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DOUBLE HYPHENATED TECHNIQUES: AN OVERVIEW

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ABSTRACT
A separation method and a spectroscopic detection method are combined to create the hyphenated methodologies like GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS to name a few. A hyphenated methodology combines or couples two distinct analytical methods with the use of an appropriate interface. The majority of these combinations use chromatographic and spectroscopic methods. The phrase "hyphenated methods" refer to the fusion of identification, separation, and identification approaches. Better component analysis results from the hyphenation of these methodologies. Hyphenated methods demonstrate sensitivity and specificity. Fractions of the chemical components of a mixture that are pure or virtually pure are produced via chromatography. Different chemical and instrumental techniques that are involved in drug estimation are created periodically so that medications would perform their intended purposes. Spectroscopic methods like NMR, MS, and IR are utilised for identification whereas chromatographic methods like GC, LC, etc. are employed for separation. Pharmaceuticals can acquire contaminants throughout the different stages of development, transit, and storage making them dangerous to administer. So, these hyphenated techniques are useful to detect the impurities and save the patients from toxicities.

KEYWORDS: GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS, GC-IR

INTRODUCTION TO HYPHENATED TECHNIQUES

Hirschfield first used the word "hyphenation" to describe the online fusion of a separation method with one or more spectroscopic detection techniques a few decades ago. This method—now referred to as hyphenated method—was created by combining a separation method and a spectroscopic detection method. Chemicals are separated from solutions and detected using hyphenated separation techniques, which combine two or more procedures. Chromatography in some form is most frequently used approaches here. In Chemistry and Biology, hyphenated methods are often employed. Sometimes a slash is used in place of a hyphen, particularly if the name of one of the methods already contains a hyphen. Combinations of the aforementioned methods result in "hybrid" or "hyphenated" methods. Existing hybrid approaches are being developed, and there are some examples that are in widespread usage now. As an illustration, we can consider GC-MS (gas chromatography-mass spectrometry), LC-MS (liquid chromatography-mass spectrometry), GC-IR (gas chromatography - infrared spectrometry), LC-NMR (liquid chromatography – nuclear magnetic resonance), LC-IR (liquid chromatography – infrared spectrometry), CE-MS (capillary electrophoresis - mass spectrometry), ICP-MS (inductively coupled plasma mass spectrometry) and so on. In order to benefit from both, hyphenated techniques combine chromatographic and spectroscopic approaches. Fractions of a mixture's chemical constituents that are pure or virtually pure are produced through chromatography. For identification using standards or library spectra, spectroscopy generates selected information.[1]

A hyphenated technique is created by combining a separation technique with an online separation approach. The marriage of two distinct analytical procedures via proper interfaces, typically with the backup of a computer linking everything together, is known as a hyphenated analytical chemistry methodology. Hirschfield is credited with coining the word "hyphenation," but the concept dates back to the pairing of GC & MS in the early 1970s. Hyphenated approaches have drawn more and more attention in recent years as the primary method for resolving challenging analytical issues. "A hyphenated technique will result from the combination of the separation approach and an online spectroscopic detection technology".[2] In order to analyse unknown substances in complicated natural

product extracts or fractions, both quantitatively and qualitatively, it is a powerful technique to combine separation methods with spectroscopic techniques. Spectroscopic detection is coupled with liquid chromatography (LC), often high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) to gain structural information leading to the identification
of the chemicals contained in a crude sample. The contemporary analytical methods include Fourier transform infrared (FTIR), UV-visible absorbance or fluorescence emission, mass spectroscopy (MS), and nuclear magnetic resonance spectroscopy (NMR). An analytical method for finding pharmaceuticals in biological materials is a critical stage in the discovery and development of drugs and this leads to the development of hyphenated techniques.

Advantages of Hyphenated Techniques

1. To facilitate quick analysis
2. More automation overall.
3. An increased sample throughput
4. More consistent replication.
5. The closed system helps to reduce contamination.
6. Simultaneous quantification and separation.[3]

Types of Hyphenated Techniques

i. Double hyphenated techniques
ii. Triple hyphenated techniques

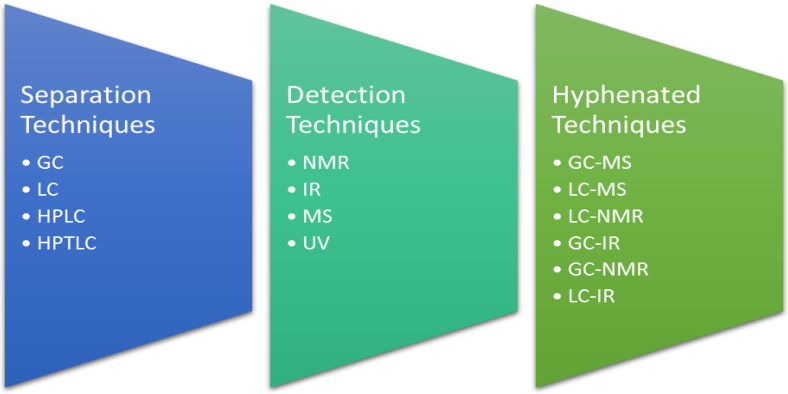

Fig. 1: Hyphenated techniques for separation and detection techniques.

