International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(REVIEW ARTICLE)

Check for updates

Hyphenated technologies: Transforming analytical approaches with combined methodologies

Daram Sushma Reddy *, Unnava Jaya Surya, Utukuri Satish, Thulugu Dhanalakshmi, Yatavelli Koushit Reddy and Yanamala Gopi Krishna

Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Dhulapally,500100, Hyderabad, Telangana.

International Journal of Science and Research Archive, 2025, 14(01), 1431-1444

Publication history: Received on 12 December 2024; revised on 18 January 2025; accepted on 21 January 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.1.0210

Abstract

The hyphenated technique was developed by combining a separation strategy with an on-line spectroscopic detection technology. Currently, a combination of a separation approach with a more sensitive detection technique is the most used method for determining the speciation of trace elements. Before the addition of a detection approach, such hyphenated techniques involved coupling the separation of a unique sample preparation offline. Currently, a separation method (chromatography) and an online spectroscopic detection method are combined to create the hyphenated technique. Over the past two decades, hyphenated analytical methods have undergone amazing advancements that have greatly expanded their applicability in the analysis of biomaterials, natural products, elemental species, explosives, trace elements, and other things. This article discusses recent developments in the use of various hyphenated techniques, such as GC-MS, LC-MS, LC-FTIR, LC-NMR, etc. in a variety of fields, including forensic science, the environment, biotechnology, the various hyphenated techniques, a brief note on their instrumentation and working principles that are used in the current setup of industries. Like-wise their remarkable improvement and efficiency over the past decade. Techniques like single quadrupole inductively coupled mass spectrometry (ICP-Q-MS), ICP- Triple quadrupole mass spectrometry (ICP-QQQ), Liquid Chromatography-Two -dimensional Gas chromatography, mass spectrometry (LC-GCXGC-MS/MS), Two-dimensional liquid chromatography (2D-LC), Fourier transform near-infrared spectroscopy (FT-NIR), etc. are considered as recent improvements in this trend—geography, and pharmaceuticals, among others, with the use of relevant examples.

Keywords: Hyphenated Technique; GC-MS, LC-MS; LC-FTIR; LC-NMR; Separation Technique; LC-MS; FT-IR; Chromatographic Techniques; Mass Spectrometry

1. Introduction

Hirschfield in 1980 presented the expression "hyphenation" to indicate the consolidation of chromatographic and spectral techniques and hence use the benefits of both. Chromatography produces almost unadulterated divisions of sample segments from the blend. Spectroscopy produces particular data for identification using standards or reference library spectra. Lately, hyphenated procedures attained considerably more important, ever-expanding and evergreen technique in the tackle of complex expositive issues. It created intensive changes in qualitative and quantitative analysis of chemical and biological substances. It also created an impact on microanalysis. When the two different principles are joined together creates more benefits to the users in the investigation of obscure mixes in complex regular item concentrates or divisions. The previous literature revealed the development in the hyphenated techniques up to the previous decade. Hence the present paper aims to update the scientific community on further development. During the last literature, there are five equipments and its related hyphenated principle with its applications were discussed in detail.

* Corresponding author: Daram Sushma Reddy.

Copyright © 2025 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

To take advantage of each method's advantages, hyphenated approaches combine chromatographic and spectroscopic methods. Chromatography is used to extract pure or almost pure fractions of a mixture's chemical constituents. Utilising standards or library spectra, spectroscopy generates selective information for identification. Hirschfeld first used the term "hyphenation" to describe the online fusion of a separation technique and one or more spectroscopic detection techniques in 1980, which was a few decades earlier. The hyphenated methodology combines or couples two distinct analytic methods with the aid of an appropriate interface. The emerging discipline of hyphenated techniques known as multi-dimensional chromatography offers some significant advantages for pharmaceutical analysis. For the analysis of a wide range of sample types, various setups including the coupling of GC, HPLC, and CE systems together in diverse combinations have been examined. Size exclusion chromatography coupled with RP-HPLC, CE, and GC coupled with LC are a few examples. Combining RP-HPLC with CE methods in a two-dimensional mode can provide exceptionally high peak capacities and extremely high resolving power, which is particularly beneficial for complex mixtures. These techniques are both capable of high-resolution separation with orthogonal separation mechanisms. The hyphenation does not always have to be between two techniques; more recently, so-called double hybrid instruments, such as LC-PDA-MS, LC-MS-MS, and LC-NMR-MS instruments have become accessible and have been used to solve pharmaceutical problems. Online coupling with solid phase extraction (SPF), solid phase micro extraction, or large volume injection can be used to create a more potent integrated system, such as SPF-LC-MS, LVI-GC-MS, in situations where the trace element is crucial and the analyte enrichment is crucial.

1.1. Types of hyphenated techniques 5-6

- Double hyphenated techniques.
- Triple hyphenated techniques.
- Double hyphenated techniques

    - LC-MS
    - GC-IR
    - GC-MS
    - LC-NMR
    - LC-IR
    - CE-MS

1.2. Triple hyphenated techniques

- LC-API-MS
- APCI-MS-MS
- ESI-MS-MS
- LVI-GC-MS
- LC-ESI-MS
- LC-NMR-MS

1.3. Advantages of hyphenated technique [7-8]

The ability to identify species other than the expected compounds is the main benefit of hyphenated speciation approaches. This has been discovered to be particularly true in the fields of drug development, biochemistry, and biotechnology, as well as the examination of drinking water and wastewater.

- Shorter analysis time
- Higher degree of automation
- Higher sample throughput
- Better reproducibility
- Reduction of contamination because it is a closed system
- Enhanced combined selectivity and therefore higher degree of information.

