

Hyphenated Techniques

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CONTENT

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INTRODUCTION

- Hyphenated technique is a combination or coupling of two analytical techniques with the help of proper interface.
- The term 'HYPHENATION' was first adapted by Hirschfeld in 1980 to refer combination of separation techniques and one or more spectroscopic detection techniques.

List of Hyphenated Techniques.

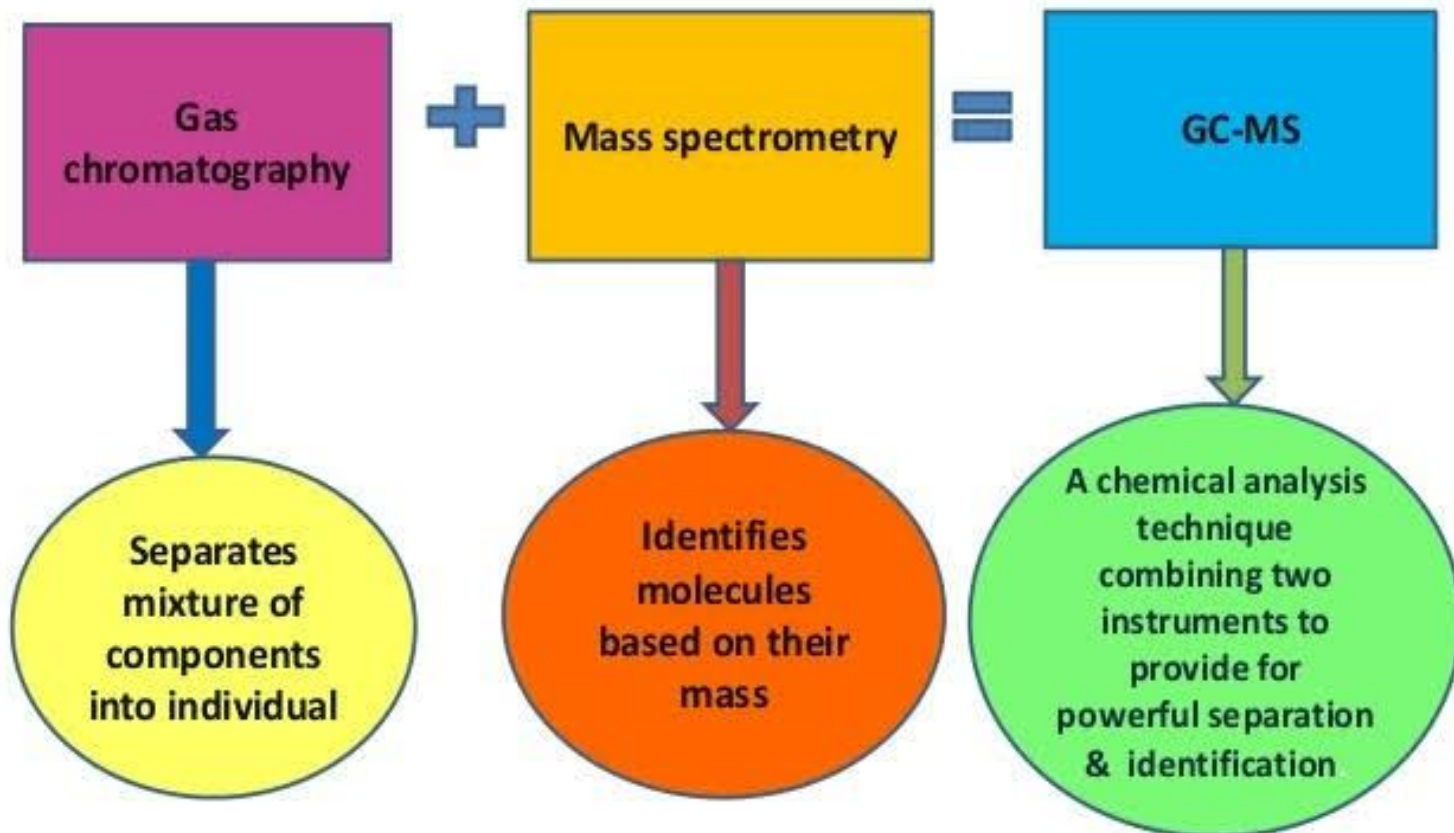
1. GC-MS
2. LC-MS
3. LC-NMR
4. GC-IR

GC-MS

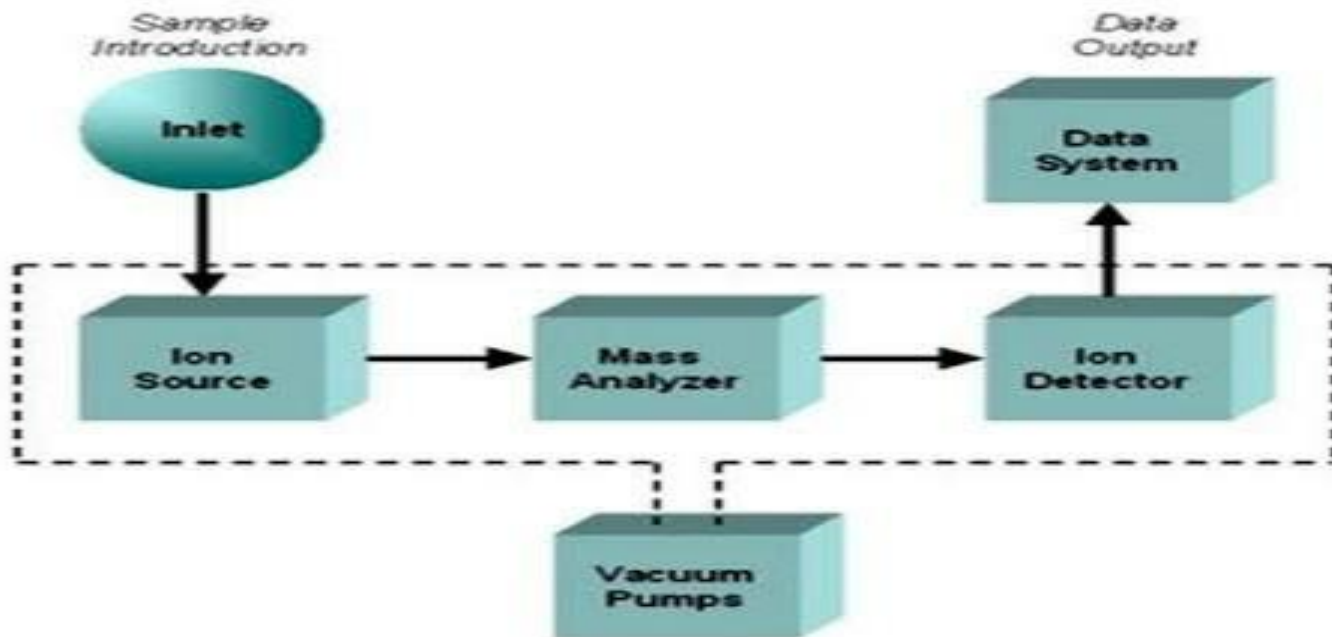


GC-MS – A BRIEF

- It's a Hyphenated Technique
