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Review Article

Hyphenated Analytical Techniques in Pharmaceutical Analysis: A Review

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ABSTRACT

Hyphenated analytical techniques have emerged as powerful tools that integrate the separation efficiency of chromatographic methods with the structural elucidation capabilities of spectroscopic or mass-spectrometric systems. This combined approach enables simultaneous qualitative and quantitative evaluation of complex mixtures within a single analytical workflow. Their high sensitivity, selectivity, reduced solvent consumption, and minimized handling errors have made them indispensable in modern pharmaceutical, environmental, biochemical, and forensic investigations. Techniques such as GC–MS, LC–MS, LC–NMR, and CE–MS provide enhanced resolution, improved detection of trace impurities, and reliable identification of metabolites and degradation products. Recent advances in instrumentation, microfluidics, automation, and AI-driven data analytics have further strengthened the performance of hyphenated systems, enabling faster, greener, and more accurate analytical outcomes. As these technologies continue to evolve, they remain central to advancing research quality, regulatory compliance, and innovation across analytical sciences.

INTRODUCTION

The general combination of different chromatographic method with the spectroscopic method was first referred in analytical methodology as Hyphenated Techniques . This combination of these two methodologies had formed a very efficient , effective analytical method in the field of Instrumental Analysis which gives the synergistic result in analysis.(Bruno, 2000a) Few decades ago, the term hyphenation was first introduced to the world by Sir Tomas Hirchfeld. He coined this term for the combination of an spectroscopic method/technique with a chromatographic method for the analysis purpose.

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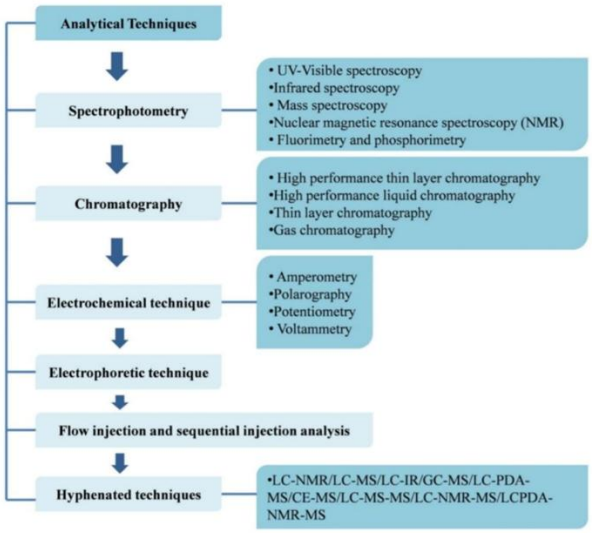

Fig 1:-Methods for Analysis

It is also referred as a marriage of a separation and a spectroscopic detection technique.

Nowadays this technique has gained a high fame or has become widely known due to its efficiency in solving complex analytical issues or detections etc.(Joshi et al., n.d.)

1.1 Principle of Hyphenation

It is basically based on principle of both spectroscopic and chromatographic technique. When these two principles are combined together this gives the person advantages of both, in investigation, separation as well as detection of complex mixture / analyte. Different hyphenated techniques have different advance principle as per the combination but the basic principle remains unchanged.(Wilson et al., 2021a) Chromatography separates a mixture into its different parts, giving almost pure components, while spectroscopy helps identify those parts by matching them with known samples or reference data. To understand the structure and identify the compound in a raw sample, techniques like liquid chromatography , or capillary electrophoresis are combined with detection method such as FTIR, UV Visible spectrometry or NMR.(Joshi et al., n.d.)

1.2 Need and Significance of Hyphenated Techniques

The advancement of analytical chemistry has led to the increasing use of Hyphenated Techniques due to their unique ability to combine the strengths of separation and detection systems. These coupled techniques enable the simultaneous qualitative and quantitative analysis of complex mixtures, providing higher accuracy, precision and reproducibility than single analytical methods.(Gashaw et al., 2025a; Meermann & Sperling, 2012a) One of the main needs for hyphenated techniques arises from the growing complexity of pharmaceutical, environmental, and biological samples. Traditional standalone methods often fail to deliver complete structural or compositional information whereas hyphenated system allows separation, detection and characterization in a single run.(Gashaw et al., 2025a) They significantly reduce analysis time, solvent consumption, and manual handling errors while enhancing selectivity and sensitivity.(Bruno, 2000a; Joshi et al., n.d.) In pharmaceutical research, these systems are indispensable for impurity profiling, metabolite identification, and quality control of drugs.(Joshi et al., n.d.; Yamarthi et al., 2024)

