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Recent Advancements and Comprehensive Review on Hyphenated Techniques

Shreyas K. Reddy[1], Anjali Nayak[1,*], Devika H.G.[1]

Abstract

Hyphenated techniques represent a powerful class of analytical methods that combine two or more established techniques – typically a separation method with a spectroscopic detection technique – to achieve enhanced analytical performance. First introduced by Hirschfeld in 1980, the term "hyphenation" refers to the online coupling of such methods, enabling more precise, sensitive, and comprehensive analysis of complex samples. These techniques exploit the strengths of individual methods while overcoming their limitations, offering superior sensitivity, selectivity, and structural insight. Hyphenated techniques are indispensable in modern analytical chemistry due to their ability to analyse intricate biological, pharmaceutical, environmental, and material samples with greater speed and accuracy. Their applications span drug development, food safety, forensic analysis, and pollutant monitoring. Common examples include GC-MS, LC-MS, LC-IR, LC-NMR, CE-MS, and ICP-MS – each with distinct advantages and drawbacks. For instance, GC-MS offers excellent separation and detection of volatile compounds but may not handle thermally labile substances. LC-NMR provides structural data but is limited by sensitivity and cost. These integrated systems address critical challenges such as limited selectivity, low sensitivity, time consumption, and destructive analysis often encountered with standalone methods. They also help overcome technique incompatibility through innovations in interface design. Ongoing developments in hyphenated technologies are driven by the need for higher throughput, automation, and real-time monitoring. Recent advancements include miniaturized systems, enhanced detectors, and software algorithms for improved data processing. These innovations are broadening the scope of hyphenated techniques, cementing their role in advancing scientific discovery across disciplines.

Keywords: Hyphenation, analytical chemistry, separation techniques, spectroscopic detection, GC-MS, interface design

INTRODUCTION

Hyphenated techniques are a potent blend of two or more analytical techniques employed in combination that provide more comprehensive and precise information about a material or sample. The integration of these techniques exploits the unique qualities of each approach, hence augmenting the total analytical capabilities [1, 2].

The word "hyphenate" or "hyphenation" was first introduced by Hirschfeld in 1980 to indicate the online merging of a method of separation with one or more spectroscopic detection techniques.

Due to their unparalleled capability to deliver accurate and thorough analysis of complicated materials, hyphenated methods are crucial to current analytical chemistry and scientific research as they are crucial for the following reasons:

***Author for Correspondence**
Anjali Nayak
E-mail: anjaliangel84.pharma@gmail.com

[1]Assistant Professor, Department of Quality Assurance, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India

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- *Superior Analytical Capabilities:* Hyphenated techniques integrate the advantages of several different analytical methods. Better sensitivity, selectivity, and accuracy in identifying and quantifying components within a sample are made possible by this integration. They make it possible for scientists to identify and analyse chemicals even in tiny amounts that could otherwise go unnoticed.
- *Sophisticated Sample Analysis:* Samples are frequently multifaceted and complicated in a variety of domains, including the biological, pharmacological, environmental, and materials sciences. Hyphenated approaches provide a deeper examination by providing comprehensive details about the composition, structure, and characteristics of these samples, thus facilitating a more comprehensive understanding.
- *Enhanced Speed and Efficiency:* These methods expedite the analytical process by carrying out several analyses concurrently or quickly one after the other. since of its efficiency, researchers may provide findings more rapidly without compromising accuracy as it saves time and resources.
- *Applications in Diverse Fields:* Hyphenated approaches are used in a wide range of academic fields. They aid in quality control, analysis, and drug discovery in the pharmaceutical industry. They support the monitoring and identification of pollutants in environmental sciences. They have applications in food safety, biochemistry, forensic analysis, and other fields, demonstrating their flexibility.
- *Research and Innovation Advancements:* The profound comprehension that hyphenated approaches often drive scientific progress. They provide major contributions to the study of intricate biological processes, the creation of novel materials, the creation of more potent medications, and the solution of environmental problems. This information stimulates new ideas and investigations across a wide range of scientific fields.
- *Challenging Analytical Demands:* Hyphenated procedures are always evolving to satisfy the more complex analytical needs. They allow scientists to take on challenging analytical issues that more traditional approaches would find difficult to properly handle.

Hyphenating procedures solves the constraints of individual analytical methods, which frequently have their own set of drawbacks. The following are some typical restrictions on distinct analytical techniques:

- *Limited Selectivity:* A combination of identical chemicals may not be able to be distinguished from one another by several analytical techniques. For instance, spectroscopic methods such as infrared spectroscopy or UV-Vis may have difficulties distinguishing closely related chemicals.
- *Sensitivity Issues:* Some techniques might not be sensitive enough to identify substances in trace amounts. This restriction is particularly crucial for domains such as environmental analysis, where contaminants might exist in extremely tiny amounts.
- *Limited Information:* various techniques offer various types of information; therefore, relying just on one approach could not provide a complete picture of a sample. For example, mass spectrometry can provide specifics about molecular weight, but it is unable to provide information about molecular structure.
- *Time-Consuming:* Some analytical techniques might take a lot of time, which makes them less useful in situations where quick analysis is essential. It might take a long time to finish using traditional techniques like titration or gravimetric analysis.
- *Destructive Nature:* Some approaches use destructive sampling, which makes it impossible to analyse the same sample in various ways. This constraint is particularly challenging when working with scarce or valuable samples.
- *Technique Incompatibility:* There are certain analytical techniques that are intrinsically incompatible. For example, due to variations in the mobile phase, combining gas chromatography with a particular detector might not be simple (Figure 1).