Types of Hyphenated Techniques

Table 1: Types of hyphenated techniques.

Sr. No.	Double Hyphenated Techniques	Triple Hyphenated Techniques
1	GC-MS	APCI-MS-MS
2	LC-NMR	SPE-LC-MS
3	LC-MS	LC-ESI-MS
4	LC-IR	LC-PDA-NMR-MS
5	CE-MS	LC-UV-NMR-MS-ESI
6	GC-IR	LC-API-MS
7	GC-FTIR	LC-MS-TSPLC-UV-NMR-MS
8	GC-NMR	LC-NMR-MS
9	LC-GC	LC-DAD-API-MS
10	ICP-MS	LC-PDA-MS
11	ICP-OES	LVI-GC-MS
12	ICP-AAS	ESI-MS-MS

1. Gas Chromatography-Mass Spectrometry (GC-MS)

Soon after gas-liquid chromatography and organic mass spectrometry were developed, Holmes and Morrell (Holmes & Morrell, 1957) successfully proved the first-time gas chromatography and mass spectrometry could be coupled. Later, computer-controlled quadruple mass spectrometer for quick acquisition was developed to accommodate the separation in gas chromatographs, leading to the commercialization of upgraded GC-MS equipment. Since then, it has become a standard method of preference for (bio)organic analysis. A gas chromatograph and a mass spectrometer are the minimum number of two primary components that make up a GC-MS apparatus, as implied by the name. A gas chromatograph is used in GC-MS to separate chemical mixtures into their constituent components, which are then identified and/or quantified at the molecular level using a MS detector. For capillary columns, the flow rate of the carrier gas is about mL/min, while for packed columns, the flow rate can reach 150 mL/min. On the other hand, with a mass spectrometer, all of the ionisation, ion transmission, separation and detection processes take place in a high vacuum system at a pressure of around 10^{-4} Pa (10^{-6} Torr). As a result, in order to produce a suitable environment for the coupling,

adequate pumping power is always necessary at the interface area of a GC-MS. Numerous techniques can be used to ionise the analytes as they go through the transfer line, transit through the length of the column, and reach the mass spectrometer. Ionization and fragmentation are complementary processes. via a mass analyser, ion separation.[4]

Electron Impact (EI) Ionization: EI source is a device that houses the ion source with a volume of about one cubic centimetre. The ion source is left open to enable the greatest possible gas conductance from the ion source into the source housing and subsequently into the high vacuum pumping system. In order to move the electron beam in a three-dimensional helical pattern and improve the likelihood of an electron interacting with an analyte molecule, the EI source is equipped with a pair of permanent magnets. Even in this scenario, only 0.01–0.001% of the molecules of the analyte are really ionised (Watson, 1997). The vaporised molecules enter the MS ion source where they are attacked with free electrons released from a heated filament during the EI ionisation.

Chemical Ionization (CI): More energy is expended during EI than CI. In the later instance, the ionising electrons accelerated to have some kinetic energy hit directly with the analyte molecules in gas phase, leading to simultaneous ionisation and fragmentation. For a singly charged analyte, the mass-to-charge ratio of the molecular ion determines the analyte's molecular weight (amu). Molecular weight, molecular ion, precise mass, and isotope distribution are all included. The most prevalent isotope of each element's mass defect is disregarded when calculating the nominal mass of an ion using the integer mass. The protons and neutrons in all of the component atoms are added together in this way, which is equal. With the use of EI ionisation, several molecular ions may be produced using soft-ionization methods (CI, FI) might be beneficial.[5]

Typical Mass Analysers And Ms Detectors In GC-MS: For the connection of GC and MS, MS instruments with various mass analysers, such as magnet/electric sectors, quadruples (linear), ion traps (Paul traps & quadruple linear ion trap), FT-ICR, and time-of-flight (TOF) mass analysers have been applied. The majority of GC-MS applications have utilised linear quadruples since they were first developed. Triple mass analyser four hyperbolic metal rods arranged in a radial array form the basis of a single-stage linear quadruple mass analyser, which is also known as a mass filter. For the purpose of capturing and storing ions in a vacuum chamber, an ion trap mass spectrometer combines magnetic and electric forces.