1.4. Hyphenated techniques

1.4.1. GC-MS: [9-10]

The 1950s saw advancements in the use of mass spectrometers as detectors in gas chromatography, after the 1952 discovery by James and Martin. Quadrupole-based GC/MS devices are among the most important tools for organic analysis and have become indispensable for chemical research. Automated GC/MS equipment is widely used for food

safety, agricultural regulation, the development of new medications, and environmental monitoring of soil, water, and air. The two main parts of the GC-MS system are the mass spectrometer and the gas chromatograph. The sample is driven through the column by a carrier gas, including argon, helium, nitrogen, or hydrogen, among others. As the gas chromatograph progressively isolates the constituents of a mixture, the mass spectrometer delivers data that facilitates the structural identification of each constituent.

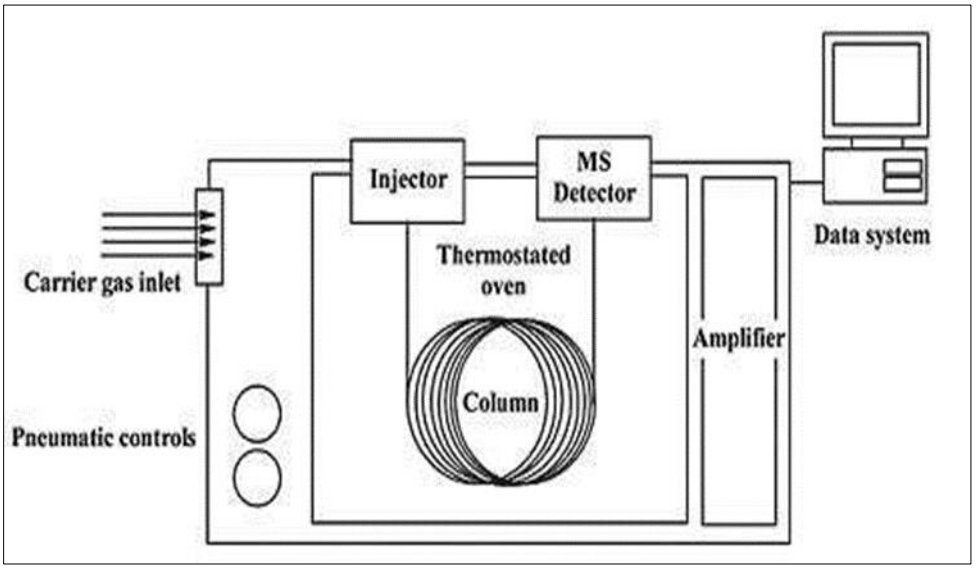

Figure 1 GS-MS

GC-MS columns are classified into two categories: capillary columns and packed columns.

Concerning the GC-MS interaction, it is essential to meticulously assess the following elements.

- The interface efficiently transfers the effluent from the gas chromatograph to the mass spectrometer.
- Analyte condensation at the interface must be absent.
- The analyte must not decompose before entering the mass spectrometry ion source.
- The MS's pumping capacity must accommodate the gas influx into the ion source.

1.4.2. LC-NMR[11-12]

NMR provides the most useful structural information while being perhaps the least sensitive spectroscopic technique currently in use. Online HPLC and NMR coupling provide the advantages that no other hyphenated method can. Rapid acquisition of a large amount of structural data from the materials. Liquid chromatography (LC) and NMR were directly connected in 1978 using a stop-flow approach. The primary detector that maintains LC operating is a UV-Vis detector. For a typical HPLC-NMR connection, magnetic field strengths greater than 9.4 T or a 1H resonance frequency of 400 MHz are advised. The LC unit in an LC-NMR system usually includes

- Autosampler,
- The LC pump,
- The column
- Non-NMR detectors, such as UV, DAD, and EC

The detector is usable as long as it doesn't alter or damage the sample. The large radiofrequency (RF) magnet that has been fitted with a vertically oriented, non-rotating flow cell makes up the NMR apparatus. This arrangement allows for laminar flow and facilitates the removal of bubbles from the mobile phase. As the RF coil is wrapped around the cell to get an appropriate filling factor, the glass that comprises the flow cell is the sole component that varies between the detection volume and coil volume.

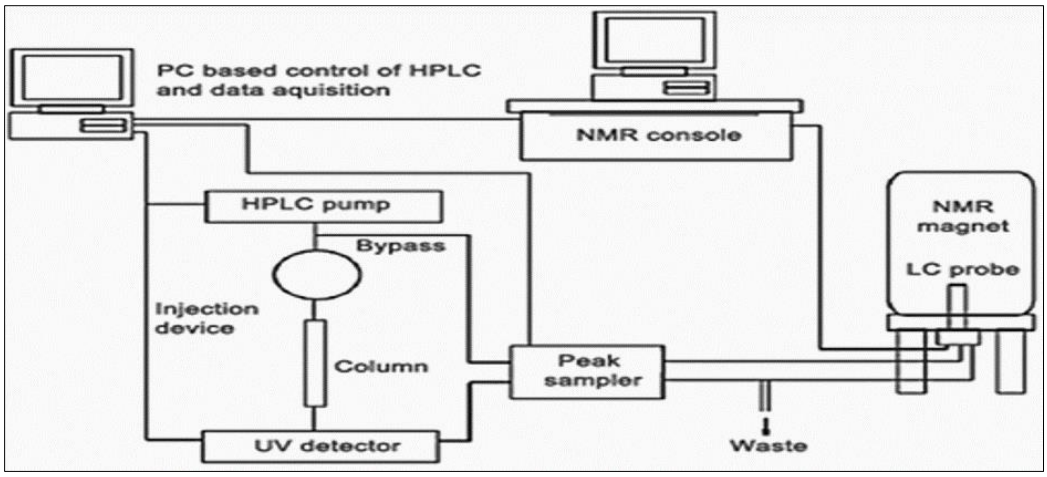

Figure 2 LC-NMR

1.4.3. LC-MS [13-14]

A technique known as liquid chromatography-mass spectrometry combines mass spectrometry for mass analysis with liquid chromatography for separation. Degradation products and contaminants may be isolated utilizing

HPLC, and we can identify them and ascertain their molecular weight using mass spectrometry. Both sensitivity and selectivity are quite high in LC-MS technique. LC-MS is called specific because it can identify and detect substances even in the presence of other molecules.

