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REVIEW ON HYPHENATED TECHNIQUE

Pallavi S. Thombare*, Ramanlal N. Kachave[2] and M. J. Chavan[3]

[1]Amrutvahini College of Pharmacy, Sangamner, Department of Quality Assurance, Savitribai Phule Pune University, Maharashtra, India.

[2]HOD of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Savitribai Phule Pune University, Maharashtra, India.

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***Corresponding Author**
Pallavi Thombare
Amrutvahini College of Pharmacy, Sangamner, Department of Quality Assurance, Savitribai Phule Pune University, Maharashtra, India.

Hyphenated separation techniques refer to a mixture of two or more methods for separating and detecting chemicals from solution. And biochemistry hyphenated methods are commonly implemented. In 1980, Hirsh Feld first adopted the term "hyphenation" to describe a probable combination of two or more instrumental research methodologies in a single run (Hirshfeld, 1980). The purpose of the pairing is to gain an information rich detection with a single analytical technique for both identification and quantification. To obtain strength of both, hyphenated technique combines chromatographic and spectral processes. In a mixture, chromatography produces pure or almost pure fractions of chemical components. Spectroscopy uses standards or library spectrums to create selective information for analysis.

The combination of hyphenated strategies varies from

1. **Separation -** separation.
2. **Separation -** Identification &
3. **Identification -** Identification techniques.

Advantages

1. For rapid and accurate analysis of the samples.
2. A greater level of automation.
3. Higher throughput of the sample.
4. Increased reproducibility.
5. Contamination avoidance due to its closed framework.
6. Separation of simultaneous quantification.

List of hyphenated techniques

- GC-MS
- LC-MS
- LC-NMR
- EC-MS
- CE-MS
- GC-IR
- LC-ESI MALDI-TOF
- GC-MS-MS
- GC-NMR
- GC-AES
- ICP-AAS
- ICP-OES

1) GC-MS

GC can distinguish the volatile and semi volatile compounds, but cannot recognise them, while MS can classify the compound by providing molecular level structural details, but cannot differentiate them. Therefore, shortly after the growth of GC, the synthesis of these two techniques took place.

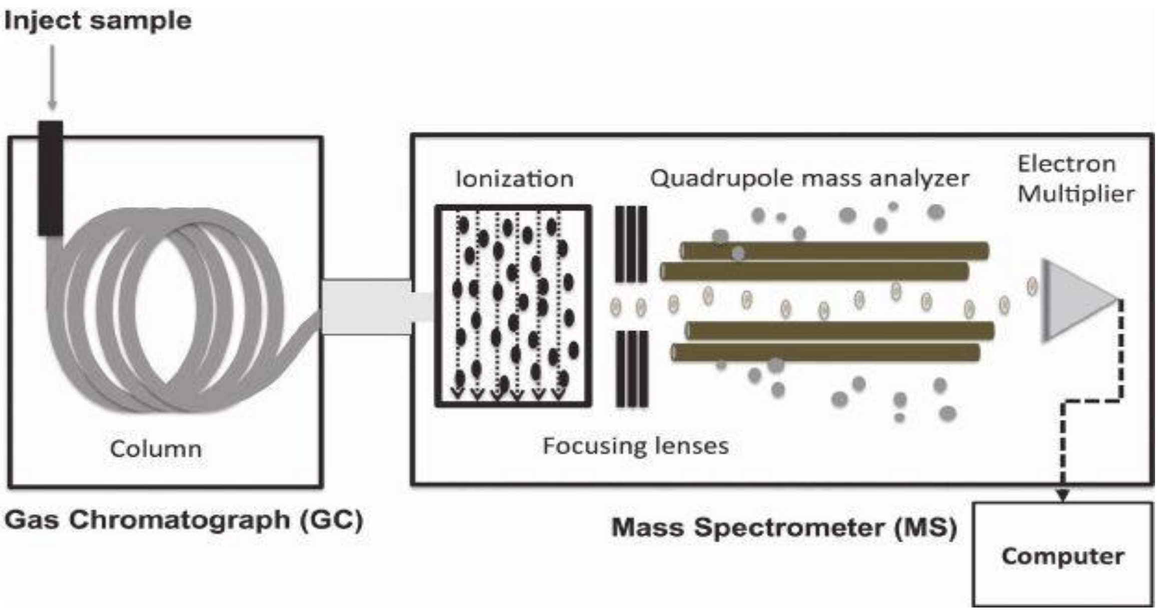
These two techniques are highly compatible with each other, the sample is in the vapour phase in both the techniques. But there is incompatibility between two techniques as GC operates at high pressure (760 torr) and in this the carrier gas is present whereas in case of mass spectroscopy it operates at a vaccum10–6 to l0–5 torr.

