**ISSN:** 2321-4902
Volume 1 Issue 3

Online Available at www.chemijournal.com

International Journal of Chemical Studies

# Review on Hyphenated Techniques

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A Hyphenated technique is combination or coupling of two different analytical techniques with the help of proper interface. Mainly chromatographic techniques are combined with spectroscopic techniques. The term hyphenated techniques ranges from the combination of separation-separation, separation-identification & identification-identification techniques. The hyphenation of these techniques leads to better analysis of the components. Hyphenated techniques show specificity and sensitivity.

*Keyword:* Chromatographic Techniques, Spectroscopic Techniques, Gas chromatography, Mass spectrometry, Liquid chromatography.

## 1. Introduction

Chromatography - Produces pure or nearly pure fractions of chemical components in a Mixture and Spectroscopy – Produces selective information for identification using standards or library spectra. "The coupling of a separation technique and an on-line spectroscopic detection technology will lead to hyphenated technique."[1] A Hyphenated technique is combination or coupling of two different analytical techniques with the help of proper interface [2]. The term hyphenated techniques ranges from the combination of separation-separation, separation-identification & identification- identification techniques [3]. The term "hyphenation" was first adapted by Hirschfeld in 1980 to describe a possible combination of two or more instrumental analytical methods in a single run (Hirschfeld, 1980). The aim of this coupling is obviously to obtain an information-rich detection for both identification and quantification compared to that with a single analytical technique [1].

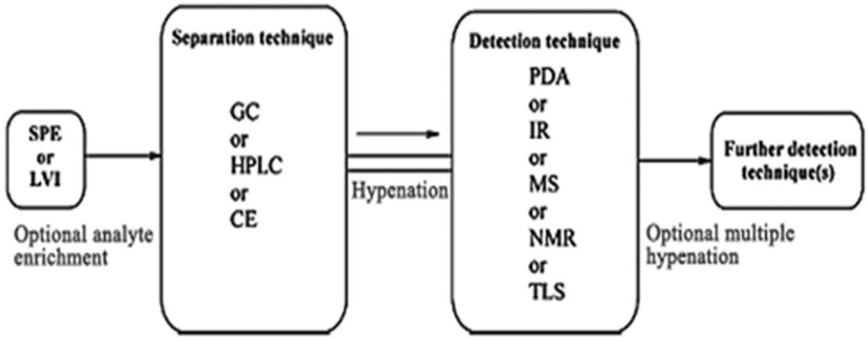


**Fig 1:** Schematic presentation of Hyphenation of chromatographic and spectrometric techniques.

### 1.1. Advantages of hyphenated techniques [4]

1. Fast and accurate analysis
2. Higher degree of automation
3. Higher sample throughput
4. Better reproducibility
5. Reduction of contamination due to its closed system
6. Separation and quantification achieved at same time.

### 1.2 List of Hyphenated Techniques

1. GC-MS
2. LC-MS
3. LC-NMR
4. EC-MS
5. CE-MS
6. GC-IR
7. LC-MS-MS
8. GC-MS-MS
9. GC-GC-MS
10. GC-NMR
11. GC-AES

## 2. GC-MS

GC is able to separate the volatile and semi volatile compounds but it unable to identify them whereas MS can identify the compound by giving its structural information at molecular level but it unable to separate them. Therefor the combination of these two techniques is took place shortly after the development of GC [5].

GC-MS was the first technique to be hyphenated and this technique can confirm the organic volatile semi volatile compounds and residual solvents with great resolution. For the analysis of the compound by GC-MS the compound should possess the property such as volatility and thermal stability [6]. These two techniques highly compatible with each other, the sample is in the vapour phase in both the techniques. But there is incompatibility between two techniques is GC is operate at high pressure (760 torr) and in this the carrier gas is present whereas in case of mass spectroscopy it operates at a vaccum10–6 to l0–5 torr [7].

