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HPLC-NMR HYPHENATED TECHNIQUE-REVIEW

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ABSTRACT

The hyphenated technique is developed from the coupling of a separation technique and an on-line spectroscopic detection technology. The remarkable improvements in hyphenated analytical methods over the last two decades have significantly broadened their applications in the analysis of biomaterials, especially natural products. In this reviewed article, recent advances in the applications of various hyphenated techniques, e.g., HPLC-NMR, GCMS, LC-MS, GC-IR etc. also including the different instrumentation required for this technique. In the context of pre-isolation analyses of crude extracts or fraction from various natural sources, isolation and on-line detection of natural products,. HPLC-NMR hyphenation offers numerous advantages compared to direct HPLC-NMR methods. Multiple trapping leads to a dramatic increase of analyte amounts available for NMR, enabling acquisition of high-quality 2D NMR data within a short time. Other new developments, including combination of solenoidal coil capillary flow-probes with microflow HPLC, are also discussed.

1] DEFINATION –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.

1.2] Chromatography - Produces pure or nearly pure fractions of chemical components in a Mixture

1.3] 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."A couple of decades ago, Hirschfield introduced the term "hyphenation" to refer to the on-line combination of a separation technique and one or more spectroscopic detection techniques.This technique, developed from a marriage of a separation technique and a spectroscopic detection technique, is nowadays known as hyphenated technique.

In recent years, hyphenated techniques have received ever-increasing attention as the principal means to solve complex analytical problems. The power of combining separation technologies with spectroscopic techniques has been demonstrated over the years for both quantitative and qualitative analysis of unknown compounds in complex natural product extracts or fractions. To obtain structural information leading to the identification of the compounds present in a crude sample, liquid chromatography (LC), usually a high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) is linked to spectroscopic detection techniques, e.g., Fourier-transform infrared (FTIR), photodiode array (PDA) UV–vis absorbance or fluorescence emission, mass spectroscopy (MS), and nuclear magnetic resonance spectroscopy (NMR), resulting in the introduction of various modern hyphenated techniques, e.g., CE-MS, GC-MS, LC-MS, and LC-NMR. HPLC is the most widely used analytical separation technique for the qualitative and quantitative determination of compounds in natural product extracts. The physical connection of HPLC and MS or NMR has increased the capability of solving structural problems of complex natural products. Because of the greater sensitivity, LC-MS has been more extensively used than LC-NMR. The hyphenation does not always have to be between two techniques; the coupling of separation and detection techniques can involve more than one separation or detection techniques, e.g., LC-PDA-MS, LC-MS-MS, LC-NMR-MS,

LCPDA-NMR-MS, and the like. Where trace analysis is vital, and the analytic enrichment is essential, on-line coupling with solid-phase extraction (SPE), solid-phase micro extraction or large volume injection (LVI) can be incorporated to build in a more powerful integrated system, e.g., SPE-LC-MS or LVI-GC-MS.

The two key elements in natural product research are the isolation and purification of compounds present in crude extracts or fractions obtained from various natural sources, and the unambiguous identification of the isolated compounds. Thus, the on-line characterization of secondary metabolites in crude natural product extracts or fractions demands high degree of sophistication, and richness of structural information, sensitivity, and selectivity. The development of various hyphenated techniques has provided the natural product researchers with extremely powerful new tools that can provide excellent separation efficiency as well as acquisition of on-line complementary spectroscopic data on an LC or GC peak of interest within a complex mixture. The main focus of this chapter is to provide an overview of basic operational principles of various modern hyphenated techniques and to present several literature examples of applications of these techniques. Detailed information on the principle, history, instrumentation, and methodology is available in li

2]WHY IT IS USED?

- Highly selective
- Highly sensitive
- Use in many Applications
- purifications
- Polymerization studies
- Natural products
- Pharmaceuticals

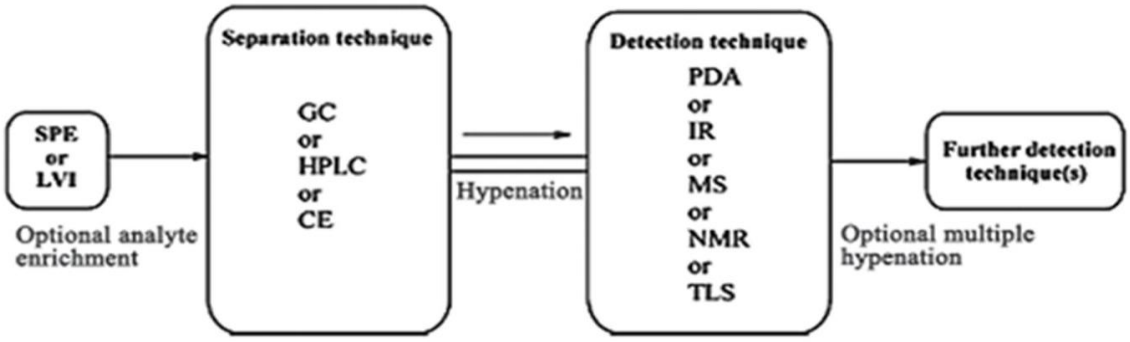

FIG:-1 Schematic presentation of Hyphenation of chromatographic and spectrometric techniques.