Their automation and non destructive nature make them suitable for bioanalytical studies and complex biological matrices.(Wilson et al., 2021a) The combine power of chromatographic separation and spectroscopic identification allows hyphenated systems to detect trace level compounds with greater reliability and accuracy(Joshi et al., n.d.; Yamarthi et al., 2024). This integrated approach enhanced analytical sensitivity and ensures minimal matrix interference, providing more dependable results.(Gashaw et al., 2025a)

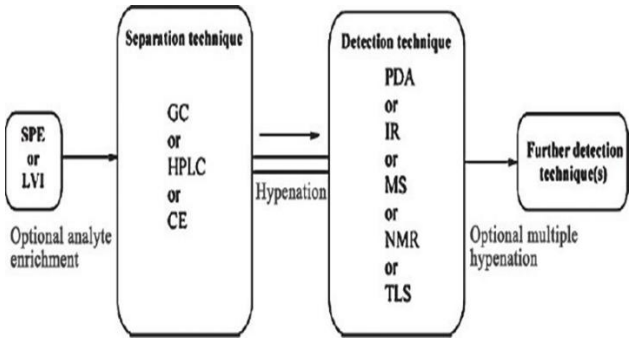

Fig 2:- Hyphenated Techniques

2. Techniques in Hyphenated Pharmaceutical Analysis

2.1 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS integrates the separation power of gas chromatography with the detection and identification capability of Mass Spectrometry. In this system,volatile analytes are separated within a heated capillary column and subsequently introduced into the mass spectrometer, where they undergo ionization and fragmentation for mass to charge (m/z) analysis. The GC-MS interface – usually a membrane, jet/orifice, or effusion separator- maintains the pressure differential between GC and MS.(Wilson et al., 2021a)

2.1.1 Advantage

- High sensitivity and selectivity for thermally stable compounds
- High selectivity and sensitivity for volatile compounds (Wilson et al., 2021a)
- Enables rapid, precise quantitative and qualitative analysis (Bruno, 2000a)

2.1.2 Limitations

- Inefficient for non volatile or thermoliable analytes
- Derivatization often required
- Requires Vacuum interface maintenance (Wilson et al., 2021a)

2.1.3 Application

- Used for pesticide residue detection
- Detection of volatile drug impurities
- Profiling essential oils and natural products (Bruno, 2000a)

2.2 Liquid Chromatography – Mass Spectroscopy (LC-MS)

LC-MS combines chromatographic separation with mass spectrometry detection to analyse complex mixtures both qualitatively and

quantitatively.(Gashaw et al., 2025a) Typical interfaces include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and thermospray (TSP), which convert liquid – phase analytes into gas-phase ions for MS detection.(Bruno, 2000a; Wilson et al., 2021a) Analyzers such as quadrupole, ion trap, or time of flight (TOF) are used depending on desired mass accuracy and resolution. Tandem configurations (LC-MS) enable structural elucidation by fragmentation studies.(Wilson et al., 2021a)

2.2.1 Advantage

- Broad analytes compatibility (polar to non polar)
- High sensitivity, selectivity, and minimal sample preparation
- Provides accurate mass, isotope and fragmentation data useful for drug metabolism and impurity profiling.(Bruno, 2000a; Gashaw et al., 2025a)

2.2.2 Application

- Bioanalysis
- Pharmacokinetic studies in pharmaceutical formulation
- Environmental monitoring in product
- Quality control in pharmaceutical formulations(Bruno, 2000a)

2.3 Liquid Chromatography – Nuclear Magnetic Resonance (LC-NMR)

LC-NMR couples chromatographic resolution with NMR spectroscopy, offering unmatched structural insight for separated analytes. The coupling may operate in on flow, stopped flow, or trapping (SPE) modes depending on sample quantity and concentration. Stopped flow mode increases sensitivity by halting the flow during quisition while LC-SPE-NMR traps analytes for solvent exchange with deuterated media. Modern LC-NMR systems employ high field (500-900 mHz) NMR and deuterated mobile phases to suppress solvent signals.(Bruno, 2000a; Wilson et al., 2021a)

2.3.1 Advantage

- Direct structural confirmation without isolation
- Complements MS by providing atomic – level data (Gashaw et al., 2025a)

2.3.2 Limitations

- Lower sensitivity
- High solvent cost
- Longer analysis time

2.3.3 Application

- Identification of unknown natural products
- Structural elucidation of metabolites
- Elucidation of impurities in complex mixtures(Bruno, 2000a)

2.4 Liquid Chromatography -Fourier Transform Infrared Spectroscopy (LC-FTUR/ LC-IR)

LC-FTIR connects HPLC with IR spectroscopyto provide functional group information through mid IR absorption spectra.