In quantitative analysis, a variety of techniques are used, which may be roughly categorized as

- Chemical/classical Techniques (Titrimetric, Volumetric, and Gravimetric Techniques)
- Instrumental Techniques (Spectrophotometry, Polarography, HPLC, GC)

The LC-MS equipment has particle beam, thermospray, and electrospray interfaces. Electrospray is a widely used interface. Mass spectrometry and liquid chromatography are connected via the spray needle. However, the independent emitter is useful and adaptable. Chromatography, interface, and spectrometry are the three parts of LC-MS. Liquid chromatography separates the samples, which are then detected using photodiode arrays, ultraviolet, fluorescent, and other detectors. These separate parts were then added to the interface. After being delivered to the MS, the liquid volatilizes at the point of contact. A mass spectrometer examines the molecule after it has been ionized using a number of ionization techniques. Quadrupoles are among the several mass analyzers used.

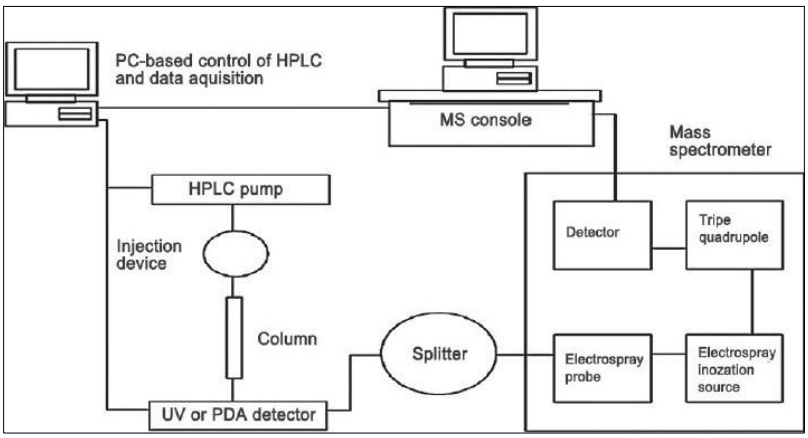

Figure 3 LS-MS

LC-MS or HPLC-MS refers to the coupling of an LC with a mass spectrometer (MS). The separated sample may be identified using the mass spectrum data that comes out of the column. The two approaches may be combined practically

with the help of a switching valve. A typical automated LC-MS system consists of a mass spectrometer, an LC system, and a double three-way diverter in line with an autosampler. The diverter usually acts as an automated switching valve to reroute undesirable elute segments from the LC system to garbage before the sample reaches the MS.

1.4.4. LC-FTIR[15-16]

LC-IR, or HPLC-IR, refers to the hyphenated technology that arises from the integration of liquid chromatography (LC) with infrared spectrometry (IR) or Fourier-transform infrared spectroscopy (FTIR) as a detection method. Although HPLC is among the most efficient separation techniques available, IR or FTIR serves as a valuable spectroscopic approach for the identification of organic compounds.

Compounds exhibit several absorption bands in the mid-infrared range, which are indicative of certain functions, such as -OH and -COOH. The extensive absorption bands of the mobile phase solvent in the hyphenated approach within the mid-IR region often obscure the minor signals generated by the sample components, rendering the integration of HPLC and IR difficult and impeding advancements in this methodology.

Furthermore, infrared detection is much less sensitive than other technologies, including ultraviolet detection. Recent advancements in HPLC-IR technology have included two essential methodologies based on interfaces used in HPLC-IR or HPLC-FTIR. One method involves a solvent-elimination procedure, while the other employs a flow-cell approach. Analogous to UV-Vis and other prevalent HPLC detectors, the technique is used with the flow cell in LC IR. In this case, mobile phase absorption obstructs the identification of sample component absorption bands, but a clear mid-IR region facilitates detection. The collection of sample components in the eluent is often performed using KBr or KCl salts, and the volatile mobile phase solvents are eliminated from the medium before IR detection. The diffuse-reflectance infrared Fourier transform (DRIFT) approach and the buffer-memory technology provide two distinct interfaces for the solvent elimination procedure. Furthermore, a unified interface for GC, HPLC, and SFC hyphenation to FTIR via the IR microscopic technique currently exists.

1.4.5. LC-IR [17-18]

By combining liquid chromatography (LC) with infrared spectrometry (IR) or Fourier transform infrared (FTIR), a detection method known as the hyphenated approach LC-IR or HPLC-IR was developed. Even though high-performance liquid chromatography (HPLC) is now one of the most successful separation techniques, infrared (IR) or Fourier transform infrared (FTIR) is also a useful instrument because the structures of organic compounds often include several absorption bands that are representative of functionality, such as -OH, -COOH, and other functions for example. Combining high-performance liquid chromatography (HPLC) with infrared spectroscopy (IR) is a tough endeavor, and the development of this hyphenated technique is especially delayed. This is because the mobile phase solvent's 237 absorption bands are so broad in the mid-IR region that they often obscure the little signal that is generated by the components.

- For this reason, the LC-IR hyphenation was used in order to develop an appropriate apparatus that is capable of producing whole mid-infrared spectra.
- Get rid of the solvent without causing the vacuum system to become too pressurized or heating the analytes, which might be harmful.
- Make certain that the spectrometer receives an effective transmission of the analyte.
- To prepare the FT-IR, deposit the analytes in a thick layer.
- Ensure that the chromatographic resolution is well preserved.

1.4.6. CE-MS [19-20]

The public first saw the automated separation technique known as CE at the beginning of the 1990s. An electric field drives the analysis of CE in tiny tubes. It has the capacity to swiftly separate hundreds of different chemicals from one another. The efficiency of CEs allows for the separation of almost all compounds using this method.