- Invented By James & Martin in 1952



Principle of GC-MS



Block diagram of mass spectrometry

COUPLING OF GC TO MS

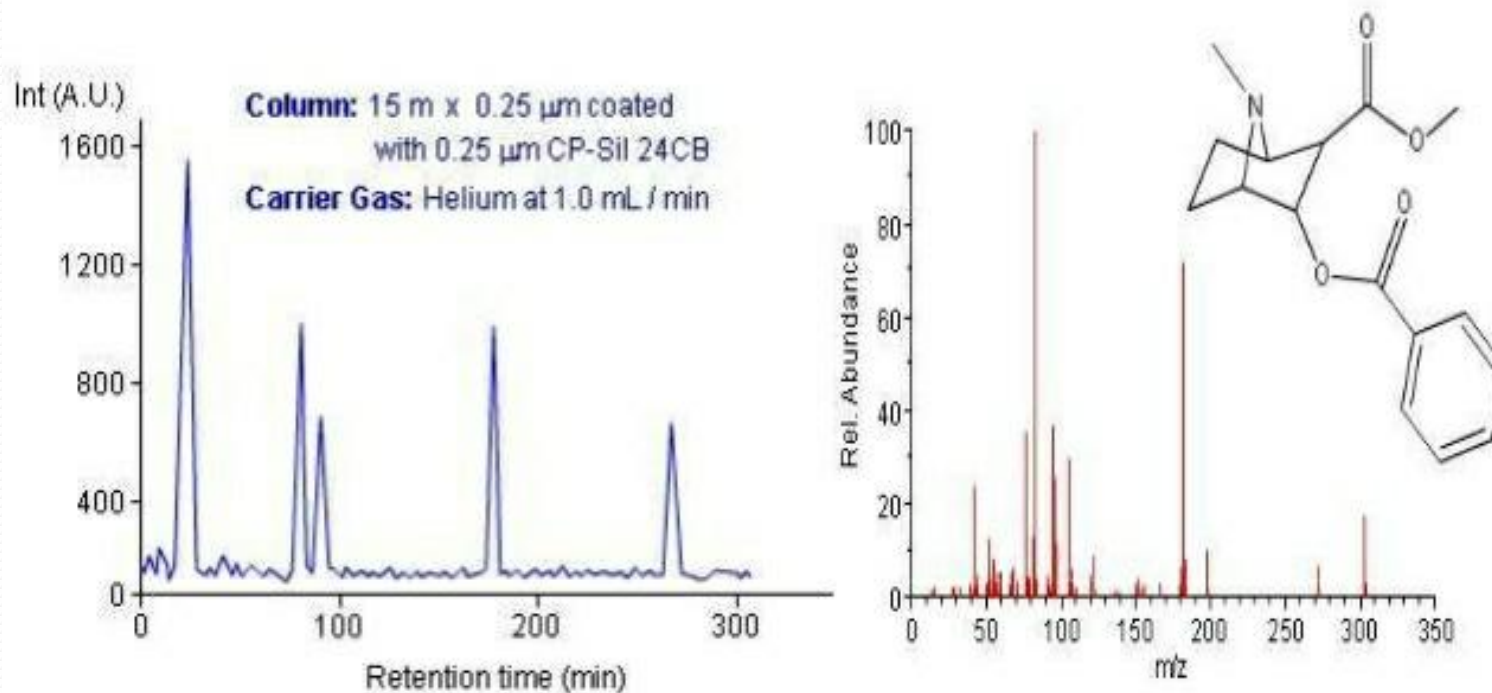


- output of the GC must be reduced to vacuum of 10^{-5} to 10^{-6} atm The interface b/w GC-MS play an important role in the overall efficiency of instrument
- Both system are heated at 200-300°C, both deal with compound in vapor state.
- Only one problem is that the atmospheric pressure

Pharmaceutical & other applications of GC-MS

1. Pharmaceutical applications

GC-MS analysis of urine sample known to contain cocaine.



GC of cocaine

MS spectrum of cocaine

What is LC-MS?

LC-MS:

- is an **analytical chemistry** technique that combines the physical separation capabilities of **liquid chromatography** (or HPLC) with the mass analysis capabilities of **mass spectrometry**.
 - LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity.
 - Its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).
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- It is the combination of liquid chromatography and the mass spectrometry.
 - In LC-MS we are removing the detector from the column of LC and fitting the column to interface of MS.
 - In the most of the cases the interface used in LC-MS are ionization source.