### 2.1 Instrumentation and Working

Vaporized analyte when carried through the GC column with the help of heated carrier gas the separation occurs in column only. Carrier can also be called as the mobile phase e.g. helium. Distinguishable interactions of analyte between mobile phase and stationary phase lead to separation of the compounds. The separation of the analyte is also depend on the column's dimensions (length, diameter, film thickness), type of carrier gas, column temperature (gradient) and the properties of the stationary phase. The sample travel through the length of column the difference in the boiling point and other chemical properties lead to separation of the components of the mixture. The components will be having differences in elution time and retention time due to their different adsorption or difference in the partition between mobile phase and the stationary phase resp. Then the separated components of the mixture will enter into the MS through an interphase. This is followed by ionization, mass-analysis and detection of mass-to charge ratios of ions generated from each analyte by the mass spectrometer. An interface like effusion separator, jet/orifice separator & membrane separator can be used to connect GC with MS. The process of ionisation not only ionise the molecule but also break the molecule into the fragments and detect these fragments with the help of electron impact ionisation and chemical ionisation The molecular ion of analyte form a finger print spectrum which is different from other analytes. GC-MS is important tool in analytical chemistry because these techniques accurately separate, identify and provide information about structure and composition from very less sample. The advantage of this technique is sometimes two different analyte will have same mass spectrum but the retention time of both the analytes is different so such type of analytes can be separated or analyses with the help of GC-MS. Two widely used Ionization techniques in GC-MS are the electron impact ionization (EI) and the alternative chemical ionization (CI) in either positive or negative modes [1].

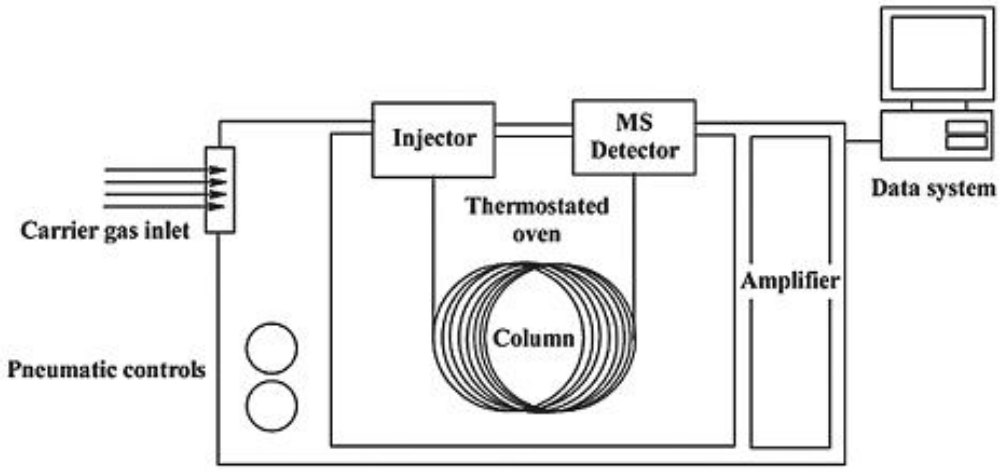


**Fig 2:** Schematic presentation of GC-MS

## 2.2 Application [5]

1. Quantitation of pollutants in drinking and waste water using official U.S. Environmental Protection Agency (EPA) methods.
2. Quantitation of drug in metabolites and urine is done for the pharmacological and forensic use.
3. Identification of unknown organic compounds in hazardous waste dumps and reaction products by synthetic organic chemistry.
4. Used for drug analysis, pesticide and herbicide detection.

## 3. LC-MS

Liquid chromatography-mass spectrometry is the technique which performs separation by liquid chromatography and mass analysis with the help of the mass spectrometry. With the help of HPLC impurities and degradation products can be separated and Mass Spectrometry allows us to obtain the molecular weight and identification of the same. LC-MS is highly selective and sensitive technique. LC-MS leads to detection and identification of chemicals in presence of other chemicals therefor it is called as specific. The flow rate of HPLC is around 1ml/min which is difficult to accommodate in mass spectrometry vacuum system also the diluent which is used has to be vaporized which leads to damage of the thermally labile compounds by excessive heating [8]. By hyphenation of these two techniques capabilities of both the techniques were improved.

## 3.1 Instrumentation and working

The LC-MS instrument can be interfaced by electrospray, particle beam, Thermospray. Electrospray is most widely used interface. The spray needle is used as bridge to connect the liquid chromatography with that of the mass. But the separate emitter is flexible as well as convenient [9-12].