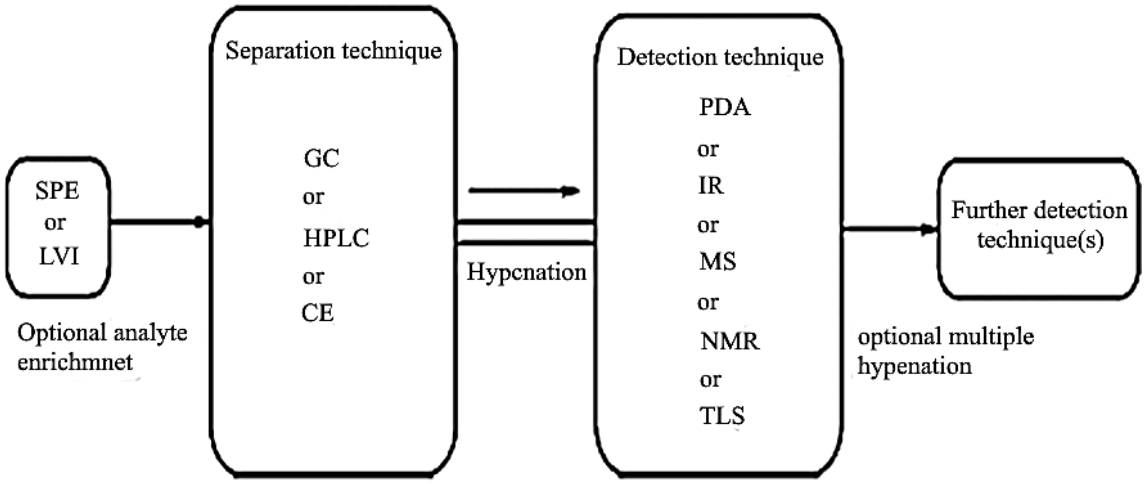

Figure 1. Hyphenation of chromatographic and spectrometric techniques [3].

Key Analytical Techniques That Are Commonly Used in Hyphenation

- Chromatographic techniques (HPLC, GC, etc.).
- Spectroscopic techniques (MS, NMR, IR, UV-Vis, etc.).
- Electrophoretic techniques (CE, SDS-PAGE, etc.).
- Nuclear magnetic resonance (NMR).
- UV-Visible spectroscopy.
- Mass spectrometry (MS).
- X-ray diffraction (XRD).
- Infrared spectroscopy (IR).

AVAILABLE HYPHENATED TECHNIQUES

GC-MS

In recent times, GC-MS and LC-MS have emerged as the predominant hyphenated techniques, where MS serves as the primary detection method. These methods make extensive use of instruments, including time-of-flight (TOF) mass spectrometers, ion traps, and single- and triple-quadrupole mass spectrometers. The pioneering, hybrid technology that unites gas chromatography and mass spectrometry acted as an initial foray into using such integrated approaches for research and development purposes. This hyphenated approach yields mass spectra that provide additional structural information through fragmentation interpretation. Library spectra can be utilized to assess fragment ions exhibiting different relative abundances. GC-MS analysis of compounds that are sufficiently tiny, stable, and volatile at high temperatures under GC conditions is straightforward. When using GC-MS for research on polar molecules, primarily those that possess multiple hydroxyl groups, derivatization is often essential. The process of changing the analyte into its trimethylsilyl derivative is the most widely used derivatization method. After a sample is loaded into the GC system, it evaporates, separates in the GC column, is examined with the MS detector, and is recorded in the GC-MS system (Figure 2). The total time taken by a compound to pass through the column and elute the components is known as "retention time" (Rt). In GC-MS, a metal column often packed with a sand-like stationary phase facilitates separation, with the detector positioned at the opposite end [4–6].

There are two types of GC-MS columns: macrobore and packed columns, as well as capillary columns. The GC-MS interaction necessitates careful consideration of the criteria below:

- The interface effectively transfers effluent from the GC to the MS.
- The interface cannot contain a condensed analyte.
- The analyte cannot break down prior to entering the MS ion source.
- The gas load that enters the ion source needs to fit inside the MS's pumping capability.

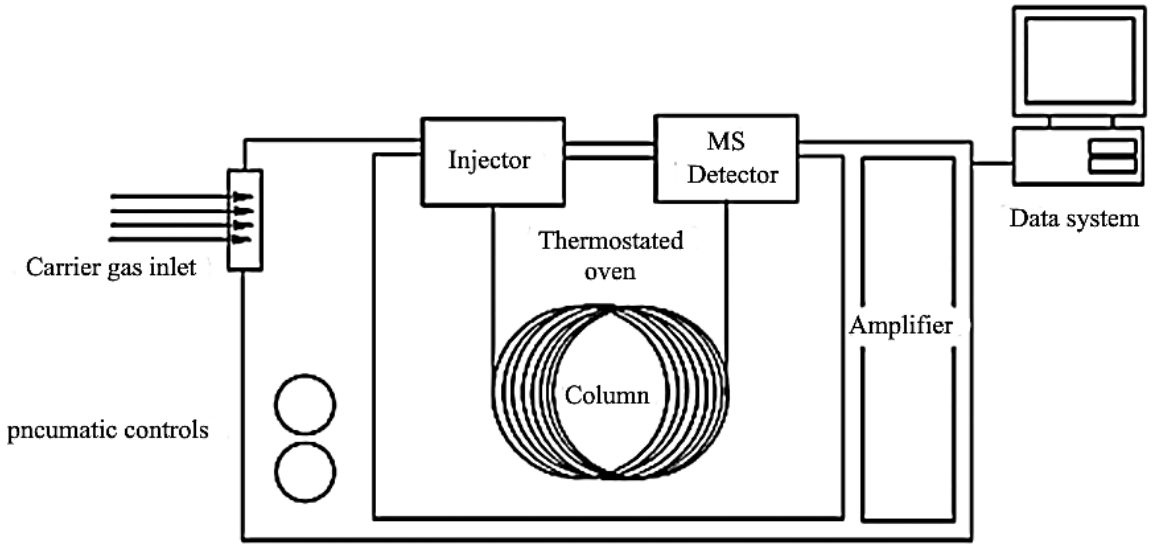

Figure 2. GC-MS [7].

The chemical ionisation (CI) and electron impact ionisation (EI) modes are the most used GC-MS interfaces. However, multiple methods of different kinds that enable molecular ion identification can be employed in modern GC-MS systems. For instance, by precisely measuring the mass and determining the elemental composition, orthogonal TOF mass spectrometry, when combined with gas chromatography, is used to confirm the purity and identification of the constituents. These days, a GC-MS system is often linked to several online MS databases containing reference spectra for numerous reference compounds, along with search features that aid in identifying separated components by matching their spectra.