It offers a wide range of applications and is highly adaptable. Generally speaking, it is used to separate ions that, when voltage is provided, move at varying speeds, depending on the size and charge of the ions. The application of voltage via buffer-filled capillaries allows for the separation of species. In the process of moving through the detector, the solutes manifest themselves as peaks. The fact that the area of each peak is proportional to the concentration of the solute makes it possible to conduct quantitative measurements from this perspective. Analyte characterization is being performed. Optimal performance in the interface between CE and MS may pose a challenge, as CE necessitates flow rates ranging from 10 to 100 milliliters per minute, achieved through the use of a make-up liquid.

1.4.7. GC-FTIR [21-22]

As a confirmation technique for the identification of organic compounds, infrared spectroscopy is considered to be a confirmation tool due to the fact that infrared spectra are unique for organic molecules that are quite similar to one another. It is possible to collect infrared spectra from the peaks as they elute from the capillary columns by using gas chromatography in conjunction with the capillary columns.

Through the use of transform Fourier infrared spectrometry, the separation capabilities of gas chromatography in conjunction with the identification capabilities of infrared spectroscopy are brought together.

1.4.8. TLC-MS [23-24]

In combination with mass spectrometry (MS), which is widely considered to be among the most efficient analytical techniques for clarifying structural connections, thin-layer chromatography (TLC) will be used. A great number of unique TLC-MS techniques have been recorded in the literature up to this point, and some of these processes are already available for purchase. The existing TLC-MS systems are capable of being segmented by dividing them into two distinct categories according to the operating methods that they follow.

- Indirect, in which the analyte on the TLC plate is scraped, extracted, purified, and concentrated before being directed into the mass spectrometer's ion source for additional analysis; and
- Direct, in which the analyte on the TLC plate is characterized.
- Directly through mass spectrometry without the need for extraction, concentration, or scraping procedures.

2. Enhancements to hyphenated instruments

2.1. Single Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-Q-MS)

ICP-Q-MS, also known as Single Quadrupole ICP-MS, which Agilent Technologies introduced the ICP MS instrument when it was first introduced in the year 2009 [25]. It is this instrument considered as the quickest equipment for establishing technique in the multi-component trace investigation? Across the globe, this device is used in greater quantities in a variety of sectors for a variety of purposes. Producing a high-temperature plasma source of around 10,000 degrees Celsius, from which the sample is transferred, is the fundamental premise behind the operation of the ICP-Q-MS procedure.

Once the atoms inside the sample have been ionized, they are subsequently sent to the mass spectrometry instrument. This MS system makes use of a mass filter system that is based on quadrupole research [26]. In liquid and solid samples, conduct a rapid and trace examination (~1000 ppm). The inductively coupled plasma is the primary source of excitation that is used in ICP-Q-MS to ionize the sample.Argon and helium are two examples of gases that are often used as plasma matrixes for the purpose of retaining samples.

After that, the ionized atoms are separated in the quadrupole, and the mass filter assists in the separation and transmission of only selected samples, which are analyte ions with a characteristic mass-to-charge ratio (m/z). These ions are then sent to the detector. In the presence of an electric field, the whole process of the ions being transferred to the detector is carried out. Figure 4 depicts the schematic representation of the representation.

Through the use of a nebulizer or a pump, the sample is permitted to enter in the form of a liquid. Through the use of argon gas, the nebulizer can transform the liquid mixture into very small droplets or aerosols. The highly ionized vapor created by the reaction between the argon gas and the sample will flow through a quartz glass and a strong magnetic field. The analytes that had been ionized were transferred to an interface that was situated between two conical disks that were constructed of either stainless steel or platinum. This is done to restrict the arrival of analyte particles into the collision cell to just those particles. The collision cell is where the ions are transferred to. The collision reaction cell is responsible for the separation of positive ions from neutral ions and photons, which not only reduces the beam widening but also eliminates polyatomic interferences. Ions will be transferred to the quadruple-tipped mass analyzer in the subsequent step of the process. Four parallel rods make up the quadrupole mass analyzer. Two of the rods have a negative charge, while the other two rods have a positive charge. The use of alternating current (AC) and direct current (DC) voltage, which may be raised or decreased, is what is used to acquire the intensity of the electromagnetic field. Because this field is responsible for the paths of the ions, it is only the analyte that has a certain mass-to-charge ratio (m/z) that is permitted to pass through to the detector. The electron multiplier detector is where the analyte ions are sent after being passed through. High sensitivity, superior chromatographic detection, outstanding precision, and

accuracy are just some of the numerous benefits that come with using ICP-Q-MS. High control of interferences, as well as analysis that is both quick and quick. Some of the limitations include the fact that it is a destructive approach, the fact that the speed of analysis may be slowed down by quadrupole scanning rates, and the amount of time that is required for washing the samples and introducing the samples. [27]: As well as other applications in the medical field, the ICP-Q-MS is primarily utilized for the characterization of atomic and polyatomic species in plasma. It is also utilized for trace analysis in bones, tissue, and blood, mineral detection in rocks, soils, and fossils, determination of toxins in the environment, and detection of metals in proteins or enzymes.[25] In the year 2009, Agilent Technologies introduced the Single Quadrupole ICP-MS (ICP-Q-MS) ICP MS equipment to the market worldwide. This instrument is considered the quickest equipment for creating techniques in the multi-component trace investigation. Across the globe, this device is used in greater quantities in a variety of sectors for a variety of purposes. Producing a high-temperature plasma source of around 10,000 degrees Celsius, from which the sample is transferred, is the fundamental premise behind the operation of the ICP-Q-MS procedure.

