on Hyphenated Technique. *World J Pharm Res* [Internet]. 2020 [cited 2025 Nov 14];9(14): [about 7 p.]

[10]. Applications of GC FTIR in pharmaceutical analysis review. *Int J Pharm Res Appl* [Internet]. 2022 [cited 2025 Nov 14];7(4): [about 8 p.].

[11] Al-Horani RA, Al-Horani H. Drug repurposing for COVID-19: A systematic review and meta-analysis. *Infect Dis Health* [Internet]. 2020 Aug [cited 2025 Nov 14];25(3):209–27.

[12] Phole PM, Ugale GA, Chakole RD, Charde MS. Applications of GC-FTIR in pharmaceutical analysis: review. Int J Pharm Res Appl. 2022 Jul–Aug;7(4):1133–40.

[13] Ranganath C, Ritchey M. The roles of the medial temporal lobe in memory: Insights from fMRI. *Nat Rev Neurosci* [Internet]. 2018 Sep [cited 2025 Nov 14];19(9):609–22.

[14] Corcoran O, Spraul M. LC-NMR-MS in drug discovery. Drug Discov Today. 2003 Jul 15;8(14):624-31.