Advantages of GC-MS

- When compared to other approaches, GC has a high-resolution power.
- This technique, when combined with thermal detectors, delivers a high sensitivity.
- Rapid sample separation and analysis an additional benefit of this technology that is used for lower-quality samples.
- This technique's precision and accuracy are very satisfactory.

Disadvantages of GC-MS

- Only samples that are volatile (or can be rendered volatile) are suitable for separation using this approach in gas phase separation (GC).

LC-IR

LC-IR, also known as HPLC-IR, is a hybrid technique that combines liquid chromatography (LC) with infrared spectroscopy (IR) or FTIR. These days, IR, or FTIR, is widely used for identifying organic molecules, while HPLC remains one of the most powerful techniques for their separation. This is since organic compounds possess numerous absorption bands in the mid-infrared (IR) region, which are distinctive for specific functional groups like –OH, –COOH, and others. However, detecting signals in the hyphenated approach is frequently complicated by the numerous absorption bands (237 in total) because mobile phase solvents in the mid-infrared (IR) region often interfere with the relatively weak signals, making the integration of IR with HPLC challenging and slowing technological progress in this area. Moreover, IR is significantly less sensitive compared to other detection methods such as UV and MS. However, recent developments in HPLC-IR technology have focused on combining two key interface methods used in HPLC-FTIR or HPLC-IR to improve performance and overcome these limitations. A solvent-elimination strategy is the other, while a flow-cell approach is the first. The method utilized in UV-Vis and other common HPLC detectors is comparable to that employed with the

flow cell in LC-IR. In this instance, the mobile phase's absorption causes interference with the sample component absorption bands' ability to be detected, but a transparent region in the mid-IR spectrum allows for detection. For instance, IR can monitor a wide range of organic compounds containing C–H structures in the molecules, if a deuterated solvent, like heavy water or perdeuterated methanol, is employed as the mobile phase. For most LC-IR operations, the solvent-elimination technique is the recommended choice. Once the solvent from the mobile phase is removed, A material that is transparent to infrared light is used for infrared detection. Usually, KBr or KCl salts are used to trap sample components from the eluent, and pre-heating the medium before infrared detection helps evaporate volatile mobile-phase solvents. The solvent-elimination method utilizes two types of interfaces: the diffuse-reflection infrared Fourier transform (DRIFT) technique and the buffer-memory approach. A unified interface is now available for coupling the gas chromatography (GC), supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC) with Fourier transform infrared spectroscopy (FTIR), using the IR microscopic method [8–10].

LC-MS

"HPLC-MS" or "LC-MS" relates to the combination of a mass spectrometer (MS) with an LC (Figure 3). The identification of the eluted sample can be achieved by utilizing the mass spectral data obtained from the column. Integrating the two approaches can be enabled to operate better by using a switching valve. A standard programmed LC-MS system comprises a mass spectrometer, an LC system, an autosampler, and a double three-way diverter. The diverter typically functions as a programmed switching valve that directs the sample before it reaches the MS, redirecting unwanted eluent from the liquid chromatography (LC) system to a waste disposal [11].

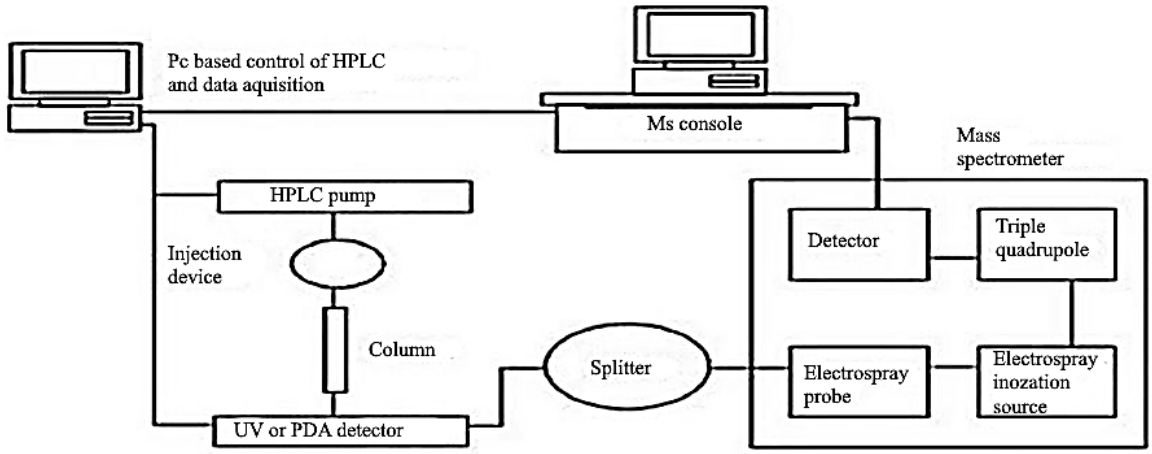

Figure 3. Schematic presentation of LC-MS [3].

The LC-MS integrates molecular identity-confirming, precise detection power of MS with the chemical separating efficiency of an LC. One of the highly sensitive and selective methods for molecular analysis that delivers information about the fragmentation pattern and molecular weight of the analyte molecule is mass spectrometry (MS). The data derived from mass spectrometry (MS) is crucial in verifying the identification of the analyte molecules. It is feasible to rebuild an unknown molecule from MS data using this qualitative technique. Most ionisation methods employed in LC-MS are soft ionisation methods, mainly displaying the molecular ion species, with a minimal presence of fragment ions. Thus, the information gathered about the compound's structure from a single LC-MS run may not be highly accurate. However, with the advent of tandem mass spectrometry (MS-MS) has been developed, this issue has been resolved. MS-MS produces fragments by splitting up the generated molecule ions during collisions. The application of LC-MS-MS is expanding quickly. When combined with biological screening, combined methods, like HPLC integrated with UV and mass spectrometry (LC-UV-MS), have demonstrated their significant utility in rapidly evaluating natural compounds. In recent years, a range of commercially accessible LC-MS systems with diverse interface types have