In GC-MS, a sample is injected, vaporised & separated in the GC column, into the GC system injection port. As the sample moves the length of the column, the differentiation in the chemical properties between various molecules in a mixture will distinguish the molecules.

By splitting each molecule into ionised fragments and distinguishing these fragments using their mass-to - charge ratio, the mass spectrometer does this.

In general, the devices used for GC-MS consist of an injection port at one end of a metal column (often filled with a sand-like material to facilitate optimum separation), a detector

(MS) at the other end of the column, a carrier gas (argon, helium, nitrogen, hydrogen, to name a few) that propels the sample down the column.



GC-MS Benefits

1. GC has the ability of high resolution to equate other approaches.
2. This form, when used with thermal detectors, has high sensitivity.
3. This approach also works by separating and evaluating sample samples very rapidly with less consistency.
4. This technique has very good precision and accuracy.

2) LC-IR

The hyphenated method developed from the LC coupling and the infrared spectrometry (IR) or FTIR detection concept is referred to as LC-IR or HPLC-IR. Although HPLC is one of the most effective separation techniques available today, IR or FTIR is a useful spectroscopic technique for organic compound identification, whereas organic compound structures has many structures in the mid-IR region.

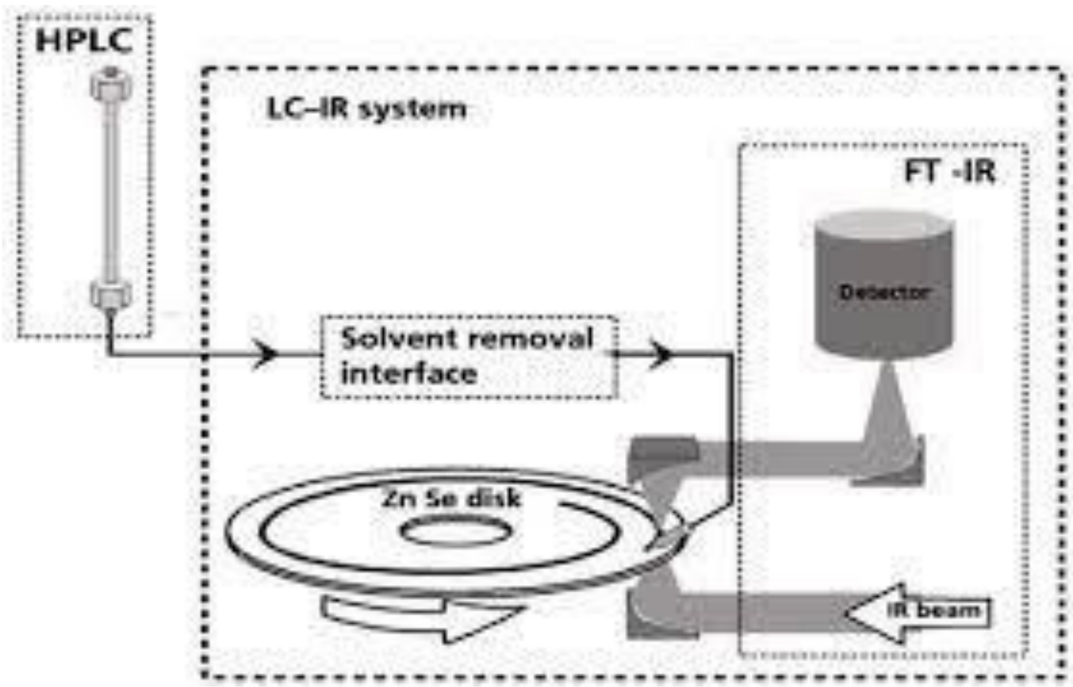
Moreover, IR is much less sensitive as a detection technique compared to various other detection techniques, e.g. UV and MS. Two simple approaches based on interfaces implemented in HPLC-IR or HPLC-FTIR have been implemented in recent developments in HPLC-IR technology.

It is difficult to combine HPLC and IR, and progress in this hyphenated technique is extremely slow because the 237 absorption bands of the mobile phase solvent of the hyphenated technique are so big in the mid-IR area that the small signal produced by the sample components is often obscured. The geometry of the sample during the measurement process matters because FT-IR is an absorbance system. Decreasing the diameter by a factor of two for a fixed mass or volume of the analyte produces a deposition of four times the thickness and four times the optical density. This deposit diameter reduction of two increases the signal-to - noise ratio by four since the IR detector is constrained by total light. Therefore, the LC-IR hyphenation process must be used to achieve a helpful instrument that generates complete mid-infrared spectra.