Time-of-flight (TOF) Mass Analyser: The basis for Duration-of-Flight Mass Spectrometry (TOF-MS) measures an ion's flight time over a defined distance which is a straightforward mass separation principle (Cotter, 1994; Stephens, 1946). A constant homogeneous electrostatic field of known intensity is used to initially accelerate pulsed ions such that they have the same kinetic energy. Kinetic Energy = $(1/2)\ mv^2$ and the time of arrival t at a detector $t = d(m/q)^{1/2} - (2U)^{1/2}$ where d is the length of the flight tube, m is the mass of the ion, q is the charge, and U is the electric potential difference utilised for the acceleration. As a result, the square of the velocity of an ion is inversely proportional to its m/z.[6]

Instrumentation and Working: With the aid of a heated carrier gas, vaporized analyte is transported through the GC column, and only the column experiences separation. The mobile phase, such as helium, can also be referred to as a carrier. The separation of the compounds occurs as a result of distinct interactions between the analyte in the mobile phase and stationary phase. The characteristics of the stationary phase, type of carrier gas, gradient in column temperature, and the column's size (length, diameter, film thickness) all affect how the analyte separates. Different boiling points and other chemical characteristics cause the mixture's components to separate as the sample moves through the length of the column. Due to the components varying adsorption or varying partitions between the mobile phase and stationary phase, respectively, the elution time and retention period will change for each component. The separated mixture elements will then pass through an interphase and into the MS. Following this, each analyte's ions are ionised, subjected to a mass analysis, and then the mass spectrometer measures the ions mass-to-charge ratios. A device such as an effusion separator, jet/orifice separator, or membrane separator can be utilised as an interface to join GC and MS. Through the use of chemical and electron impact ionisation, the ionisation process not only ionises the molecule but also breaks it into pieces that may be detected. A finger print is created by the molecular ions of the analyte.

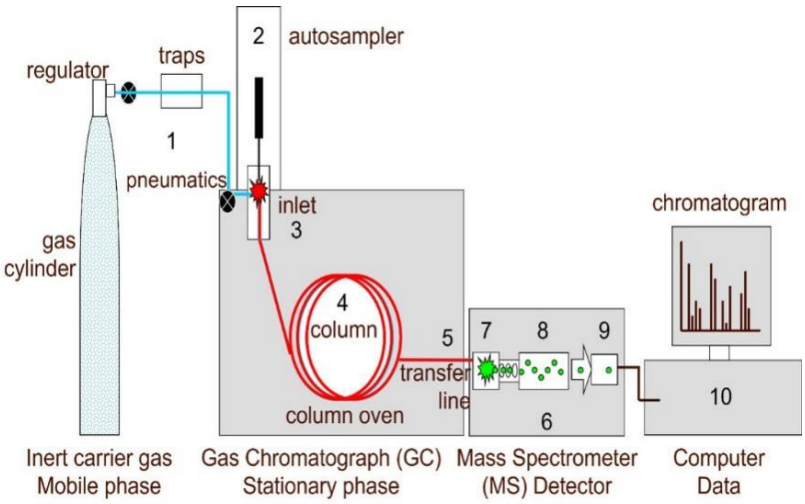

Fig. 2: GC-MS.

Applications of GC-MS

1. This technique is used for the quantification of contaminants in drinking water. The Environmental Protection Agency (EPA) upgraded these two systems by hyphenating them, and they are now both officially capable of waste water management approaches.
2. It is also used for quantitative analysis of drugs or metabolites in urine and is performed for screening pharmacological activity.
3. It is also used for labelling of unidentified organic substances. The most common contact is electro spray. When using synthetic organic liquid chromatography, the substances in hazardous waste dumps spray needle are employed as a link between the reaction products and the mass. It is convenient to use the separate emitter for pesticide and drug analysis.[7]

2. Liquid Chromatography- Nuclear Magnetic Resonance (LC-NMR)

Principle: They have been widely used in the analysis of complex mixtures that contain unknown components, such as impurities and metabolites in pharmaceuticals, natural products, and synthetic polymers (3,4), ever since they were first reported in 1978. This technique combines high-performance liquid Chromatography (HPLC) and Nuclear Magnetic Resonance spectrometers (NMR).[8]

The first online LC-NMR experiments were carried out by Watanabe and Niki in the late 1970s. The addition of a thin-walled Teflon capillary within a typical NMR tube converted the normal NMR probe into a flow-through probe, and spectra were collected as the sample was rotated. Theoretically, saving a lot of time by physically linking LC and NMR has been suggested for more than 20 years. The last ten years have seen the first successful and useful use of LC-NMR coupling.[9]