Interfaces include flow cell and solvent – elimination types, depending on solvent transparency and analyte volatility.(Wilson et al., 2021a)

2.4.1 Advantages

- Offers direct information on functional groups complementary to MS.

2.4.2 Limitations

- Strong solvent absorption in the IR region limits sensitivity
- Requires solvent removal interfaces
- Requires deuterated mobile phases

2.4.3 Application

- Verification of structural isomers
- Confirmation of specific functional groups in pharmaceutical intermediates and natural products.(Wilson et al., 2021b)

2.5 Capillary Electrophoresis – Mass Spectrometry (CE-MS)

CE-MS unites high efficiency electrophoretic separation with mass detection for charged and polar species. Because CE operates at extremely low flow rates, sheath liquid, or sheathless electrospray interface are used to stabilize ionization. Sheathless interfaces enhance sensitivity by minimizing dilution and ion suppression.

2.5.1 Advantages

- Exceptional separation efficiency for ions and small biomolecules
- Requires minimal sample volume and reagents

2.5.2 Limitations

- Interface complexity
- low absolute sensitivity

(Improving with one line pre concentration)

2.5.3 Application

- Metabolomics, chiral separations
- Peptide mapping
- Profiling of ionic drug metabolites(Gashaw et al., 2025b)

2.6 Liquid Chromatography – Gas Chromatography

LC-GC sequentially combines LCs broad separation range with GCs superior resolution for volatile fractions.(Wilson et al., 2021b) Transfer interfaces such as loop, retention gap and programmed – temperature vaporizer (PTV) injectors remove solvent before GC analysis.(Bruno, 2000b) Comprehensive (LC×GC) and heart cutting configurations further enhance peak capacity and analytical depth (Gashaw et al., 2025b)

2.6.1 Advantages

- Combines LCs loading capacity with GCs high resolution.
- Minimizes manual handling and contamination.

2.6.2 Limitations

- Requires careful solvent removal
- Requires interface optimization

2.6.3 Application

- Used for petrochemicals analysis
- Used for food and pharmaceutical analysis of multi component samples such as mineral oils and polycyclic aromatic hydrocarbons.(Gashaw et al., 2025b)

2.7 Advanced Hyphenated and Multidimensional Techniques

Modern analytical research increasingly relies on multi hyphenated systems like LC-MS-NMR, LC-SPE-NMR, 2D-LC, LC×GC and GC×GC which provide complementary separation and detection data. LC-MS-NMR delivers orthogonal data – chromatographic retention, mass spectra, and NMR structure for unambiguous compound identification LC-SPE-NMR improves sensitivity by trapping analytes, exchanging solvent and acquiring high field (600-900MHz) spectra.(Gashaw et al., 2025b; Wilson et al., 2021b) 2D-LC and LC×GC employ orthogonal mechanisms to increase resolving power for highly complex matrices.(Meermann & Sperling, 2012b)

2.7.1 Advantages

- Maximal structural and compositional information from a single workflow
- Ideal for natural products dereplication, metabolomics, and impurity profiling.(Wilson et al., 2021b)

2.7.2 Limitations

- High system complexity
- Higher cost
- High data processing demand

2.7.3 Application

- Comprehensive drug – metabolite elucidation
- Quality Control
- Identification of trace level components in pharmaceutical and biological matrices(Meermann & Sperling, 2012b)

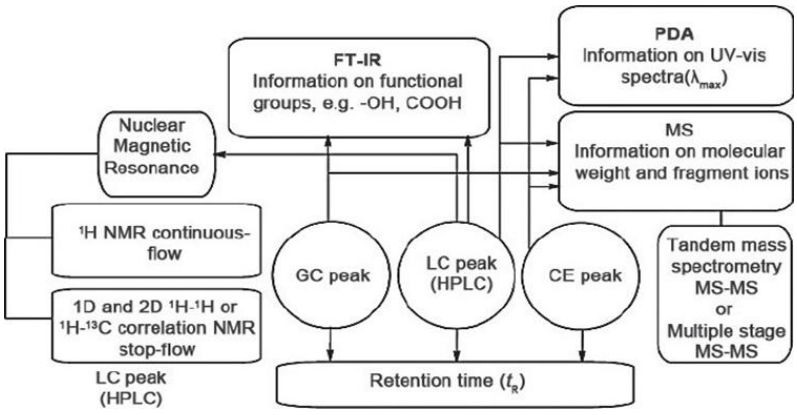

Fig 3:- Application of Hyphenated Techniques

3. Recent Innovations and emerging Hyphenated Techniques

3.1 Expansion to Multi Hyphenated Techniques

- Analytical research is moving beyond two techniques combination such as LC – MS or GC – MS.
- Modern instruments now integrate three or more detectors like LC – MS – NMR or LC-SPE – NMR – MS to obtain complementary molecular information in a single workflow.
- These setups allow simultaneous measurement of chromatographic retention, mass to charge ratio and detailed structural data reducing the need for repeated analyses and sample loss.