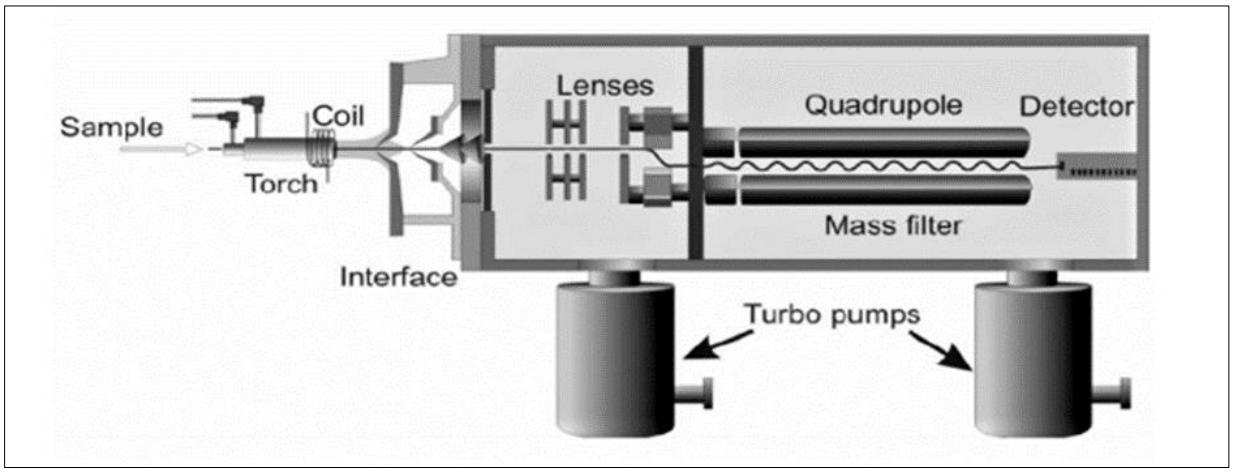

Figure 4 Schematic diagram of ICP-Q-MS

2.2. Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-QQQ)

The triple quadrupole ICP-MS was introduced by Agilent Technologies in 2012[ ]. This apparatus is more efficient than the standard single quadrupole ICP-MS due to the extra quadrupole positioned before the collision cell. The primary principle involves the utilization of a tandem mass spectrometer (MS/MS), which effectively selects ions based on their mass-to-charge ratio through two quadrupole mass analyzers, referred to as Q1 and Q2, separated by a collision reaction cell, also known as the Octupole Reaction System (ORS).

The triple quadrupole ICP-MS has an extra quadrupole system compared to the single quadrupole ICP-MS. The instrument schematic diagram is seen in Fig. 5. The Q1 mass filter regulates the target analyte while excluding extraneous ions, thereby directing the selected ions into the collision cell. Any ions lacking the requisite mass-to-charge ratio (m/z) will be prohibited from traversing Q1.

The ions are next transferred to the collision cell, where they are broken into daughter ions by inert gases such as nitrogen or argon.

Subsequently, the fragmented ions traverse to the second Quadrupole, where they are reselected based on their mass-to-charge ratio (m/z) before being sent to the detector. The positives include enhanced efficiency in trace analysis, ease of fitting and disconnecting interference, significantly better detection limits, rapid operation, and reduced time consumption. Conversely, the downsides include the complexity of instrument setup for laboratory workers from diverse backgrounds [28]. This approach is mostly used in sectors such as ultra-trace analysis, Quantitative proteomics, phosphoproteomics, and environmental analysis.

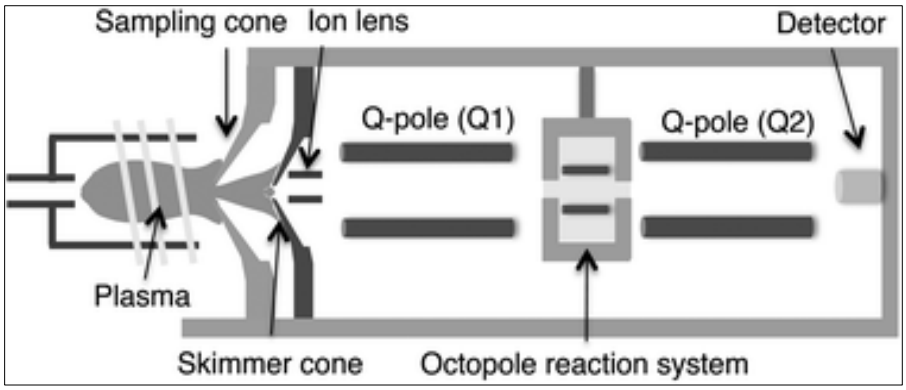

Figure 5 Schematic representation of ICP-QQQ

2.3. Quadrupole Time-of-Flight Liquid Chromatography Mass Spectrometry (QTOF-LC-MS)

The Quadrupole Time-of-Flight LCMS has been introduced & Authored by Agilent Technologies [29]. It is an advanced ionization method that provides enhanced sensitivity.

This device operates on the premise of using two mass analyzers: Quadrupole and Time of Flight (TOF). The quadrupole filters sample ions according to their mass-to-charge ratio, while the time-of-flight (TOF) analyzer detects the ions' flight duration. After traversing the reflectron, the ions are directed to a plate detector, where the ion signals are transformed from electrons to photons and then back to electrons before reaching the detector.The advantages include elevated sensitivity, enhanced mass accuracy, ultrahigh resolving power, swift data collecting rates, and quick polarity switching[30].