Instrumentation: A typical online LC-NMR system comprises of an LC device that is coupled to an NMR detection device into which a flow probe is put. The LC apparatus is made up of a pump mechanism that forces liquid solvent through the system, a column where separation occurs, and a detector with a flow cell that uses light to measure the components as they elute. Refractive index (RI), ultraviolet/visible light (UV/VIS), or infrared light (IR) detectors are three different types of light detectors that can be used in this system.[10] Diode Array Detector (DAD), a form of UV/VIS detector that can measure many light wavelengths simultaneously, is the one that is most frequently used in modern times. It basically doesn't matter what kind of detector is used as long as the sample is not changed or destroyed.[11] The NMR system is composed of out of a massive radiofrequency (RF) magnet where a vertically oriented, non-rotating flow cell has been installed This orientation makes room for laminar flow and makes it simple to remove bubbles from the mobile phase. The glass that makes up the flow cell is the sole variation between the detection volume and coil volume since the RF coil is wrapped around the cell to get a suitable filling factor.[12]

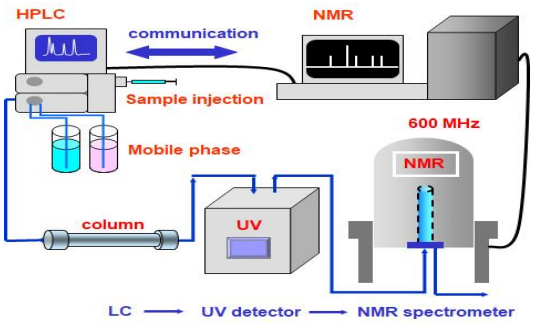

Fig. 3: LC-NMR.

Applications of LC-NMR

1. Recognizing medication deterioration by-products.
2. It is possible to separate and recognise low level contaminants.
3. Using this method, it is possible to monitor the environmental impact of pesticides, herbicides, and organic pollutants.

4. The analysis with this method is quick, effective, and requires little solvent or sample. Long capillaries used to link the CE to the MS will lengthen analysis times and prevent the CE from having an appropriate volatile buffer that is compatible with MS.[13]

3. Liquid Chromatography-Mass Spectroscopy (LC-MS)

Technical difficulties have prevented a solution to the combination of mass spectrometry and HPLC separation for the analysis of non-volatile substances for decades. It was difficult to create a suitable interface for the effective ionisation of thermally and non-thermally stable and non-volatile chemicals, as well as to overcome the solvent and flow rate issues that prevented HPLC and MS from working together.[14] Liquid chromatographers are quickly making LC/MS their primary method of analysis. The combination of mass spectrometry's detection specificity with liquid chromatography's resolving capability creates a potent analytical approach. After separating the sample's components using liquid chromatography (LC), the mass spectrometer is then used to analyse them (MS). Ions with charges are produced and found by the MS. Information may be provided using the LC/MS data.[15]

Instrumentation: An auto-sampler, an LC system, a mass spectrometer, and a double three-way diverter are all parts of a typical automated LC-MS system. The diverter typically functions as an automated switching valve to send unwanted components of the elute from the LC system to garbage before the sample enters the MS.[16]

An unknown molecule can be recreated from MS data using this qualitative approach. The ionisation processes utilised in LC-MS are typically soft ionisation processes, which primarily reveal molecular ion species with a small number of fragment ions. As a result, the knowledge of the compound's structure gained from a single LC-MS run is somewhat limited. However, tandem mass spectrometry (MS-MS) has since been developed to address this issue and yields fragments by inducing collision. Different LC-MS system types with diverse interface are now commercially available. The interfaces are made to provide proper nebulization and vaporisation of the liquid, ionisation of the sample, elimination of the surplus solvent vapour, and extraction of the ions into the mass spectrometer. Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) are the two most often employed interfaces, especially in connection to the examination of natural products. Due to its high solvent flow rate capacity, sensitivity, response linearity, and areas of use, the latter is known as "the chromatographer's LC-MS interface."[17]