2.1] Advantages of hyphenated techniques

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.

2.2] 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

3] 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. 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 .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 l0–5torr **.**

3.1] 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 GCMS are the electron impact ionization (EI) and the alternative chemical ionization (CI) in either positive or negative modes.

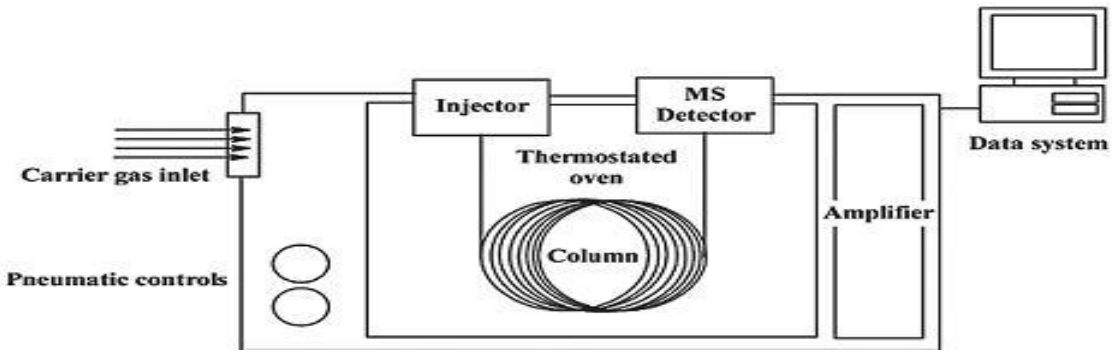

Fig 2: Schematic presentation of GC-MS

3.2] Application GC-MS

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 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.

4] HPLC- NMR

HPLC-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. In LC-NMR LC does the separation and NMR does the identification of the separated components.

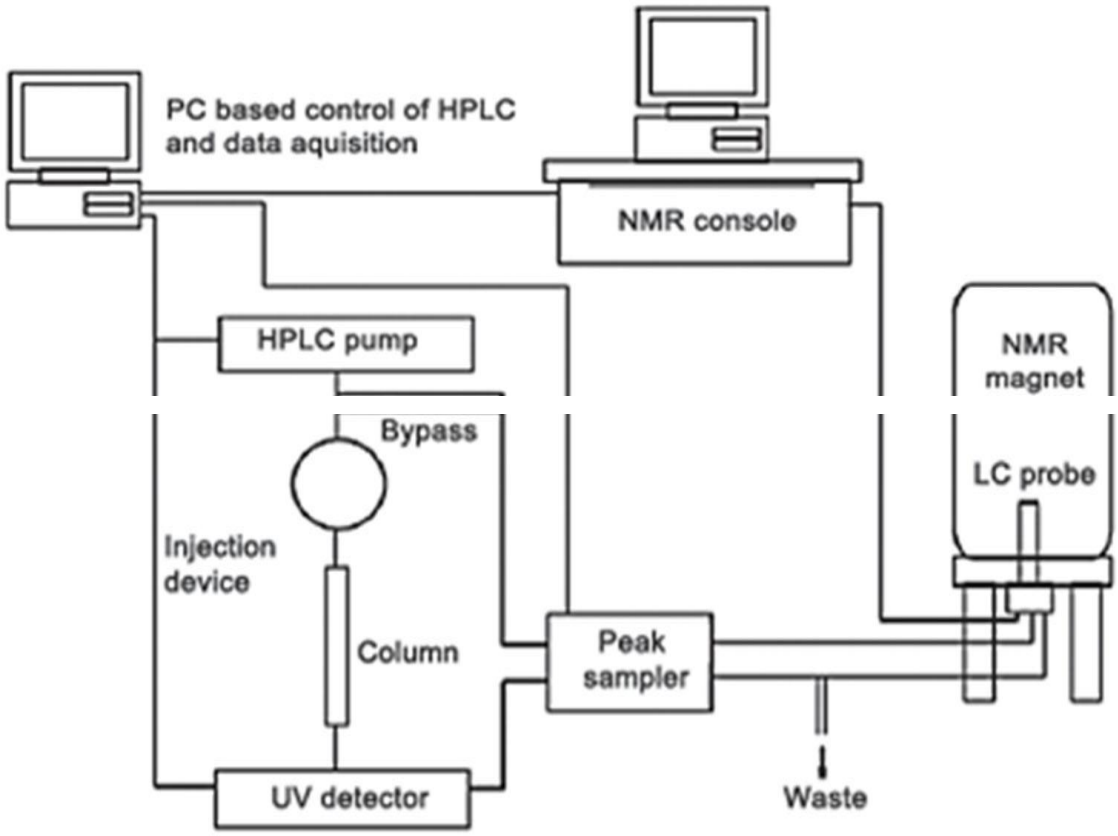

Fig:3 Schematic presentation of HPLC-NMR

4.1] Instrumentation and working

HPLC-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. The improvement in sensitivity has made for a large red uction 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.