Toxicological analysis Its many applications include food safety, environmental analysis, pesticide identification, characterisation, quantification of biomolecules, and the confirmation and identification of proteins, all of which are facilitated by QTOF-LC-MS.The sample acquired from the LC system is directed via the nebulizer to generate tiny droplets, thereafter entering the MS system, and is then transferred to a second nebulizer for enhanced mass accuracy. The high-volume drying gas counterflow reduces noise, after which the ions are sent via a sampling capillary. Additionally, a skimmer aperture is used to mitigate beam widening. Subsequently, they are sent via an octupole ion guide, where the lenses enhance ion transmission and improve sensitivity over a broader mass range. The sample is thereafter sent to the first mass analyzer, the Quadrupole. Figure 6 illustrates the many components of the system. Quadrupole mass filters let the passage of just target ions with precise mass. Subsequently, the precursor ions enter the collision cell, where they produce product ions and neutral fragments, which are sent to a slicer that optimizes the ion beam for enhanced sensitivity and improved mass accuracy. The sample is then introduced into the flight tube, where the reflectron adjusts for variations in velocity [31-32]

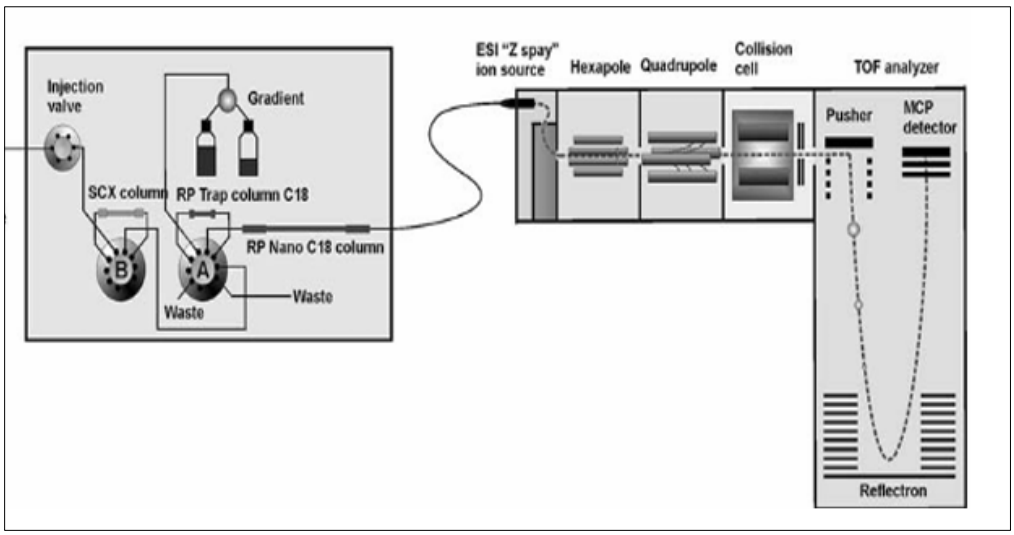

Figure 6 Quadrupole time of flight LCMS (QTOF-LC-MS)

2.4. Orbitrap Fusion LC-MS (Tribrid MS) MS (Tribrid LC)

The MS (Tribrid LC-MS) system, namely the Orbitrap Fusion LC-MS, was introduced by Thermo Fisher Scientific in 2013[33]. This constitutes a fusion. The LC-MS system integrates three mass analyzers: Quadrupole, Orbitrap, and Linear Ion Trap, providing comprehensive analysis of complex analytical samples. The operational concept involves trapping, injection, excitation, and a rapid mass analyzer, which has two exterior electrodes and a focal terminal, enabling it to function as both samples. The operational concept involves trapping, injection, excitation, and detection. It is a particle trap mass analyzer that consists of two exterior electrodes and a focal terminal, enabling it to function as both an analyzer and a detector.The hardware comprises an ion source, lens, active beam guide, quadrupole mass analyzer, Orbitrap, dual pressure linear ion trap filter, ion routing multipole, and a detector, as seen in Fig.7. Particles entering the Orbitrap are captured by "electrodynamic crushing," thereafter oscillating around the focal anode and between the two exterior electrodes [34].

Diverse particles oscillate at distinct frequencies, resulting in their separation. By calculating the oscillation frequencies generated by particles on the external electrodes, the mass spectra of the particles are obtained by image current detection. The quadrupole consists of four parallel metallic rods. Each pair of opposing rods is electrically interconnected, and a radio frequency (RF) voltage, accompanied by a direct current (DC) offset voltage, is applied between one rod pair and the other. Ions traverse the quadrupole between the rods based on their distinct m/z ratio. The linear ion trap analyzer retains ions along a predetermined linear path [35]. Its primary benefit is the enhanced ability for ion trapping. This allows a wider scope and enhanced quantification.The advantages include superior and efficient performance, ultrahigh resolution of up to 500,000 full width at half maximum (FWHM) that eliminates interferences, exceptional selectivity and sensitivity achieved through the simultaneous use of three analyzers, precise determination of the detailed structure of small molecules, high accuracy, and precision, and enhanced sensitivity for comprehensive analysis of low-abundance proteins [36]. This approach is used in domains including glycoproteomics analysis, peptide fragmentation pattern determination, ultra-trace analysis, multi-elemental analysis, protein analysis, and drug metabolite identification.