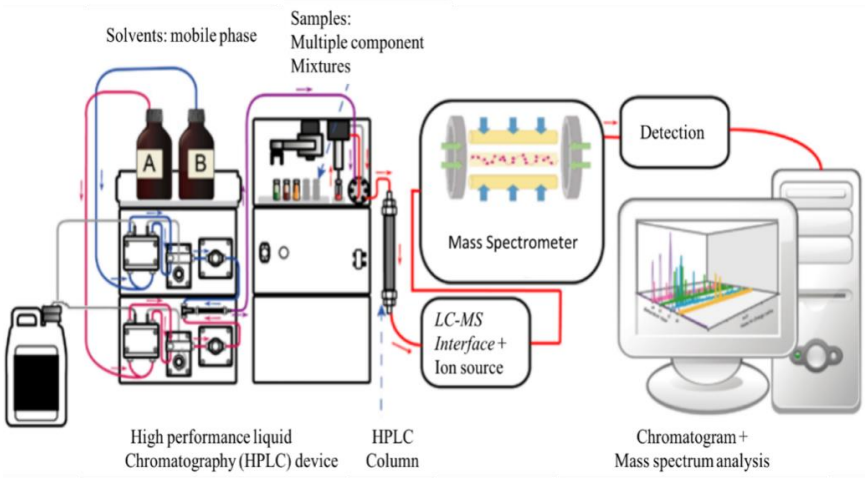

Fig. 4: LC-MS

4. Liquid Chromatography-Gas Chromatography (LC-GC)

Principle: A stationary phase and a mobile phase are used in all types of chromatography. The mobile phase is a liquid in all other types of chromatography that you will encounter at this level. In gas liquid chromatography, the stationary phase is a liquid, whereas the mobile phase is a gas, such helium. The amount of time of a compound spends travelling with the gas as opposed to being bonded to the liquid will determine how quickly it moves through the machine.

Instrumentation and working: With a tiny syringe, very minute amounts of the sample that will be examined are injected into the apparatus. The injector is housed in a thermostatically controlled oven. The sample is hot enough to boil completely, and the helium (or other carrier gas) carries the gas into the column. In gas-liquid chromatography, there are primarily two types of columns. The stationary phase is contained in one of these long, thin tubes, while it is bound to the inner surface of the other, which is even thinner. The column normally has an internal diameter of up to 4 mm and is between 1 and 4 metres long. It is composed of stainless

steel. It is wound up so that it will fit inside an oven with a thermostat. Diatomaceous earth, an extremely porous rock, is packed tightly into the column. This has a coating made of a highly flammable liquid, usually a waxy polymer. The column's temperature may be adjusted from around 50°C to 250°C. As it is colder than the injector oven, certain combination ingredients may condense at the start of the column. The retention time of a certain chemical is the amount of time it takes for that compound to move from the column to the detector. This period of time is determined by timing the injection of the sample and the moment the monitor displays the compound's highest peak height.

Retention times for various substances vary. The retention period for a certain molecule varies according to the compound's boiling point. The beginning of the column will be where a substance that boils at a temperature greater than the column temperature will spend almost all of its time condensed as a liquid. High boiling point hence indicates a lengthy retention duration. A molecule will spend less time being transported by the gas if it is more soluble in the liquid phase. A high retention time is a result of high solubility in the liquid phase.

Higher temperatures have a tendency to stimulate molecules into the gas phase because they either evaporate more easily or are so energetic that they are no longer attracted to the liquid phase. Everything in the column has shorter retention periods when the column temperature is high. We can't do much about the compounds' boiling points or their solubility in the liquid phase for a given sample and column, but we can change the temperature.

The separation will be better if the temperature of the column is lower, but it may take a very long time to get the chemicals through that are condensing at the start of the column. On the other hand, everything will go along the column considerably more rapidly when heated up, but less effectively. The solution is to start the column at a reasonably low temperature and then gradually and often raise it. Compounds that spend the majority of their time in the gas phase will first move fast through the column and be visible. Several detectors of various kinds are in use. The flame ionisation detector is a widely used device**.**

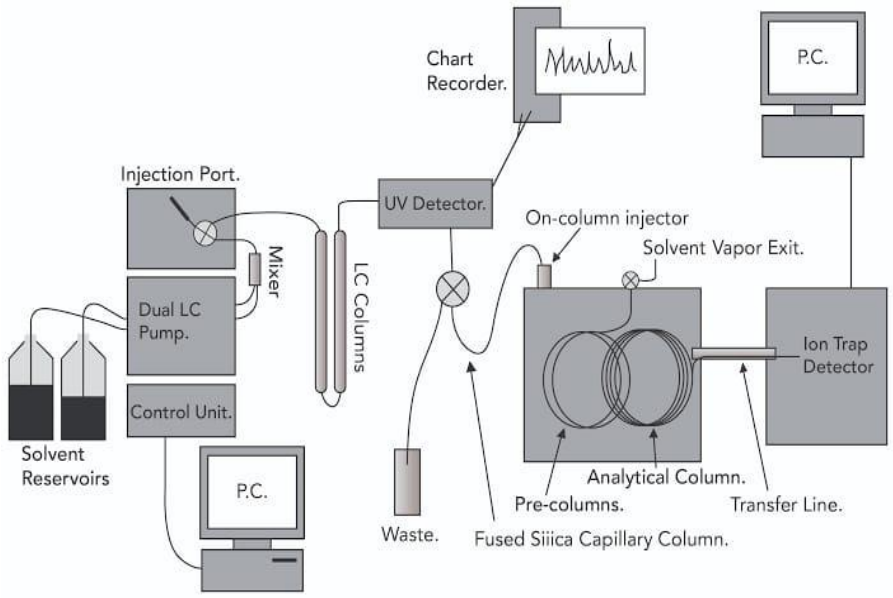

Fig. 5: Instrumentation of LC-GC.