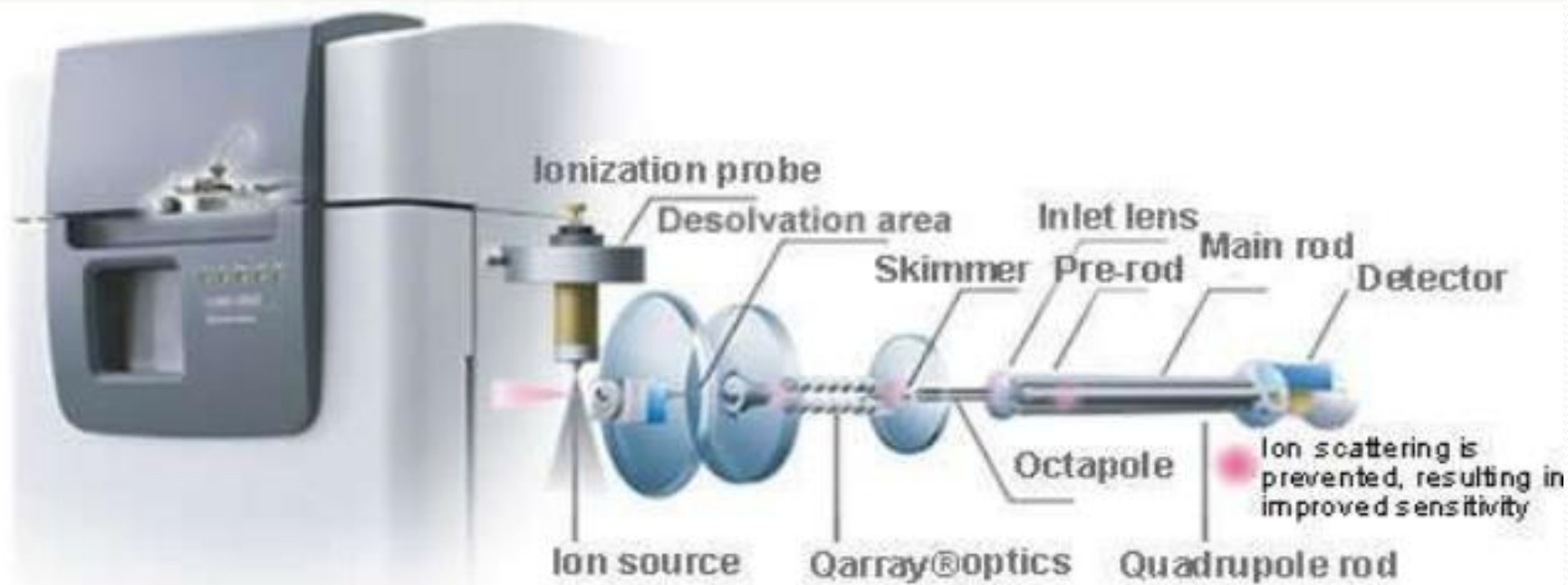


WHY WE COMBINE THESE TWO?

- Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a liquid chromatograph and a mass spectrometer.
- Therefore, when an identifying mass spectrum appears at a characteristic retention time in a LC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.



Modern LCMS (LCMS 2020 SHIMADZU)