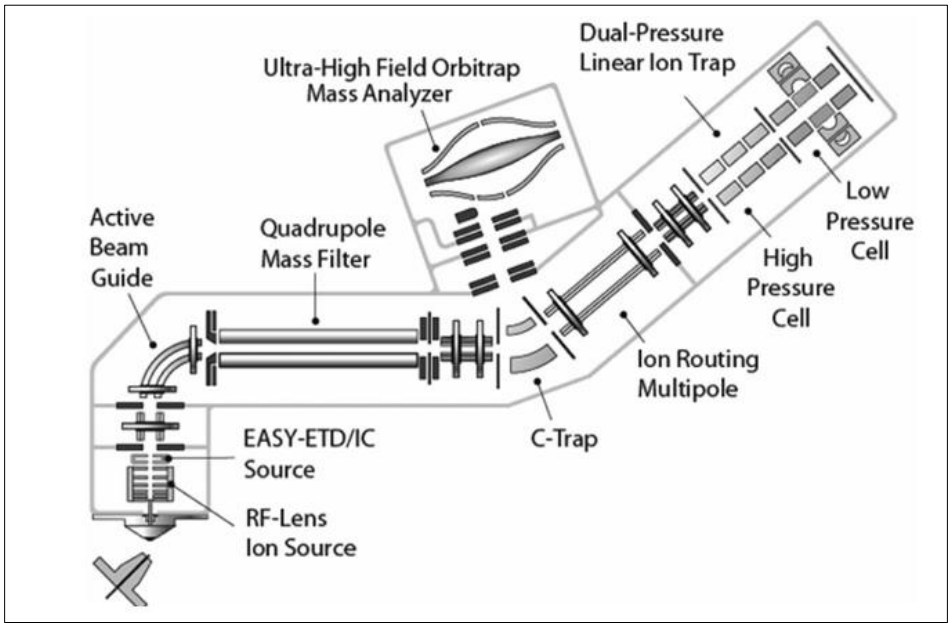

Figure 7 Diagram of Tribrid LC-MS instrumentation

2.5. LC-GCXGC-MS/MS (5D Ultra-e)

The Shimadzu Company introduced LC-GCXGC-MS/MS (5D Ultra-e) (5D Ultra-e) in 2013 [37], In Italy, Luigi Mondello works at the College of Messina and Cromaleont S. r. l. This device uses a special method that combines a triple quadrupole mass spectrometer, an HPLC system, and complete two-dimensional gas chromatography. When the HPLC and 2D-GC systems are used together, the automation is improved, increasing power and productivity. Target analysis of constituents in several complex analytes is made possible by the triple quadrupole mass spectrometer. The analyte may be separated from any complicated matrix using the HPLC. They may be configured especially for certain analytes. After being extracted from the LC, the eluent—which only contains the necessary target 63—is injected into the GC

system components. Since there are no loops or valves, any amount of sample may be injected. Effective temperature programs get rid of the solvent residues.

2.5.1. Eluted parts are added to the GC apparatus.

Compared to the one-dimensional GC system, the two-dimensional GC system separated components more effectively. After that, they are sent through the very effective MS/MS, which uses a triple quadrupole technology to accurately read the analytes by analyzing their mass-to-charge ratio [38]. All of these connected systems are then controlled as a single unit using 5D integrated software. Benefits include the following: LC and 2D-GC make complex sample characterization much simpler and provide more detailed results; high speed and high sensitivity; the triple quadruple MS system provides specific analysis within trace concentrations; large volumes of samples can be injected; simple software controls all integrated systems with a single click; powerful analysis of trace components; no need for liquid nitrogen; and high accuracy and precision [39-40]. The primary use is in the thorough examination of sulfur compounds and coal tar.

2.6. 2D-LC (Comprehensive Two Dimensional Liquid Chromatography)

Shimadzu introduced Dimensional Liquid Chromatography, or 2D-LC, in 2014 [41].

Two separate separation phases are utilized in two-dimensional chromatography, a kind of chromatographic technique, to extract the injected sample [42]. Two separate chromatographic columns are connected in succession, and the material from the main framework is transferred to the next column. This is the basic idea of 2D LC. Groups that are ineffectively resolved from the initial column may be completely segregated in the second column because the latter column often contains an additional separation component. Four components make up the two-dimensional liquid chromatography system: pumps, autosampler, columns, and detector. Figure 6 shows this system's schematic depiction. An auto sampler is essentially a robotic device that either transports the sampling apparatus to the trays with the other samples or transports the samples to the sampling station.

It is then identified after being transmitted to the separating column. The procedure is then carried out once again, this time pumping the first column's effluent into the second column and detecting it [43]. UV detectors, mass spectrometers, fluorescence detectors, and refractive index detectors are the types of detectors that are used. The result is a two-dimensional chromatogram. Benefits include high peak capacity increase, high resolving power, the ability to resolve first-dimension peak overlap, the ability to separate closely related compounds, and the ability to separate combinations that one-dimensional LC is unable to separate efficiently. Its primary drawback is undoubtedly the very lengthy timeframe (often a few to many hours) of comprehensive 2D-LC. Among the uses of 2D-LC include chiral analysis, trace analysis, impurity profiling, herbal medicine, bioanalysis, food testing, etc.

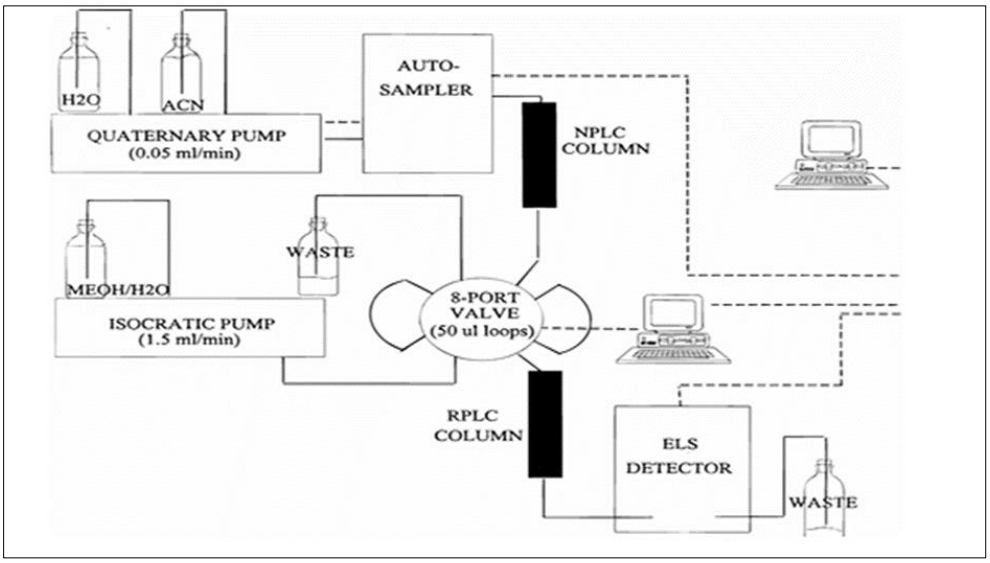

Figure 8 Schematic representation of 2D-LC

2.7. Fourier transform near infrared spectroscopy, or FT-NIR, [44-45]

FT-NIR was introduced by Thermo Fisher Scientific in 2011. The overtone and combination bands of the near-infrared spectrum, which include wavelengths between 12,000 and 4,000, exhibit mid-infrared absorption frequencies.