Applications of LC-GC

i) Fossil fuel analysis
ii) Food sampling
iii) Enviromental samples analysis
iv) Biological/pharmaceutical samples monitoring

5. Capillary Electrophoresis-Mass Spectroscopy (CE-MS)

Principle: With the use of the online separation method known as CE-MS, molecules are identified based on the structural data and variations in their electrophoretic motilities. The components are separated using capillary electrophoresis (CE), and the mass will reveal which components were successfully separated. This method allows for quick analysis with less solvent and sample needed. Long capillaries used to link the CE to the MS can lengthen analysis times, and there is a dearth of adequate volatile buffers that can work with MS.

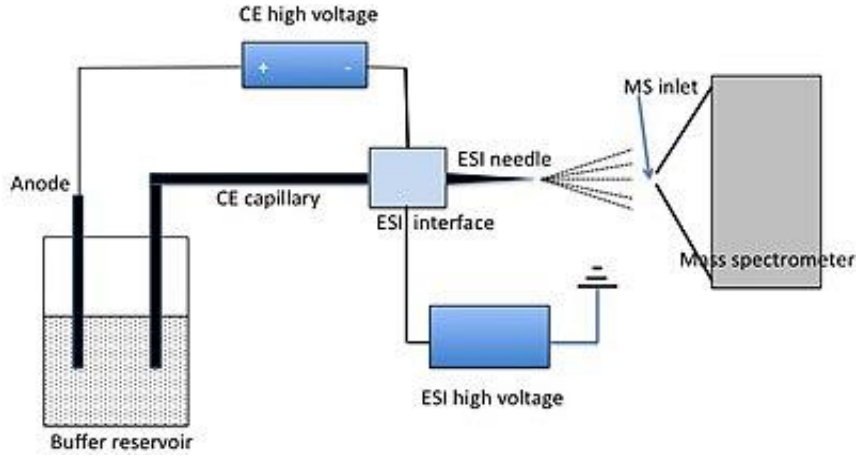

Fig. 6: CE-MS.

Instrumentation and Working: The UV and DAD detectors and inorganic, non-volatile electrolytes employed in this approach are for the CE analysis. This method's shortcomings include a lack of selectivity, repeatability, and reproducibility. By using the buffer system, which will double-coat the inner wall of the fused silica capillaries, these issues can be resolved. The connectors allow a very tiny volumetric flow into the MS. Electrospray, rapid atom bombardment interface, and ion spray ioniser are common ionisation MS. The TOF, ion trap and quadrupole detectors are employed in mass spectroscopy.

Applications

1. It is possible to distinguish between basic and acidic chemicals using non-aqueous CE and non-aqueous CE-MS.
2. Analysis of complex arabinose oligosaccharides.
3. A technique for drug bioanalysis and biomarker identification is the CE-MS.[20]

6. Gas Chromatography-Infrared Rays Spectroscopy(GC-IR)

Instrumentation and Working: It takes many analytical scale separations to identify each component in a mixture; this is because measuring retention data for this purpose is frequently too ambiguous for the identification of molecules eluting from a capillary gas chromatography (GC) column which has the capacity to resolve hundreds of components.[25]

The identification process may be aided by prior knowledge of the components' chemical structures and by spiking the mixture with one or more reference standards. However, a less ambiguous identification can be achieved by connecting the chromatograph to a sensitive, rapid scanning spectrometer to obtain the distinct signatures of each component. The chromatographic resolution of this device ought to enable for the real-time detection of each component. The most widely used method for this is mass spectrometry (MS), although it has certain drawbacks, especially when it comes to differentiating between structural isomers like ortho-, meta-, and para-xylene, whose electron-impact and chemical-ionization mass spectra are similar. A method that is supplementary to MS is sought for such compounds. A common alternate method for this is Fourier transform infrared (FT-IR) spectroscopy, which produces distinctive spectra for the majority of structural isomers.[26]