3.2 High Resolution and Accurate Mass detection

- The introduction of high resolution mass analyzers such as Orbitrap, quadrupole – time of flight (Q – TOF) and Fourier transform ion cyclotron resonance (FT – ICR) has markedly improved accuracy and sensitivity.
- These systems help identify trace impurities and isobaric compounds that earlier instruments could not distinguish.

3.3 Two dimensional and Comprehensive Chromatography (2D LC, GC – GC)

- Two dimensional separation, where two columns with different selectivities operate sequentially, offers far greater peak capacity and resolution.
- Techniques such as LC×LC and GC×GC are now used for metabolomics, petrochemicals, and natural product pofiling, enabling visualization of hundred of components in a single run.

3.4 Ambient Ionization Methods

- New soft ionization approaches – like desorption electroscopy ionization (DESI), direct analysis in real time (DART), and laser ablation electroscopy ionization (LA ESI) – allow direct sampling of solids or surfaces with almost no preparation.
- These techniques shorten analysis time and make field portable MS feasible for rapid screening.(*Mass Spectrometry*, 2019)

3.5 Microfluidic and Miniaturized Platform

- The trend towards miniaturization has produced micro LC – MS and lab on chip systems that handle nanoliters volumes.
- They reduce solvent consumption, cost and waste white maintaining analytical performance an important step towards sustainable ‘green’ analytical chemistry.(Reddy, n.d.)

3.6 Hyphenation with Spectroscopic and Elemental Detectors

- The integration of chromatography with atomic spectroscopic detectors such as ICP – OES (eg. LC – ICP – MS) has become crucial for trace – metal and metalloprotein analysis.
- This combination supports both qualitative identification and quantitative elemental profiling in pharmaceuticals and biological matrices.

3.7 Automation and Software Driven Data Fusion

- Modern systems feature intelligent control software capable of synchronising multiple detectors and merging datasets.

- Automated deconvolution and chemometric tools translate the combined outputs of MS, NMR, and IR into unified molecular fingerprints, improving reproducibility and speeding up decision making.(*Mass Spectrometry*, 2019)

3.8 Integration with Artificial Intelligence and Machine Learning

- Emerging work flows use AI algorithms for spectral matching, compound identification, and predictive modeling.

- These tools enhance the interpretation of complex LC – MS and GC – MS data support real time quality assessment in drug development.(Sharp & Marquez, 2006)

3.9 Portable and Field – Deployable Hyphenated Systems

- Advances in compact MS and micro – GC devices have made on site environmental and forensic analysis possible.

- Battery powered or handheld units combine separation and detection in small formats while maintaining acceptable sensitivity.(Reddy, n.d.)

DISCUSSION

Hyphenated techniques combine the strengths of chromatographic separation with the structural identification abilities of spectroscopic methods. This combination helps analysts obtain faster, more accurate, and more detailed information from complex samples. Techniques such as GC–MS, LC–MS, and LC–NMR have become essential in pharmaceutical analysis because they can detect impurities, identify unknown compounds, and support quality control with high sensitivity. Although these techniques offer major advantages, they also require high-cost instruments, expert handling, and careful maintenance, which may limit their use in smaller labs. Still, the benefits—such as improved accuracy, reduced analysis time, and better reliability—make hyphenated techniques an important part of modern analytical science. As technology improves, these methods will continue to play a key role in research and pharmaceutical development.

CONCLUSION

Hyphenated techniques have greatly improved the quality and reliability of analytical testing by combining chromatographic separation with strong spectroscopic detection. This integration allows analysts to study complex samples with higher sensitivity, faster analysis time, and better clarity in identifying unknown compounds or impurities. Because of these advantages, techniques such as GC–MS, LC–MS, and LC–NMR have become essential tools in pharmaceutical analysis, impurity profiling, environmental monitoring, and natural product research. Although these systems require higher investment, trained personnel, and proper maintenance, their contribution to accurate and efficient analysis is undeniable. As technology continues to evolve, hyphenated techniques are expected to become even more powerful, more automated, and more accessible. Overall, they represent a major step forward in modern analytical science and will continue to support quality control, research innovation, and regulatory compliance in the future.

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