For the selection of sample components in the eluent, KBr or KCl salts are usually used, and heating up the medium before IR detection removes the volatile mobile phase solvents. The solvent-elimination solution has two types of interfaces: the diffuse-reflectance infrared Fourier transform (DRIFT) solution and the buffer-memory technique.

LC-IR Benefits

1. Remove the solvent without the analytes being thermally compromised or diluent gas overloading the vacuum system.
2. Have successful transmission to the spectrometer of analytes.
3. Present the FT-IR in a dense deposition of analytes.
4. Preserve the resolution of chromatography.



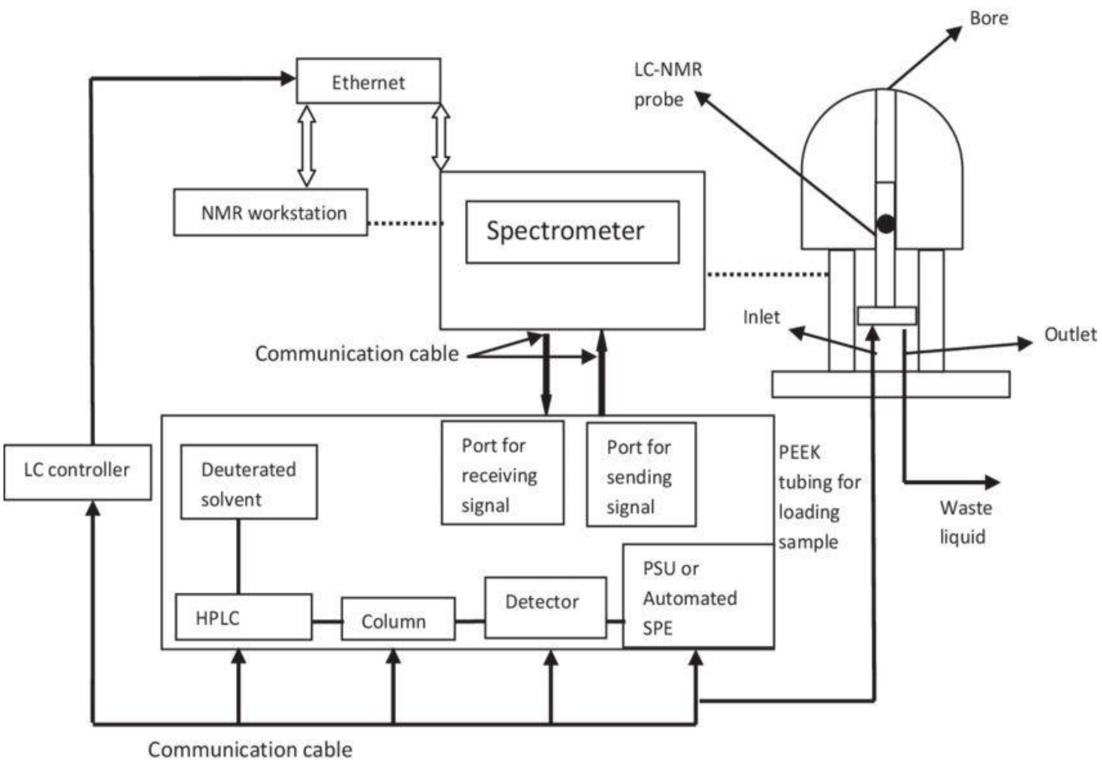
3) LC-NMR

The hyphenated technique in which HPLC and NMR are combined is LC-NMR. This approach is generally used to analyse complex mixtures containing unidentified impurities, natural products and synthetic polymers. In LC-NMR, LC does the separation and the separated components are identified by NMR.

The sensitivity of the NMR can be improved by the use of highly magnetic field magnets and highly sensitive probes and by the maturation of peripheral technologies such as solvent removal technology and automated multi-component analysis measurement software. LC-NMR is a sensitive process. The higher the sensitivity, the stronger the external magnetic field is. The increase in sensitivity has resulted in a major reduction in time for measurement. With the aid of enhanced magnetic fields, compounds displaying complex spectra can also be easily analysed to enhance signal resolution.

LC-NMR Benefits

1. Identifying products for drug degradation.
2. Impurities at low levels can be isolated and detected.
3. For environmental monitoring, this technique is used to track pesticides, herbicides and organic contaminants.



4) LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is now a standard technique providing a simple and robust interface for the design of electrospray ionisation (ESI). It can be applied to a wide range of biological molecules, and the use of tandem MS and stable internal isotope standards allows the production of highly sensitive and precise assays, although some optimization of the method is needed to reduce the effects of ion suppression.A high degree of multiplexing is enabled by quick scanning speeds, and many compounds can be measured in a single analytical run.