Ultra Fast UFswitching

Rapid polarity switching
between positive/negative
ionization modes

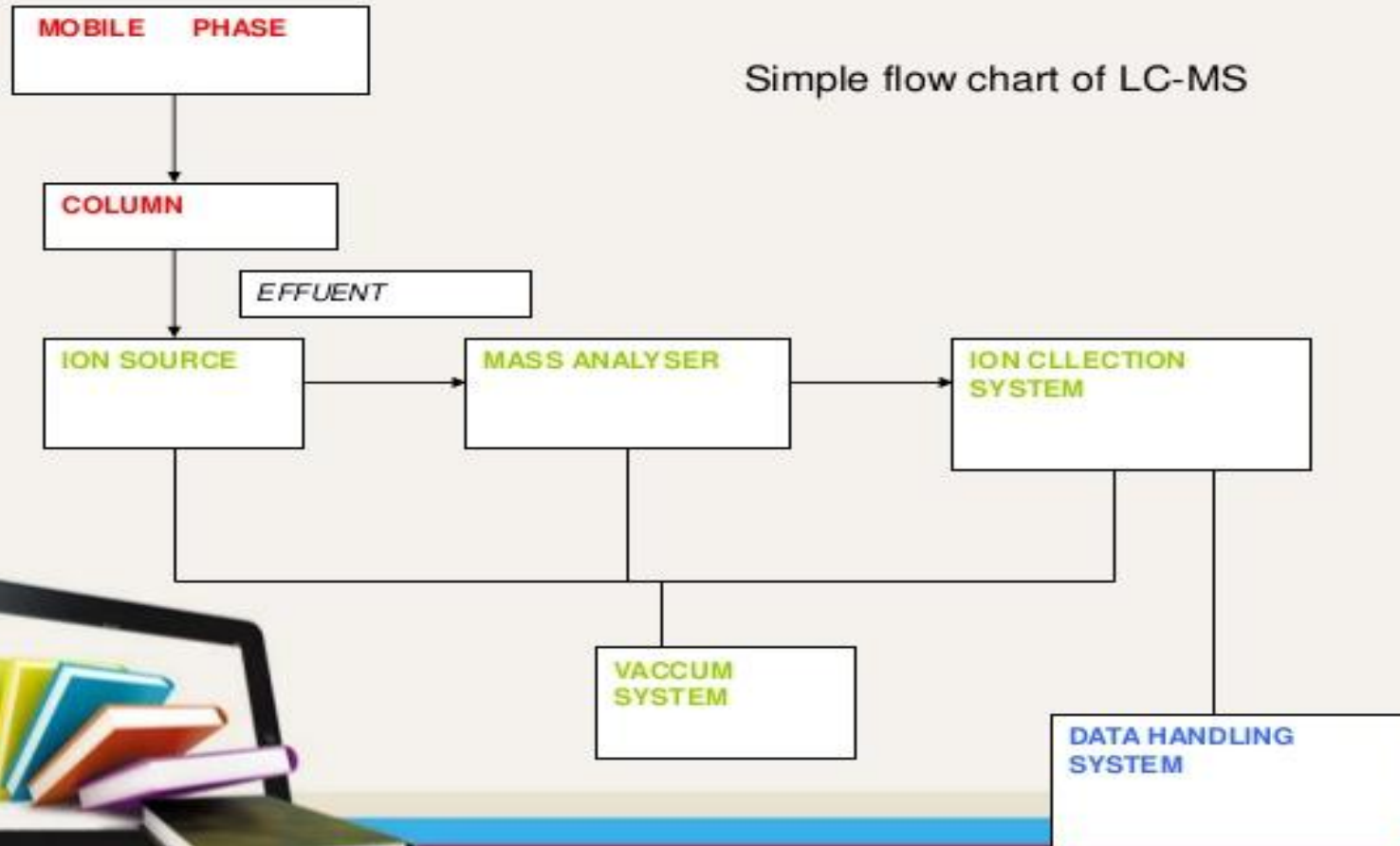
Ultra Fast UFsensitivity

Superior sensitivity,
especially for ultra
high-speed analysis

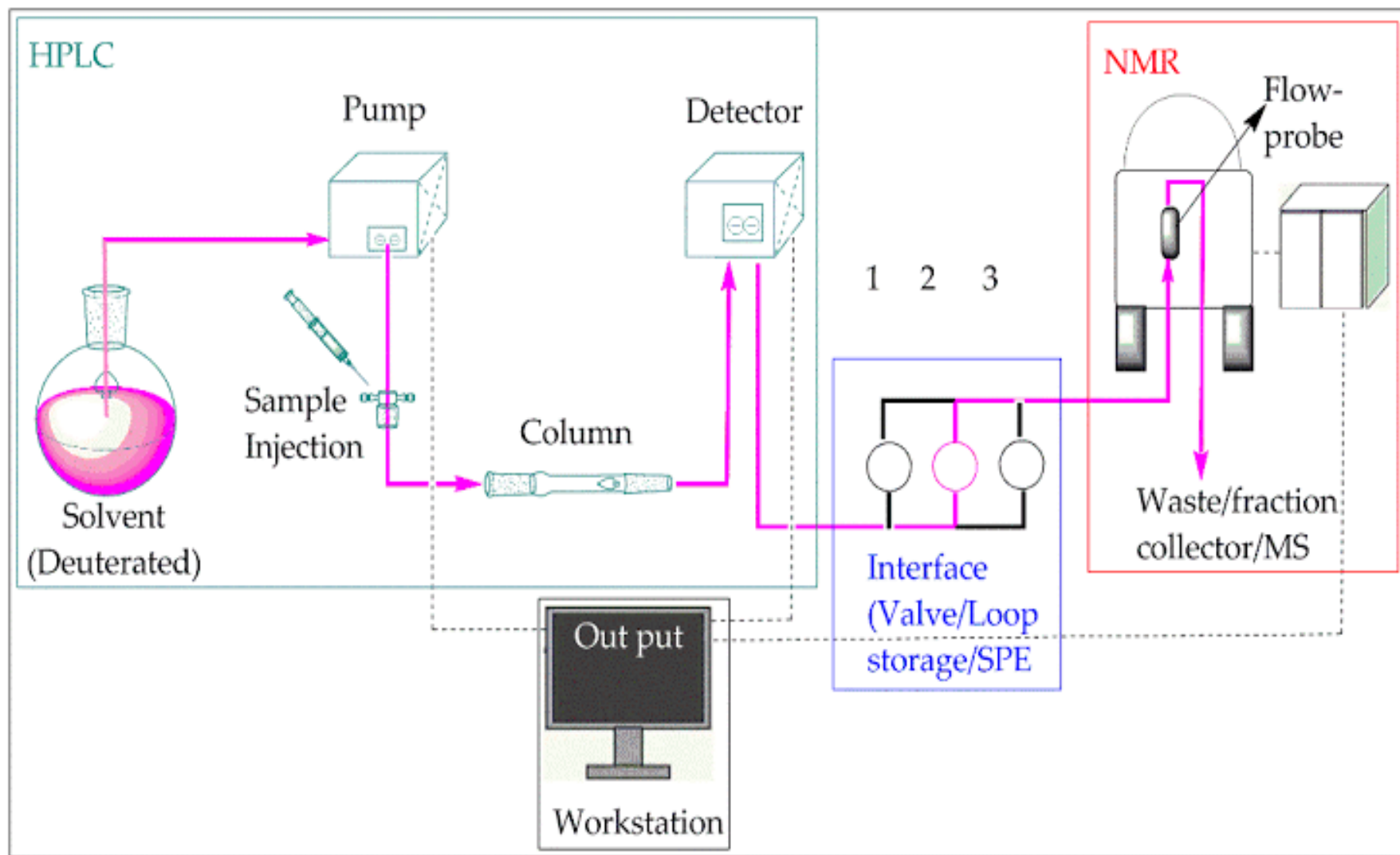