become available. The design of the interfaces makes sure that adequate liquid nebulization and vaporization, sample ionization, solvent vapor removal, and ion extraction within the mass analyser are offered. When it comes to natural product analysis, electrospray ionisation (ESI) and atmospheric pressure chemical ionization (APCI) are the two extensively used interfaces. The ladder is referred to as "the chromatographer's LC-MS interface" because of its broad application, sensitivity, linear response, and high solvent flow rate capabilities. Several analyzer types, including quadrupole, ion trap, and TOF, are used with these interfaces. However, the mass accuracy and resolution offered by each of these analyzers vary. The LC-TSP-MS (Thermo spray) and LC-CF-FAB (continuous-flow FAB) interfaces are alternative options for use in LC-UV-MS mode. The TSP interface, particularly, has demonstrated suitability for phytochemical analysis by allowing the introduction of an aqueous phase into the MS system at a flow rate of 1–2 ml/min, aligning well with common practices in phytochemical analysis. For LC-MS, it is advisable to employ a reversed-phase system with a gradient or isocratic solvent combination involving water, acetonitrile (ACN), or methanol (MeOH) as the preferred choice for LC operation. The mobile phase can also contain trace quantities of ammonium acetate, ammonium hydroxide/ammonia solution, formic acid, and acetic acid. Different types of analysers, such as quadrupole, TOF, or ion trap, can be utilized in combination with these interfaces. These analysers offer different levels of mass accuracy as well as MS-MS capabilities. Except for well-known natural products, LC-MS methods prevent a comprehensive and unambiguous online component detection when complementing online spectroscopic data that is readily available in databases. A reversed-phase system utilising a gradient or isocratic solvent combination of water, ACN, or MeOH is the recommended choice for LC operation for LC-MS. The mobile phase can also contain trace quantities of ammonium acetate, ammonium hydroxide/ammonia solution, formic acid, and acetic acid. several kinds of analysers, such as quadrupole, TOF, or ion trap, are suitable to be used in combination with these interfaces. These analysers offer different levels of mass accuracy and MS-MS possibilities. Except for widely recognized natural products, LC-MS methods do not enable a thorough and clear-cut online detection of a component, especially when additional online spectroscopic information is readily accessible in databases [12–14].

LC-NMR

Nuclear magnetic resonance (NMR) is one of the least sensitive spectroscopic techniques but remains the most powerful for structural elucidation of natural compounds. With technological advancements, high-performance liquid chromatography (HPLC) can now be coupled directly with NMR, forming the LC-NMR (or HPLC-NMR) technique, which has been in use for over 15 years. The first online HPLC-NMR experiments using superconducting magnets were published in the early 1980s, but practical use in analytical labs became more widespread in the late 1990s.

LC-NMR is particularly valuable for analysing complex mixtures such as drug metabolites and natural products in biological fluids. It operates in two primary modes: continuous-flow**,** which allows real-time analysis, and stopped-flow, which enables in-depth structural studies of specific components. Modern LC-NMR systems, operating at 500, 600, or 800 MHz, and using a variety of probes (^1H, ^13C, ^2H, ^19F, ^31P), support a wide range of bioanalytical applications.

Key components include a continuous-flow NMR probe, a switching valve for toggling between flow modes, the HPLC and NMR instruments, and a UV-Vis detector for initial chromatographic detection. A magnetic field strength of at least 9.4 Tesla (400 MHz for ^1H) is recommended for optimal performance. While continuous-flow mode is suited for rapid screening, stopped-flow mode is ideal for detailed structural characterization of unknown or novel compounds. Its integration with advanced 2D and 3D NMR techniques has significantly enhanced the utility and automation of LC-NMR systems, particularly for natural product research and metabolomics (Figure 4) [15, 16].

The LC unit of an LC-NMR system typically consists of an autosampler, a column, an LC pump, and a non-NMR detector (such as UV, DAD, radioactivity, or EC). This detector transmits the flow to the LC-NMR interface, which may require numerous loops to retain some LC peaks temporarily.

Subsequently, the flow-cell NMR probe head or the waste container receives the response from the LC-NMR interface. Once the flow has passed through the probe head, it is directed into a fraction collector for the purpose of retrieving and thoroughly analysing different fractions analysed by NMR. A splitter at the LC-NMR interface's output can also be used to incorporate an MS into the system.