Light-Pipe-Based GC-IR Instruments: Three methods have been used to couple gas chromatography with FT-IR spectrometers (GC-IR). The GC column is connected directly to a heated cell. This cell is typically constructed for capillary GC using a 10-cm length of heated glass tubing with an internal diameter of 1 mm. This tube's interior bore has a thick enough ring of gold coating to be extremely resistant to infrared (IR) radiation. The tube has IR transparent windows at both ends (formed, for instance, of potassium bromide). When IR radiation enters one window, it is multiple resected down the inner bore that is coated with gold before coming through the opposite window. Through heated fused silica transfer lines, the effluent from the Column is transferred into one end of the tube and out of the other. To prevent the condensation of semi-volatile compounds, the entire unit is maintained at a temperature of between 250℃ and 300℃. Each wavelength in the spectrum is modulated at a distinct frequency when infrared light from an incandescent source, such as a Sic Global, is collimated and fed through a rapid-scanning interferometer. The radiation beam is then directed to the light-rest pipe's window, and the infrared beam originating from the second window is refocused onto a sensitive detector, MCT photoconductive detector (usually a mercury cadmium telluride detector chilled by liquid nitrogen). The signal obtained by measuring it in this way is referred to as an interfere-program, and a single beam spectrum is produced by the interfere organ's Fourier transform. The transmittance spectrum of the component, T(v), is calculated as the ratio of a single beam spectra measured with the component present in the light pipe to one measured with only the helium carrier gas. The standard Beer's law operation is typically used to immediately convert the transmittance spectrum to an

absorbance spectrum, a (v), because the relative intensities of bands in absorbance spectra are independent of the concentration of the analysis, enabling spectral library searching. For GC-IR systems that utilise light pipes and the formed spectral bands are fairly broad, it is rarely necessary to analyse spectra at high precision. The standard resolution at which GC-IR spectra are taken is 8 cm since the majority of bands in the spectra of molecules in the vapour phase have a width of at least 10 cm^{-1}.[28]

Matrix-Isolation GC-IR

In this method, argon is added to the helium mobile phase either at the end of the GC column or as a small (1%) component of the carrier gas. The column effluent is then sprayed onto a revolving gold-plated disc that is kept at a temperature of less than 15 K from a heated fused silica transfer line. While argon condenses at this temperature, helium does not. Argon is deposited as a track with a width of about 300m by positioning the transfer line's end at the proper distance from the cooled disc. Any component that emerges simultaneously from the transfer line gets caught in the argon matrix. After the separation is finished, the disc is rotated to the position, so that the focused beam from an FT-IR spectrometer transmits through the argon track, deflects from the gold-coated disc, and then passes through the argon once more before being collected and focused onto an MCT detector. In theory, the argon matrix will isolate each analyte molecule from other molecules of a similar kind if the concentration of any analyte in the matrix is sufficiently low. Although the concentration is typically a bit too high to enable genuine matrix isolation in GC-IR measurements, this method is nevertheless referred to as matrix isolation GC-IR. A sequence of spectra that can be measured by gently rotating the disc is comparable to the series of spectra that are collected in real-time during a light-pipe-based GC-IR run, and from these data, either GS or FG chromatograms may be created. Each component may be examined using spectral library searching, but this requires a particular library of spectra from standards that have been separated using the Matrix.

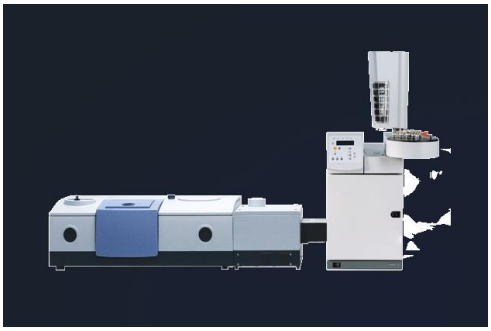

Fig. 7: GC-IR

Applications

1. This technique is used in both Pharmaceutical and Industrial areas.
2. It is used in DNA testing on blood and other fluid samples.[21]

7. Gas Chromatography-Fourier Transform Infrared Spectroscopy (GC-FTIR)

It is conceivable that no matched reference spectra may be identified in MS databases for novel structures or new chemical entities. It is sometimes impossible to offer any potential structures when mass spectra are manually interpreted since it takes thorough understanding of organic mass spectrometry and years of dedication. Information on functional groups or structural moieties with particular infrared absorptions is supplementary to MS and can be highly helpful for structure elucidation using molecular spectroscopy (FT-IR), which is already being employed as a stand-alone method. Each peak separated by GC has its elemental makeup analysed.[22]

Instrumentation: In this procedure, analytes are first atomized at high temperatures, either by Inductively Coupled Plasma (ICP) or microwave irradiation, where the atoms are then moved to an electronically exited state. When these electrons return to their lower energy levels, photons with specific wavelengths that are unique to the individual element are released. Since the sample in both methods is in the gas phase, they are interchangeable. Through a heated nickel transfer line, the GC effluent is immediately injected into the Quartz atomization furnace. The interface is straightforward, but in order to get adequate sensitivity and selectivity, the circumstances must be optimised, such as utilising a quartz furnace, flame heating, a thermostat, or a graphite furnace as the atomization device.[23] Applications are same as GC-IR as mentioned above.