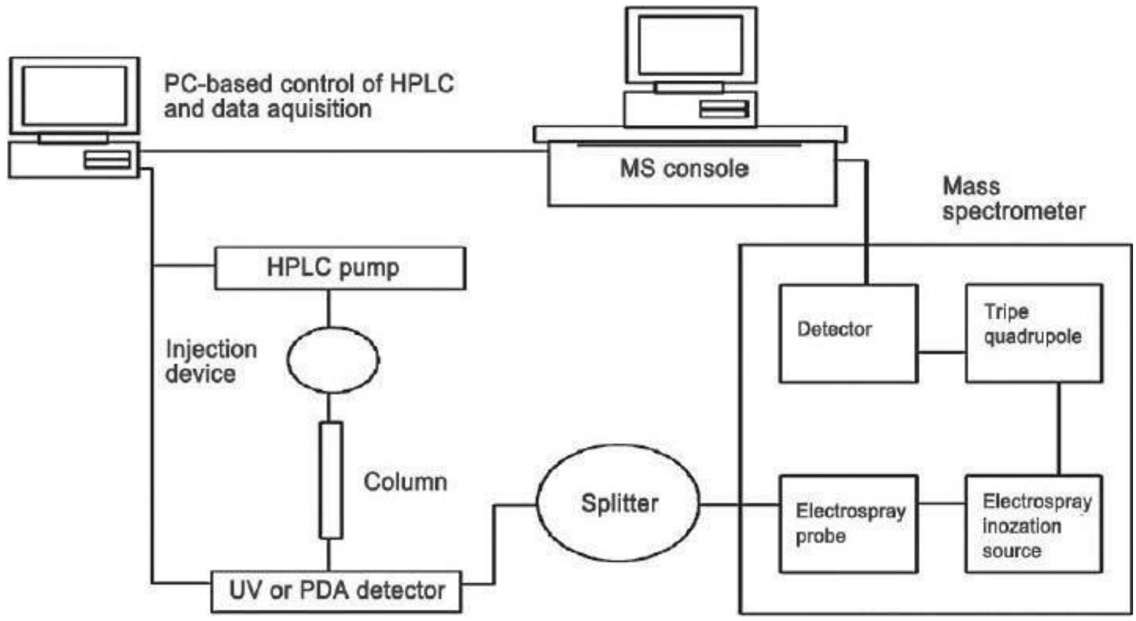
Due to the sensitive and highly precise nature of MS compared to other chromatographic detectors, coupling of MS to chromatographic techniques has always been desirable. In the 1950s, the fusion of gas chromatography with MS (GC-MS) was achieved with commercial instruments available from the 1970s onwards. Relatively inexpensive and effective GC-MS systems are now a staple of many clinical biochemistry laboratories and are invaluable in a variety of areas where complex mixtures need to be analysed and unambiguously detected, such as screening urine samples for inborn metabolic or drug errors.

Mass spectrometers work on the basis of their mass to charge ratio (m / z) by converting the analyte molecules to a charged (ionised) state, with subsequent analysis of the ions and any fragment ions formed during the ionisation process. For both ionisation and ion analysis, many different technologies are available, resulting in several different kinds of mass spectrometers with different combinations of these two processes. In operation, some configurations are much more robust than others and the following explanations concentrate on the key types of ion sources and mass analyzers that are likely to be found throughout clinical laboratories in LC-MS systems.

Lc-ms/ms benefits

1. The development of LC-MS assays as alternatives has been stimulated by frustration with the high cost of commercial immunoassays used in therapeutic drug testing and their variable cross-reactivity with metabolites.
2. Due to the variable responses of commercial immunoassays to various types of vitamin D and its metabolites, there is considerable interest in using LC-MS for vitamin D measurements.
3. For several areas of steroid biochemistry, LC-MS analysis is relevant. Difficulties with the measurement of low testosterone and dihydrotestosterone levels observed in women

and children using traditional immunoassays has prompted the creation of many highly sensitive LC-MS assays capable of providing accurate measurements in these classes.



CONCLUSIONS

It is also possible to assume that hyphenated techniques are far stronger and more useful than regular single techniques. Hyphenation requires isolation as well as labelling, which makes it easier to examine samples. The hyphenated techniques are more used nowadays than standard techniques of spectroscopy or chromatography. GC, LC, etc. chromatographic techniques are used for separation and spectroscopic techniques used for identification purposes, such as NMR, MS, IR. To solve various complex analytical issues in various fields, hyphenated techniques such as LC-MS, GC-MS, LC-NMR, CE-MS and ICP-MS have been developed.

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