Ultra Fast UFscanning

High-speed scanning

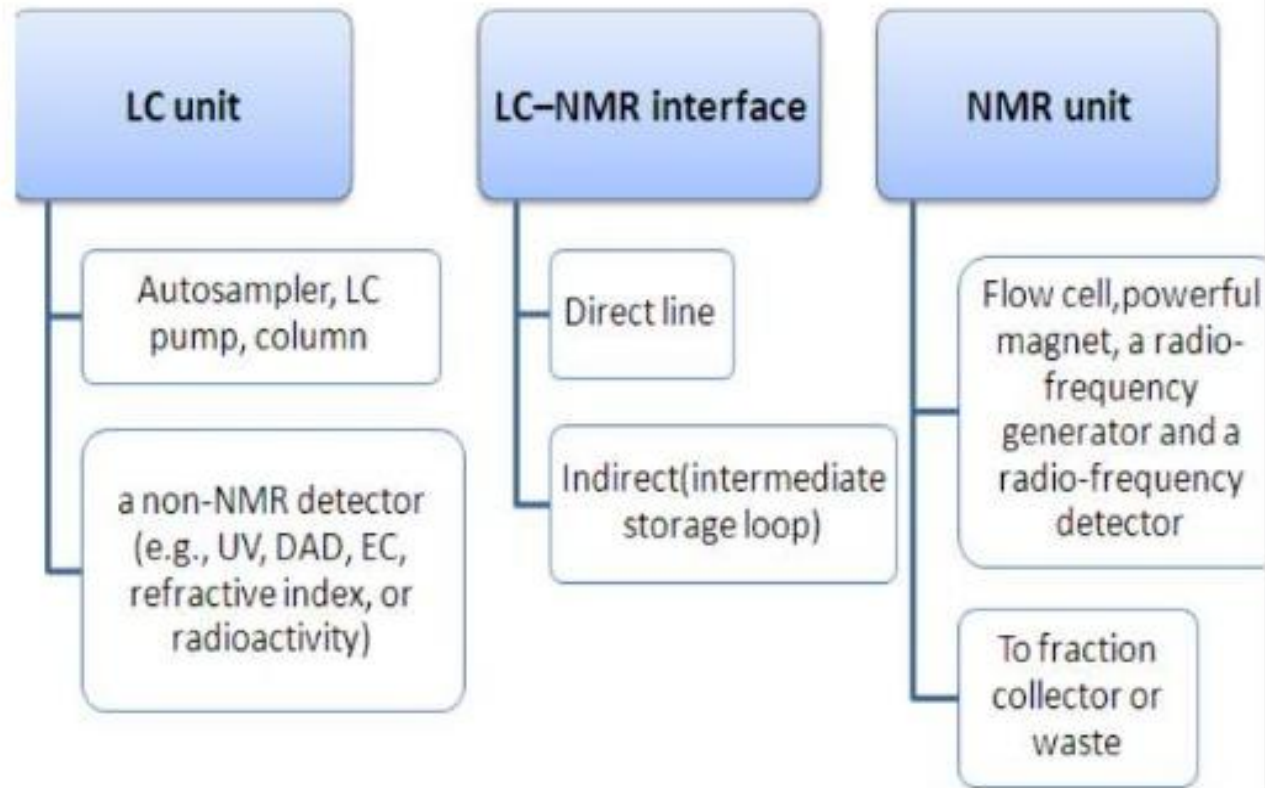
Simple flow chart of LC-MS



HPLC-NMR



Instrumentation of LC-NMR



LC-NMR Interface

- 1) Direct coupling:** It include direct flow of LC effluent in to NMR flow cell and continuous recording of spectra
 - ✓ **post-column splitter**
 - ✓ **valve-switching interface** i.e BNMI (Bruker NMR-Mass Spectrometry Interface)
- 2) Indirect coupling:**
 - ✓ intermediate **storage loop** which transfer outlet of lc to NMR flow cell at specified time interval
 - ✓ **SPE unit**