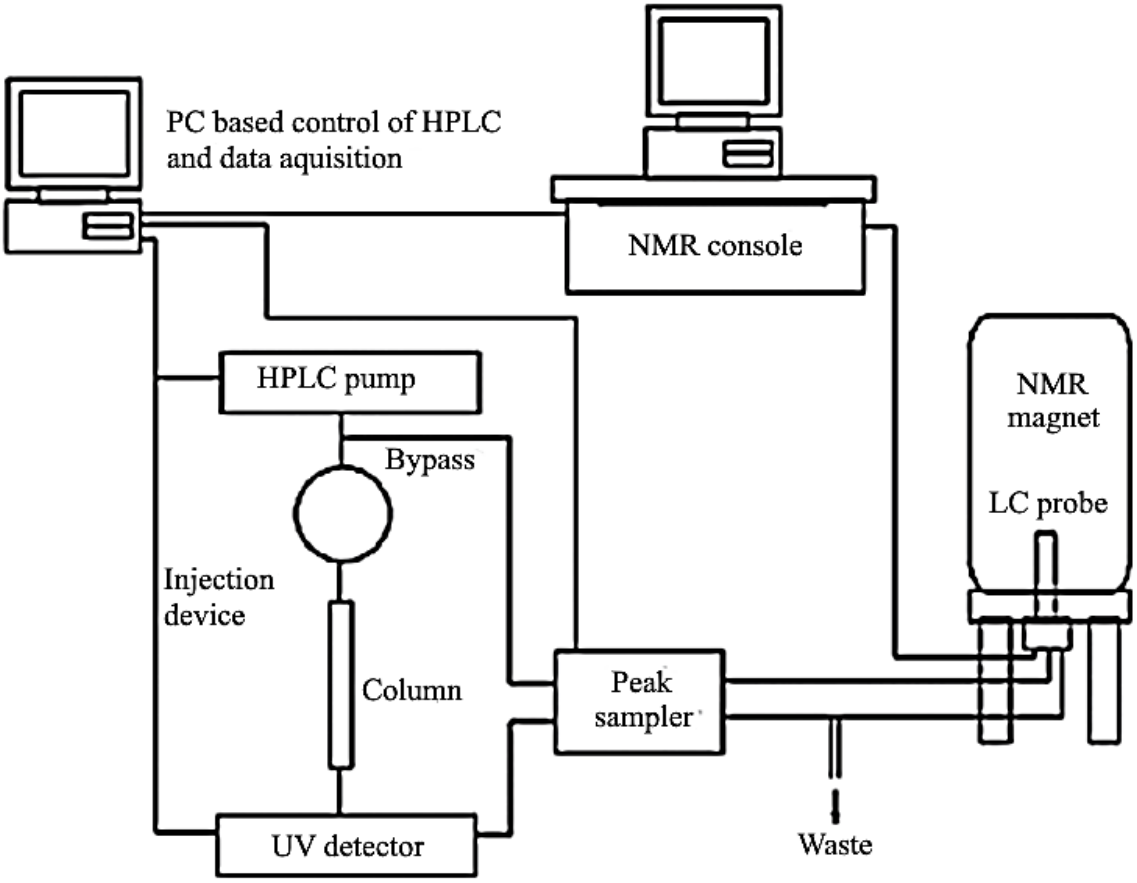

Figure 4. A typical LC-NMR system [2].

Reversed-phase columns with binary or tertiary solvent mixtures and isocratic or gradient elution are often utilized in most LC-NMR applications. The protons in the mobile phase's solvents make it extremely difficult to get a good NMR spectrum. The receiver of the NMR spectrometer cannot process the strong signals from the solvent and the weak signals from the material at the same time. To resolve this issue and minimize the solvent signal, pre-saturation, soft-pulse repeated irradiations, and water-suppression enhancement through T1 effects (WET) pre-saturation using a z-gradient are the three main strategies that may be used. Additionally, this issue can be reduced by considering the following recommendations:

- Using eluents, like H_2O, ACN, or MeOH, that have the fewest 1H NMR resonances feasible.
- Using a minimum of one deuterated solvent, such as MeOD (about $3000/L), ACN-d3 (approximately $1600/L), or D2O (roughly $290/L).
- Utilizing buffers with the minimum available 1H NMR resonances as possible, such as ammonium acetate or TFA.
- Utilizing ion pair reagents with the lowest possible 1H NMR resonances, for example, ion pairs containing t-butyl groups provide an extra resonance.

There are now three primary forms of data capture modes available: time-sliced acquisition, stopped-flow acquisition, and continuous-flow acquisition. For every LC-NMR analysis, an optimal HPLC separation is essential, regardless of the acquisition modality. Since LC-NMR has far lower sensitivity

than other hyphenated methods, such as LC-PDA or LC-MS, it is essential to design an appropriate LC separation in which the amount of an isolated component is concentrated in the lowest elution volume. As a capable, fascinating supplement to LC-UV-MS for in-depth online structural investigation, LC-NMR is worth considering. In fact, LC-NMR has received a fresh lease on life thanks to recent advancements in NMR technology, and it is quickly becoming a potent analytical instrument. The development of efficient solvent suppression methods makes it possible to get an excellent LC-1H-NMR spectrum in reversed-phase HPLC conditions, both continuous-flow and intermittent-flow. You can use nondeuterated solvents, like MeOH or MeCN, and D2O, can be used in place of water. The hyphenated approach of direct-connecting LC and NMR has been revitalized by recent advancements in hardware and software. Advancements, such as new coil and flow-cell designs, aimed at enhancing sensitivity, an improved RF system catering to diverse solvent suppression needs and offering enhanced dynamic range gradient elution performance, along with automated peak-picking and storage capabilities, represent notable progress in this field. This makes the approach an effective tool that may be used on a wide range of goods, including organic compounds, natural products, biomolecules, drug degradation products, reaction mixtures, and drug impurities. The advancement of robust solvent suppression techniques, integrated with a variety of homo- and heteronuclear 2D NMR experiments, like 2D nuclear overhauser enhancement spectroscopy (NOESY) or 2D total correlation spectroscopy (TOCSY), has significantly broadened the capabilities of HPLC-NMR in structural elucidation and exploration of novel natural products. Despite being around for the previous 20 years, LC-NMR has not yet gained widespread acceptance. This is mostly due to its greater cost and lesser degree of sensitivity when compared to other hyphenated methods. But recent technological developments – particularly in the areas of solvent suppression techniques and pulse field gradients. In addition, this method has been revitalized by advancements in probe technology and the development of elevated-frequency magnets (800–900 MHz).

CE-MS

The early 1990s saw the introduction of the automated separation technique known as CE. CE analysis can separate hundreds of different chemicals quickly. It is operated via an electric field and conducted in small tubes. Since CE is so versatile and has so many applications about every type of molecule can be divided utilizing this efficient method. It operates by applying an electric potential across capillaries filled with a buffer to separate various species. It is typically used to split ions that move at varying rates when voltage is applied, based on their size and charge. As the solutes flow by the detector, they occur as peaks, with the area of each peak corresponding to their concentration, accounting for quantitative measurements. The study includes assays, trace-level estimates, and purity tests. CE-MS is the combination of an MS detector and a CE system used to obtain online MS data of an isolated molecule (Figure 5).