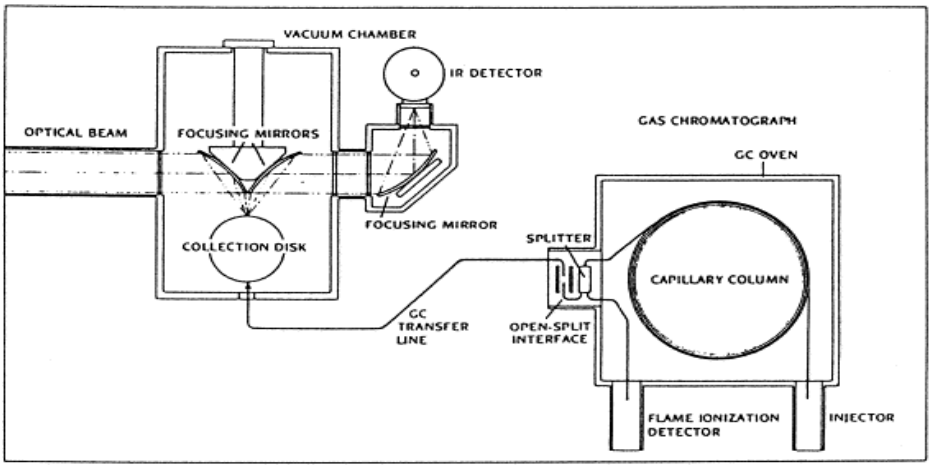

Fig. 8: GC-FTIR

8. Gas Chromatography-Nuclear Magnetic Resonance (GC-NMR)

In this method, GC and NMR are coupled. NMR is utilised to identify the components, and GC is employed to separate the components. This method of hyphenation gives molecules structural information on the divided components.[24]

Instrumentation: The issue with combining these two techniques is that the materials used for analysis by NMR are in a liquid or solid form, whereas those utilised for analysis by GC are in a gaseous condition. If the carrier gas is employed for NMR analysis, the signal obtained at atmospheric pressure will have a poor Signal-to-Noise Ratio. Microcells and computers are utilised to increase the signal-to-noise ratio in order to solve the sensitivity issue. Stronger magnets and more sophisticated microprobes are used, among other improvements. The capillary connection, or transfer capillary and probe head, experienced condensation of the analytes with boiling points more than 65℃. This coil's construction includes zero susceptibility wire and a powerful magnetic field.[27]

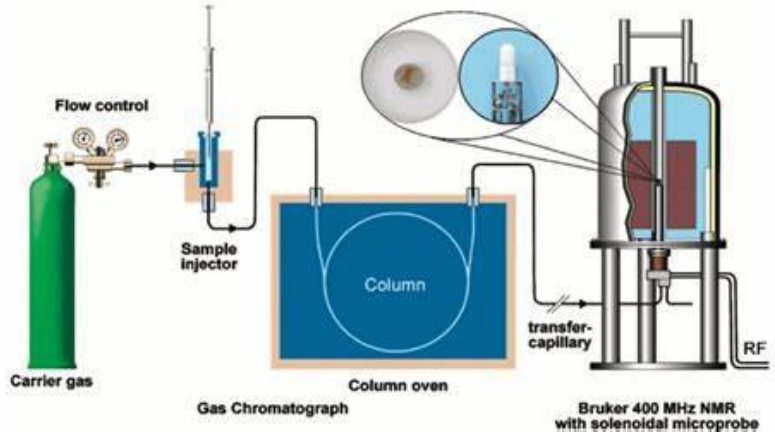

Fig. 9: GC-NMR

Applications

1. **It is Possible to Distinguish** the constitutional and configurational isomers. The same spectra are seen by enantiomers at various retention times.
2. Finding stereoisomers in a complicated combination.[29]

CONCLUSION

The aim of this review article is to supply information taken from sources believed to be valid and reliable. This is not an attempt to render any type of professional advice, or analysis, nor is it to be treated as such. While much care has been taken to ensure veracity and currency of the information presented within, neither the publisher, nor its authors bear any responsibility for any damage arising from in advertent omissions, negligence, or inaccuracies (typographical or factual) that may have found their way into this review article. A hyphenated technique is combination or coupling of two different analytical techniques with the help of proper interface. The term hyphenated techniques ranges from the combination of separation, separation-identification and identification-identification techniques. The hyphenation of these techniques leads to better analysis of the components. With the help of this technique, we can analyse the drug in short period of time.

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