LC-MS is mainly separated into the three parts chromatography, interface and spectrometry.in liquid chromatography separation is performed which is detected with the help of Photo diode array, Ultraviolet, fluorescent etc detectors. These separated components then transferred to the interface. In interface the liquid is volatilized and transferred to the MS. With the help of various ionization techniques the compound is ionized and then it is analyzed by mass analyzer. various mass analyzers are used *viz.* Quadrupoles, quadrupole ion traps, time-to-flight (TOF), time-to-flight reflection (TOFR), and ion cyclotron resonance (ICR) mass analyzers.in this technique LC separate due to which clear

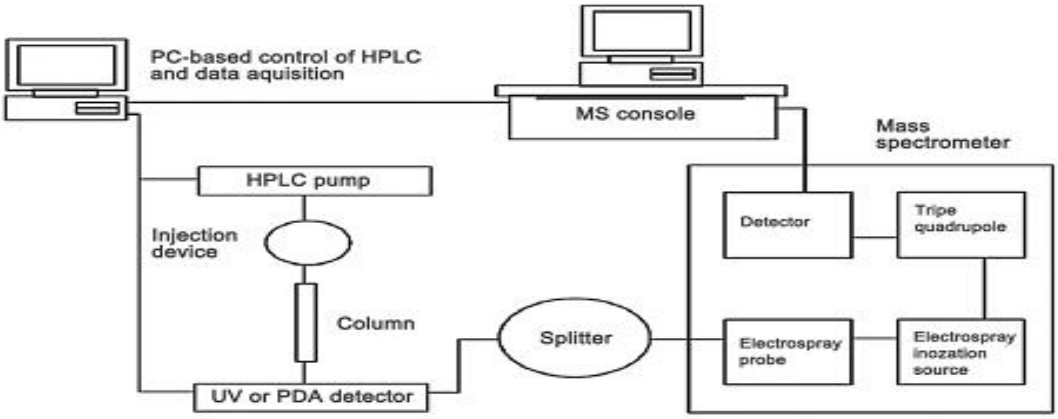


**Fig 3:** Schematic presentation of LC-MS

mass spectra is obtain by suppression of the mutual signal.

### 3.2 Applications

1. LC-MS used to detect compounds from poly-aromatic (non-polar) to peptide and proteins.
2. LC-MS used for compounds identification and purity.
3. Used for determination of pesticides, herbicides & organic pollutant for environmental monitoring.
4. proteome analysis is done by this technique [13].

## 4. LC- NMR

LC-NMR is the hyphenated technique in which HPLC is combined with the NMR. This technique is widely used for the analysis of complex mixtures which contain unknown impurities, natural products and synthetic polymers [14-16]. In LC-NMR LC does the separation and NMR does the identification of the separated components.

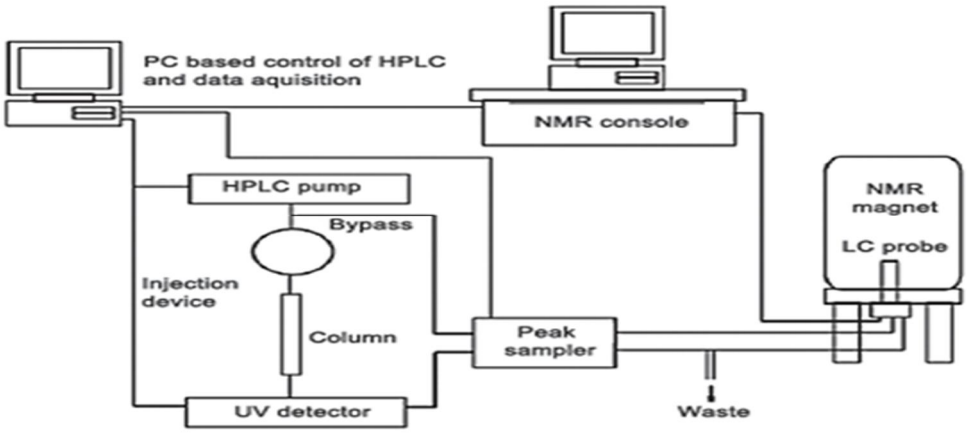


**Fig:** 4 Schematic presentation of LC-NMR

### 4.1 Instrumentation and working

LC-NMR is the sensitive method because the sensitivity of the NMR can be increased by use of highly magnetic field magnets and highly sensitive probes, and maturation of peripheral technologies, such as solvent elimination technology and automatic measurement software suitable for multicomponent analysis. Stronger the external magnetic field is, the higher the sensitivity is [17]. The improvement in sensitivity has made for a large reduction in measurement time. The compounds showing complex spectra can also be easily analyzed with the help of increase in magnetic field which will improve the signal resolution [18].