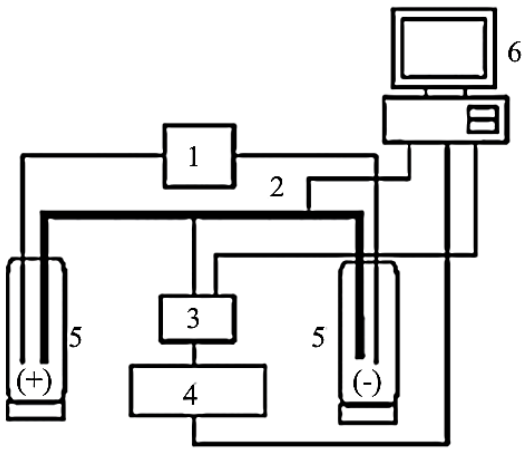

Figure 5. A typical CE-MS system: 1= High voltage supply; 2= Capillary; 3= UV-Vis or PDA detector; 4= MS detector; 5= Buffer solution; 6= PC control.

In CE-ESI-MS, the sample is delivered through capillary channels connected to a high-voltage source, enabling separation with high sensitivity and selectivity. The coaxial sheath liquid interface allows seamless switching between CE-MS and LC-MS on the same mass spectrometer, with a pump supplying sheath liquid to stabilize the ion spray and prevent current leakage. Both CE separation and ESI ionization are powered simultaneously, ensuring stable operation. ESI is widely used for biomolecule analysis, enabling molecular weight and structural characterization. However, optimizing the CE-MS interface can be challenging due to low CE flow rates (10–100 µL/min), requiring a make-up liquid for effective ionization [17–19].

ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive analytical technique used for detecting trace elements in various sample types. It combines the ionization power of an inductively coupled plasma with the detection precision of a mass spectrometer. Samples are typically prepared by converting them into liquid form through digestion methods like acid treatment. The liquid is then aerosolized and introduced into an argon plasma, which reaches temperatures around 10,000 K. This high-temperature environment atomizes and ionizes the sample, primarily producing singly charged ions. These ions are directed into the mass spectrometer, where magnetic and electric fields separate them based on their mass-to-charge ratio (m/z). Only selected ions pass through the mass analyzer – commonly a quadrupole or time-of-flight (TOF) detector – which measures ion intensity to determine element concentrations. The resulting data is processed to identify and quantify the elements present in the sample.

ICP-MS is widely used due to its exceptional sensitivity, precision, and ability to perform multi-element analysis quickly. It plays a vital role in fields such as environmental monitoring, geochemistry, pharmaceuticals, food and beverage safety, and clinical diagnostics – especially for detecting elements at ultra-trace levels (Figure 6) [20, 21].

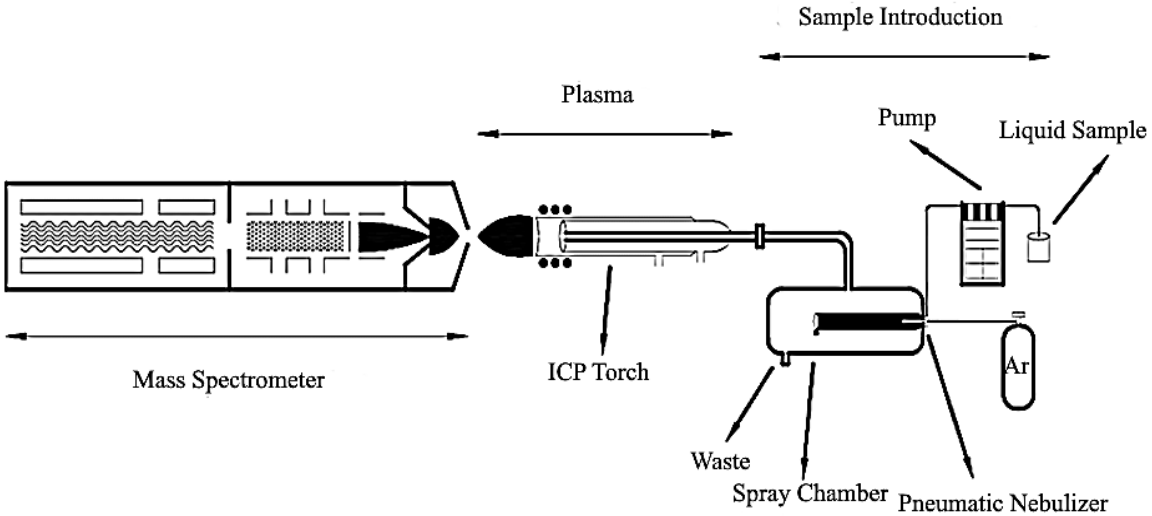

Figure 6. A typical ICP-MS [22].

APPLICATIONS OF HYPHENATED TECHNIQUES IN VARIOUS FIELDS LIKE

Environmental Analysis

- A variety of polar and non-volatile compounds can be analysed using LC-MS, which is widely used for the analysis of volatile organic compounds (VOCs) in air, water, and soil samples.
- Halogenated flame retardants and chlorinated hydrocarbons are among the volatile organic compounds that can be analysed using GC-MS in conjunction with atomic emission detection or mass spectrometry.
- While inorganic ions, such as anions and cations, are determined by IC-ICP-MS in water samples.

- LC-FLD is frequently used to determine polycyclic aromatic hydrocarbons (PAHs) and other fluorescent chemicals in environmental samples.

Pharmaceutical Analysis

- Pharmaceutical substances are often analysed using LC-MS because of its excellent sensitivity and specificity. It is useful for the detection and quantification of drug substances, impurities, and metabolites in a variety of matrices, including pharmaceutical formulations and biological samples.
- It is especially helpful for analysing tiny chemical compounds. The HPA flexible hyphenated method for the identification and separation of medications is LC-MS. LC-UV/Vis is used to quantify pharmaceutical chemicals based on their absorbance characteristics.
- It is frequently used for the study of peptides, proteins, nucleotides, and polar molecules that may not be readily accessible to GC-MS.
- CE-MS is a widely used analytical technique that evaluates the purity as well as the number of pharmacological drugs and related chemicals.
- NMR may be hyphenated with chromatographic methods like HPLC or GC to provide structural information about pharmaceutical substances. It offers high resolution and sensitivity and has proven helpful for biomolecule analysis in pharmaceutical research.