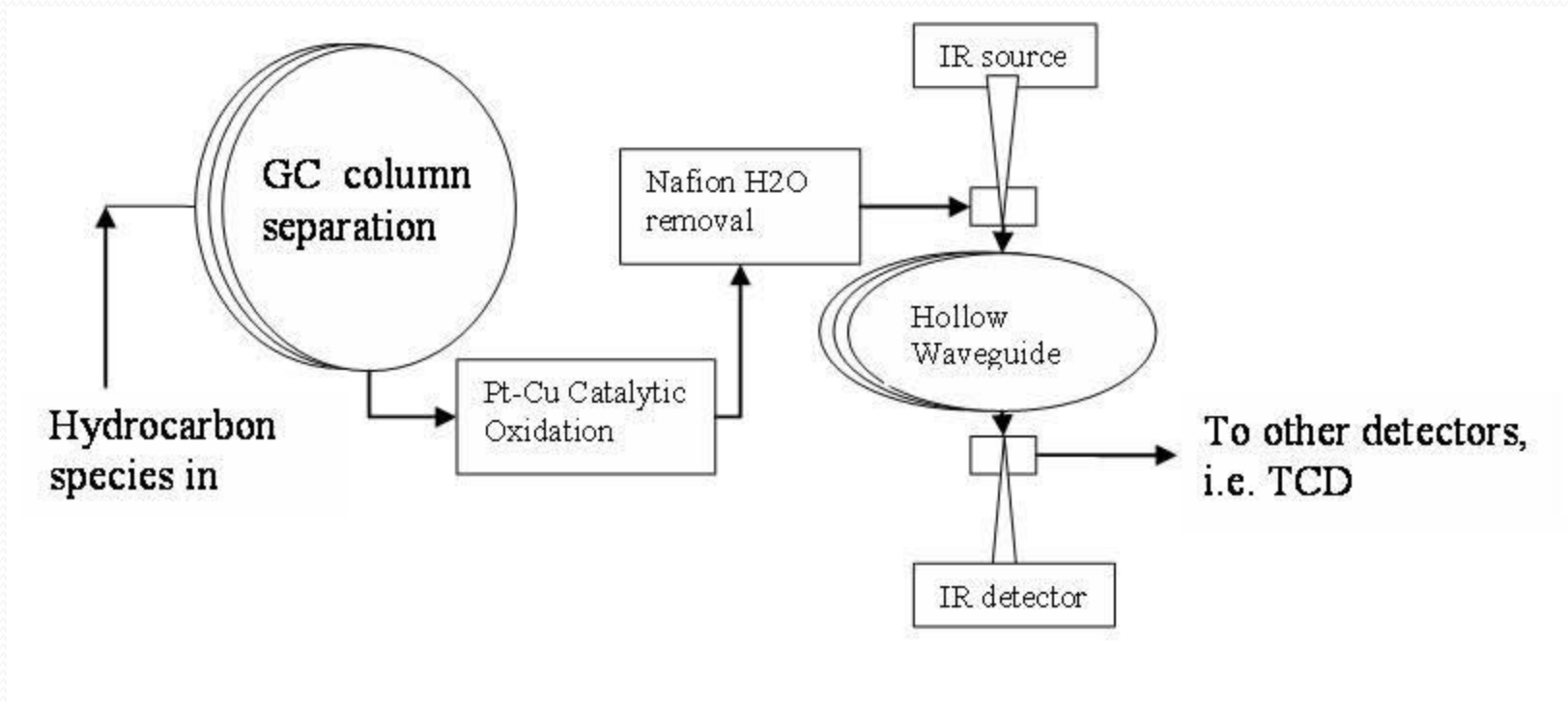


fig: 36 Loop Cassette

GC-IR (Gas Chromatography + Infra red spectrometry)

- 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.
- Effluent from GC is directly forwarded into the heated pipe of IR at atmospheric pressure.

GC-IR Working



LC- NMR

- Direct coupling of liquid chromatography to NMR using stop flow method was reported in 1978.
- This method is a powerful tool used in many areas such as a natural products, organic molecules, biomolecules, drug impurities, by - product, reaction mixtures, and drug degradation products.
- Giving structural information no other techniques can.

Advantages of Hyphenated Techniques

- Fast and accurate analysis.
- Higher degree of automation.
- Higher sample throughput.
- Better reproducibility.
- Reduction of contamination due to its closed system.
- Separation and quantification achieved at same time

CONCLUSION

- Advances in the hyphenated technique such as LC-MS , GC-MS, LC-NMR, CE-MS and ICP-MS have been made to excellently solve various complex analytical problems in different fields.
- These techniques solve such problems in time efficient manner, higher degree of automation, higher sample throughput better responsibility.

References

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Thank U!