4.2] Application OF HPLC-NMR

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] GC-IR

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 **.**Light pipe is heated in order to rid condensation and maximize path length for enhanced sensitivity.

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

5.1] Application GC-IR

1. Pharmaceutical, Industrial

2. DNA Analysis of blood samples, other fluid.

6] APPLICATION OF HYPHENATED TECHNIQUES IN NATURAL PRODUCT ANALYSIS

6.1] Isolation and analysis of natural products

Crude natural product extracts, which represent extremely complex mixtures of numerous compounds, can be analyzed successfully by using appropriate hyphenated techniques. Among the various hyphenated techniques, LC-PDA and LC-MS are the two most extensively used for natural product analysis. LC-NMR, as well as different multiple hyphenated techniques like LC-PDA-NMR-MS have also become popular most recently. LC-MS, if the ionization technique is chosen appropriately, can be an extremely powerful and informative tool for screening crude plant extracts. The currently available various types of

LC-MS systems allow the analysis of small nonpolar compounds to large polar constituents like oligosaccharides, proteins, and tannins present in natural product extracts.

6.2] Alkaloids

Alkaloids are a large group of nitrogen-containing secondary metabolites of plant, microbial, or animal origin. Various hyphenated techniques have been used in the analysis of several types of alkaloids to date. With the development and wider availability of bench-top systems, GC-MS has become the method of choice for the analysis of various pyrolizidine and quinolizidine types of alkaloids. Quinolizidine alkaloids, the main class of alkaloids found in the family Leguminosae, have been analyzed by GC-MS recently.Most of these alkaloids are sufficiently volatile and thermostable under GC conditions to permit analysis without chemical modification. However, some hydroxylatedpyrolizidine alkaloids need to be analyzed as their trimethylsilyl derivatives. Ephedrine-type alkaloids, in dietary supplements containing the Chinese herb ma huang, were analyzed by GC-MS and GC-FTIR.

6.3] Coumarins

The coumarins are the largest class of 1-benzopyran derivatives that are found mainly in higher plants. HPLC-PDA can be used successfully in the analysis of various phenolic compounds, including coumarins, because of the presence of significant amounts of chromophores in these molecules. The HPLC-PDA determination of coumarins, where absorption spectra are registered with a PDA detector, provides useful information about the identity of the molecule including oxidation pattern. The retention time together with the UV spectrum of individual peaks can be considered characteristic, and can easily be used to detect known coumarins in a crude extract. The coupling of MS to LC-PDA provides further structural information that is helpful for on-line identification of individual coumarins in any crude extract. Various coumarins together with other oxygen heterocyclic compounds, e.g., psoralens and polymethoxylated flavones, present in the nonvolatile residue of the citrus essential oils of mandarin, sweet orange, bitter orange, bergamot, and grapefruit, were analyzed by atmospheric pressure ionization (API) LC-MS system equipped with an APCI probe in positive ion mode.[27] Recording MS spectra at different voltages provided information on molecular weight as well as fragment ions, and this allowed the identification of the main components in the extracts. In this study, cold-pressed citrus oils were analyzed by a Shimadzu LC system coupled with UV and MS detector with an APCI interface. The LC separation was carried out on a C18 Pinnacle column (250 × 4.6 mm, 5 mm), eluted isocratically or using a gradient at a flow rate of 1 mL/min with the solvent mixture: solvent

A (THF:ACN:MeOH: water¼15:5:22:58) and solvent B (100% ACN). As coumarins are UV-absorbing compounds, they could be detected at 315 nm. The MS acquisition conditions were as follows: probe high voltage, 4 kV; APCI temperature, 400°C; nebulizing gas (N2) flow rate, 2.5 L/min; curved desolvation line (CDL) voltage, 25.5 V; CDL temperature, 230°C; deflector voltage, 25 and 60 V; and acquisition mode SCAN, 50–500 m/z.

6.4] Carotenoids

This group of natural products includes the hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). LC-TLS has been applied successfully for the determination of carotenoids in four marine phytoplankton species, and a good degree of separation of diadinoxanthin, diatoxanthin, and other carotenoids has been achieved by isocratic HPLC elution with a greater sensitivity and selectivity than UV detection. This technique has allowed the monitoring of the interconversion of diadinoxanthin to diatoxanthin, and changes of other carotenoids under different light conditions LC-TLS has also been found to be an ultrasensitive method for determination of b-carotene in fish oil-based supplementary drugs.

7] CONCLUSION

The technique developed from the coupling of a separation technique and an on-line spectroscopic detection technology is known as hyphenated technique. The remarkable improvements in hyphenated analytical methods over the last two decades have significantly broadened their applications in the analysis of biomaterials, especially natural products. In this article, recent advances in the applications of various hyphenated techniques, e.g., GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS, etc. in the context of preisolation analyses of crude extracts or fraction from various natural sources, isolation and on-line detection of natural products, chemotaxonomic studies, chemical fingerprinting, quality control of herbal products, dereplication of natural products, and metabolomic studies are discussed with appropriate examples. Particular emphasis is given on the hyphenated techniques that involve LC as the separation tool.

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