Food Science

- GC-MS is extensively utilized in the analysis of volatile substances in food such as Flavors, aromas, and contaminants. It is valuable for the identification and quantification of compounds like pesticides, flavor compounds, and fatty acids.
- LC-MS is adaptable in food analysis, enabling the separation and identification of a diverse array of compounds, including pesticides, mycotoxins, food additives, and contaminants.
- CE-MS is employed for the separation and detection of charged molecules in food, including amino acids, peptides, and organic acids.
- NMR coupled with chromatography provides structural information about food components, including identification of sugars, organic acids, and other small molecules. It is valuable for elucidating complex mixtures.

Forensic Analysis

- GC-MS is frequently employed in forensic investigation for the identification of volatile and semi-volatile compounds such as drugs, explosives, and accelerants.
- LC-MS is versatile and widely applied in forensic toxicology for the detection and quantification of drugs, metabolites, and poisons in various biological samples.
- CE-MS is employed for the separation and detection of charged molecules such as drugs, peptides, and metabolites. It offers high resolution and sensitivity and is useful in forensic toxicology and drug analysis.
- LC-MS/MS is generally used for targeted analysis of specific compounds in forensic toxicology, including drugs, pharmaceuticals, and metabolites.

Biotechnology

- LC-MS is widely utilized in studies related to proteomics and metabolomics for identifying and quantifying proteins and metabolites. It allows for the analysis of complex biological samples like blood, urine, and cell extracts.
- GC-MS is employed in the analysis of volatile compounds such as fatty acids and small metabolites.
- CE-MS is used for the separation and analysis of charged biomolecules, including peptides, proteins, and nucleic acids.
- LC-NMR integrates the separation efficiencies of liquid chromatography with the structural information provided by NMR spectroscopy.
- LC-MS/MS is applied in targeted analysis of specific biomolecules, including peptides, proteins, and metabolites.

RECENT ADVANCES AND DEVELOPMENTS IN HYPHENATED TECHNIQUES

Single Quadrupole ICP-MS (ICP-Q-MS)

Agilent Technologies introduced the ICP-Q-MS instrument in 2009 for rapid, multi-element trace analysis. It uses an inductively coupled plasma (ICP) source, reaching ~10,000°C, to ionize sample atoms, which are then filtered by a quadrupole mass analyzer. Argon or helium gas forms the plasma matrix, converting liquid samples into aerosols via a nebulizer. Ionized analytes pass through a magnetic field and an interface of nickel or platinum cones, entering a collision cell to reduce interferences. The quadrupole mass filter separates ions by mass-to-charge ratio, guiding them to an electron multiplier detector. ICP-Q-MS is widely used in biological and industrial applications for its high sensitivity, precision, and accuracy, though it is destructive and influenced by scan rates and sample handling time (Figure 7) [23].

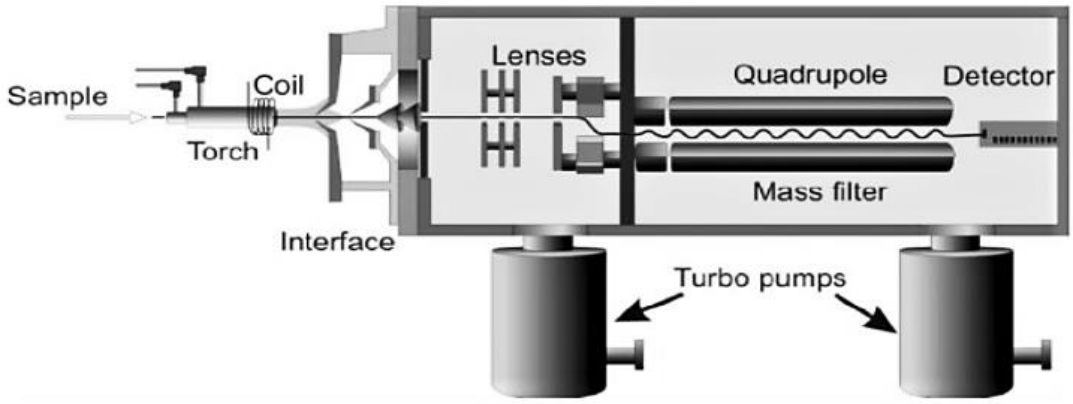

Figure 7. Schematic diagram of ICP-Q-MS [23].

Triple Quadrupole ICP-MS (ICP-QQQ)

Launched by Agilent Technologies in 2012, Triple Quadrupole ICP-MS (ICP-QQQ) is a highly efficient instrument featuring an additional quadrupole before the collision cell. It uses two quadrupole mass filters (Q1 and Q2) separated by an Octupole Reaction System (ORS) to enhance ion separation based on mass-to-charge ratio. Q1 selects target analyte ions, excluding unwanted ones, which are then fragmented using inert gases like argon or nitrogen. The resulting ions pass through Q2 for further filtering before detection. This method is widely used in trace analysis, proteomics, phosphor proteomics, and environmental testing. Its benefits include improved interference removal, enhanced detection limits, and faster analysis, though setup can be complex for users from varied technical backgrounds (Figure 8) [24, 25].

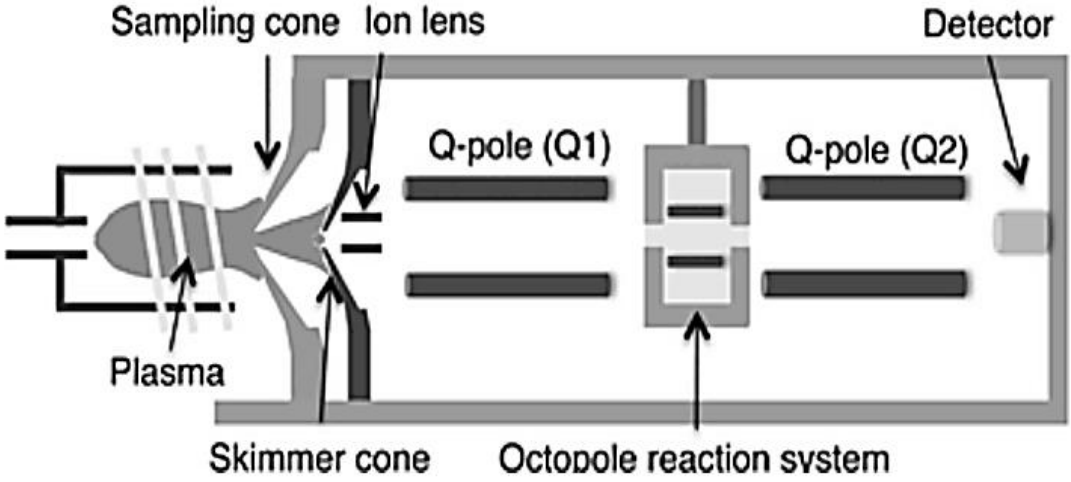

Figure 8. Schematic presentation of ICP-QQQ [24].