### 4.2 Application

1. Identification of drug degradation products.
2. Low level impurities can be isolated and identified.
3. This technique is used for tracking pesticides, herbicides & organic pollutant for environmental monitoring.

## 5. EC-MS

EC-MS is the combination of electrochemistry (EC) mass spectrometry (MS). In this technique by EC one can do the electrochemical oxidation which will directly be introduced into the ESI interface for monitoring short lived intermediated with the help of MS.

### 5.1 Application[19]

1. EC/MS provides an easy and rapid means to predict possible oxidation products of xenobiotics in the liver.
2. More recent applications include the bio affinity screening of electrogenerated oxidation products, and attempts to predict the allergenic potential of xenobiotics by investigating the peptide and protein adduct formation of their electro-generated products.

## 6. CE-MS [20, 21]

CE-MS is online separation technique in which molecules are distinguished according to their differences in electrophoretic mobilities and structural information [22]. Capillary electrophoresis (CE) is used for the separation of the components and mass will identify the components separated by CE. This technique is speedy efficient and low solvent and sample is required for the analysis. The CE is connected to the MS with the help of the long capillaries which will increase the analysis time also there is lack of suitable volatile buffer which has to be compatible with MS.

### 6.1 Instrumentation & working

The detectors used for the CE analysis are UV and DAD end the electrolytes used in this technique are inorganic and non-volatile. This technique has some drawbacks which include lack of reproducibility, repeatability, selectivity. These problems can be counteract by applying the buffer system which will coat the inner wall of the fused silica capillaries with double layer. Very small volumetric flow is passed through the interfaces into the MS. Ionisation interfaces used are electrospray; fast atom bombardment interface and ion spray ioniser. Detectors used in mass spectroscopy are TOF, ion trap and Quadrupole. Main advantage of quadrupole detector is sensitivity [23].

### 6.2 Application

1. Using non-aqueous CE and non-aqueous CE-MS basic and acidic compounds can be identified.
2. Complex arabino oligosaccharides are analyzed.
3. CE-MS is a tool for drug bioanalysis & biomarker discovery.

## 7. GC-IR [24]

GC-IR technique is hyphenation of gas chromatography and Infrared spectroscopy. This technique is very sensitive, very expensive, sample recovery is also possible because IR is non-destructive technique in this technique the GC does the separation part where as IR perform the function of identification.

Gas chromatography separate's components of the analyte. These components will travel through the column. These two techniques are linked through glass column or vacuum tubes.

Interface used in this technique is internally gold coated small glass pipe connected to column by narrow tubing [25]. Light pipe is heated in order to rid condensation and maximize path length for enhanced sensitivity.

### 7.1 Instrumentation & working

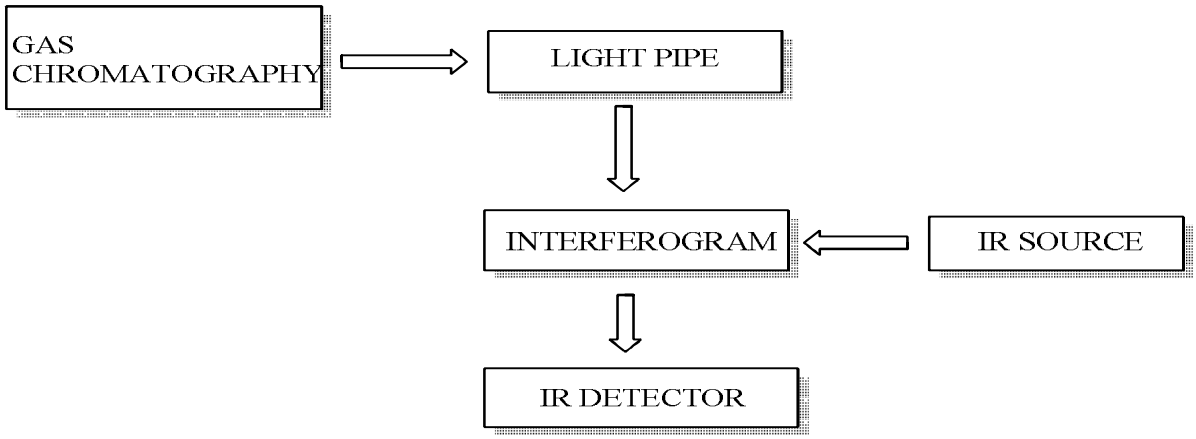


**Fig:** 5 Schematic presentation of GC-IR

Effluent from GC is directly forwarded into the heated pipe of IR at atmostpheric pressure. Infrared red spectroscopy identifies the compound by identifying the functional groups.