Quadrupole Time of Flight LCMS (QTOF-LC-MS)

Agilent Technologies introduced the Quadrupole Time-of-Flight LC-MS (QTOF-LC-MS), a high-sensitivity ionization system combining a quadrupole and a time-of-flight (TOF) analyzer. The quadrupole filters ions by mass-to-charge ratio, while the TOF measures their flight time for precise mass analysis. The system includes components such as nebulizers, a sampling capillary, a skimmer, an octupole ion guide, a collision cell, a flight tube, and a plate detector. It offers high sensitivity, accurate mass detection, rapid data acquisition, high resolving power, and fast polarity switching. QTOF-LC-MS is widely used in toxicology, food safety, environmental testing, pesticide detection, and biomolecule and protein identification (Figure 9) [26].

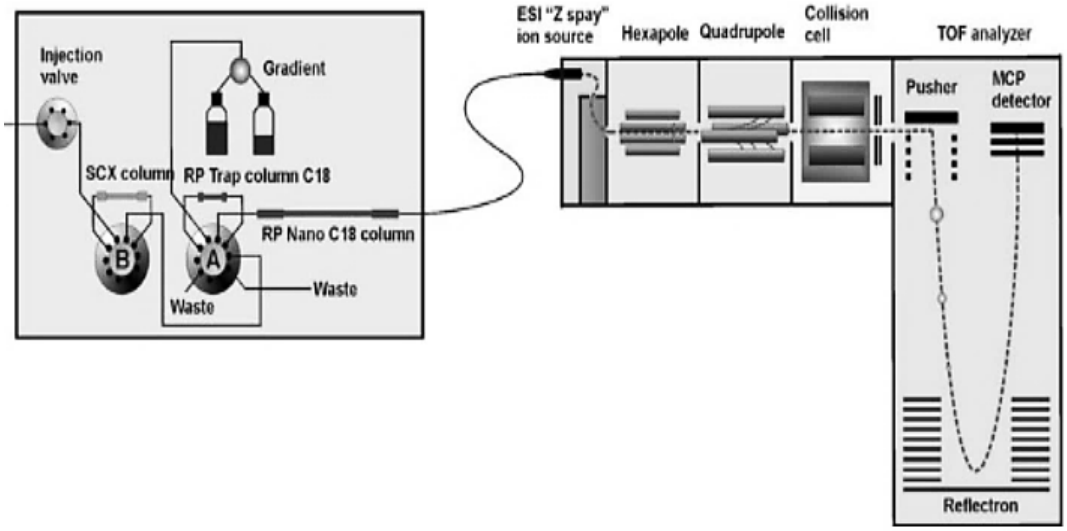

Figure 9. Schematic presentation of QTOF-LC-MS [26].

Triple Quadrupole GC-MS

Agilent Technologies introduced the Triple Quadrupole GC-MS in 2011**,** featuring two quadrupole mass analysers and a collision cell designed for collision-induced dissociation (CID). This setup enables the fragmentation of parent ions into daughter ions for enhanced specificity. Ions are generated from the GC outlet at the ion source, and the first quadrupole isolates analyte ions by removing unwanted matrix ions via vacuum pumping. A gold-plated quartz inert source maintains a high-temperature environment to prevent contamination. In the collision cell, nitrogen and helium gases are used to control ion fragmentation while minimizing metastable helium interference. Helium is removed, allowing only analyte ions to reach the second quadrupole for mass-to-charge (m/z) analysis. This technique is widely used for trace analysis in fields such as food safety, pesticide detection, environmental monitoring, forensics, chemical warfare agent detection, soil analysis, and clinical toxicology studies [27].

CONCLUSIONS

To sum up, this examination of hyphenated procedures highlights how important they are to the advancement of analytical techniques in a variety of scientific fields. Overcoming the shortcomings of individual procedures has been shown to be an effective tactic achieved via the synergistic combination of various analytical methodologies. The collective benefits of hyphenated methods have significantly expanded the scope and depth of analytical applications and scientific investigations. The applicability of hyphenated approaches is apparent in their capacity to improve sensitivity, selectivity, and efficiency in a variety of fields, including pharmaceutical research, forensic investigations, and environmental studies. These integrated techniques have yielded more accurate and dependable findings due to their complete insights, which have also enabled a better comprehension of complicated sample matrices. Improved resolution, more analytical power, and the capacity to examine a wide variety of chemicals

in a single analytical run are some benefits of hyphenated procedures. Many hyphenated systems have automation and integration capabilities that improve data dependability, repeatability, and efficiency. Hyphenated approaches seem to have a bright future as long as technology keeps developing. Continued advancements in data processing, instrumentation, and technique optimisation will probably allow these integrated systems to function even better. Scholars can expect hyphenated methodologies to continue evolving, providing fresh approaches to analytical problems and broadening the purview of science. Essentially, hyphenated methods are complementary and comprehensive, making them essential instruments in contemporary analytical chemistry. The combined understanding that results from their use advances scientific understanding as well as the creation of solutions that have an influence on a range of industries, including healthcare, environmental monitoring, and beyond. Without a doubt, hyphenated technique development and investigation will be crucial in determining the direction of analytical science in the future.

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