### 7.1 Application

1. Pharmaceutical, Industrial
2. DNA Analysis of blood samples, other fluids.

### 8. LC-MS/ (MS)

LC-MS/ (MS) can detect over 300 compounds of different class by low injection volume. Minimal sample pre-treatment is required and it also reduce the time of analysis.LC-MS is the first step of LC-MS/MS. This technique is more sensitive and specific than that of the LC-MS.it around 20-100 times sensitive than LC-MS. More specific because in this technique second filtering process is also involved.

### 9. GC-MS/MS

In this technique the gas chromatography is coupled with tandem mass spectrometry. This technique is sensitive as well as specific and can be used for ultra-trace analysis. For qualitative identification with MS/MS, product ion scan, precursor ion scan and neutral loss with a triple quadrupole or product scan with an ion trap can be used. In recent years the sensitivity of the quadruplole is increased instead of loss also the scanning speed is also high [1].

### 9.1 Application

1. Identification of trace unknown impurities.
2. Used for determination of contaminations in environment and foods such pesticides and PCBs in foods and biological samples [26]

### 10. GC×GC-MS

In this technique two dimensional GC is coupled with mass spectroscopy. This coupling will lead to the better resolution of the peaks in GC. Sometimes in one dimensional GC the analyte is unable distributed along the whole retention time. The two dimensional GC lead to the better resolution. with the help of two dimensional GC components of the analytes are properly separated due to which in the MS even trace amount components will be identified [27].

### 10.1 Application

1. Large number of samples can be analyzed at the same time.
2. Analysis of petroleum, PCBs, complex extracts and food samples is performed by this technique [28].

### 11. GC-NMR

In this technique the GC is combined with NMR.NMR perform the identification of the components and GC is used for the separation of the components. The hyphenation of this

technique provides the molecules structural information of the separated components [29,30].

### 11.1 Instrumentation

The problem involved in combination of this two technique is for NMR the samples used for analysis are liquid or in solid state whereas in GC it is in the gaseous state. If the carrier gas is used for analysis in NMR it will show the low Signal-to-noise ratio of the signal obtained at atmospheric pressure. To overcome this sensitivity problem microcells are used and computers are used to improve the signal to noise ratio [31]. some other modifications are also performed which include use of stronger magnets and advanced microprobes [32]. The analytes having boiling point above 65 °C condensed in the capillary connection i.e transfer capillary and probe head. This problem can be solved by use of transfer capillary which will be heated by bifilar coil. This coil is constructed from zero susceptibility wire which is combined with strong magnetic field [33].

### 11.2 Application

1. Constitutional and Configurational isomers can be separated. Enantiomers shows the same spectra at different retention time.
2. an identification of stereoisomers in a complex mixture

## 12. GC-AES

This technique is combination of gas chromatography with atomic emission spectroscopy. Atomic emission spectroscopy is one of the elemental analysis techniques. GC performs the separation of the components and with the help of AES the elemental identification of the components is performed. Elemental composition of every peak separated by GC is determined.

### 12.1 Instrumentation

In this process, analytes are first atomized using either ICP or microwave irradiation (high temperatures), where the atoms are transferred to electronically exited state. Then these electrons are return to the lower energy levels at that time photons are emitted at certain wavelengths that are characteristic of the particular element. In both the techniques sample is in gas phase so the techniques are complementary to each other. The GC effluent is directly introduced into the Quartz atomization furnance via heated nickel transfer line [34]. The interface is simple but in practice the conditions has to be optimised eg. Quartz furnace, heating with flame or a thermostat, or using the graphite furnace as the atomization device for obtaining good sensitivity and selectivity [34].

### 12.2 Application

1. Identification and Quantification of the compounds.

## 13. Conclusion

The review Hyphenated technique include various hyphenated techniques which are used nowadays for analysis. Chromatographic techniques GC,LC etc are used for separation and spectroscopic techniques such as NMR,MS,IR used for identification purpose. Combination of these techniques gives better analysis of the components. In this review introduction, instrumentation, working and applications are explained for every technique.

## 14. Acknowledgment

The authors gratefully acknowledge the contributions of Prof. Navale, M.N. President and Dr. (Mrs.) Navale, S. M. Secretary, Sinhgad Technical Education Society, Pune, for constant motivation and encouragement.

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