These bands provide the vibrations of the atoms that comprise the material. This is largely because various materials cannot create the same near-infrared Fourier transform since each substance is made up of a distinct and exact atomic combination. The main purpose of the transform near-infrared spectroscopy technique was to overcome the limitations of the dispersive NIR apparatus. Because this method examines all infrared wavelengths at once, it takes less time. The frequencies contained in them are already present in an interferogram that this spectrometer uses. The Fourier transform method is then used to translate it into a frequency vs. intensity spectrum. The primary difference between the two FT NIRs is that the latter uses a QTH (quartz, tungsten, halogen) source that is more adapted for the near infrared spectrum, whilst the former uses a silicon carbide source. However, the process is the same for both instruments. An example of the instrument in operation is shown in Fig. The source is a halogen light source, and a beam splitter splits the radiation it generates into two, transforming the beam into an interferometer. The beam is reflected into the beam splitter by two mirrors, one stationary and the other movable, before being mixed and sent to the detector.

The constructive or destructive joining of the two beams changes the optical path. An interferogram is subsequently found. The data collected by the interferogram, which uses a mathematical process to produce an infrared spectrum, is referred to as the Fourier Transformation. The instrument's mechanical simplicity, non-destructive nature, low sample preparation requirements, reliability, robust mechanism, extreme accuracy, and precision, lack of optical throughput degradation, internal calibration errors, quick method, ability to analyze multiple components simultaneously, and cost-effectiveness are among its advantages. The interferometer is controlled by an internal reference laser. The food sector finds the technology very intriguing, and it may also be used for rapid chemical analysis and raw material identification.

Both methods use the same methodology. The operational representation of the instrument. An interferometer is used as the beam source, and it features a beam splitter that splits the radiation into two halves. The beam is reflected into the beam splitter, where it is mixed and sent to the detector, using two mirrors, one stationary and the other movable. The constructive or destructive joining of the two beams modifies the optical path. Following that, an interferogram is found. The Fourier data collected by the interferogram, which generates an infrared picture, is referred to as transformation. An internal reference laser controls the interferometer. The device's advantages include being mechanically destructive, requiring no sample preparation, being very accurate and precise, without compromising optical throughput, having an internal calibration system that reduces mistakes, being quick, producing findings that can be replicated, and analyzing a wide range of components. One of its other uses is as a rapid raw material, which is appealing to the food industry.

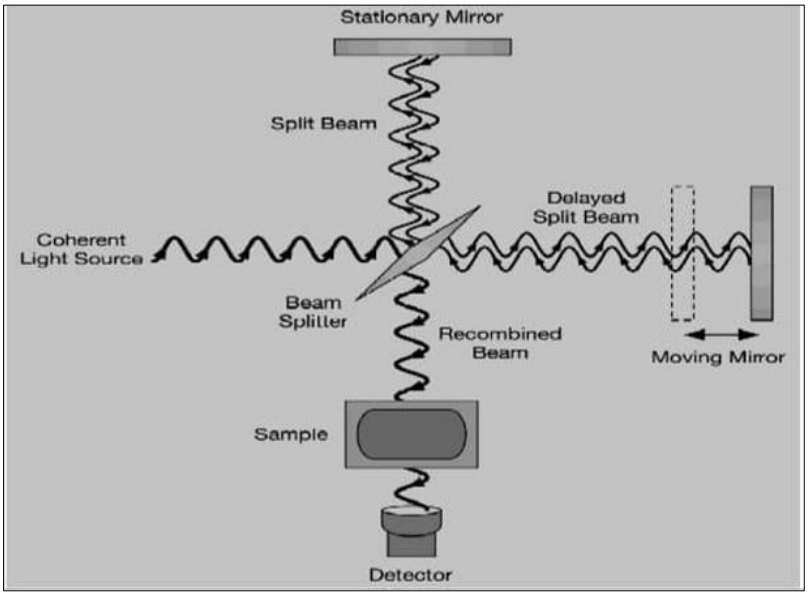

Figure 9 Working of FT-NIR

2.7.1. Applications of the hyphenated technique: [46-47]

- Identifying the byproducts of drug breakdown.
- Low-level pollutants may be recognized and differentiated.
- This technique is used to monitor organic contaminants, pesticides, and herbicides in the environment.
- Characterization and isolation of peptide libraries
- Combinatorial chemistry, photochemical analysis, and drug discovery
- Identification of drug impurities
- Acid glucuronide isomers and vitamin A derivatives are described
- Direct identification of endogenous and xenobiotic compounds produced from body fluids
- The combination of LC-NMR and LC-MS
- Evaluation of polymers
- LC-NMR enabled isomer separation and identification without reference compounds.
- Drug metabolism (for the analysis of biofluids like urine or plasma); a) 19F (a selective tracer with little background); NMR detection of drugs containing 19F is very clean and selective. b) Following the administration of a therapeutic dosage of ibuprofen, we identified the molecules of unmetabolized ibuprofen, carboxy-ibuprofen, and 2 hydroxyibuprofen in a small urine sample. (A micro-coil NMR probe with an active capacity of three microliters was used.)
- Nine closely eluting and isomeric aporphine alkaloids from the Taiwanese plant genus Lit aria were found using 50 times less material than conventional NMR studies using 5 mm tubes.
- Two instances of how LC-NMR provides quick multiparametric information on microbial biotransformation are the discovery of new warfarin metabolites from Streptomyces rimosus and the identification of the antibiotic azithromycin from broth supernatants of Streptomyces citric lour.
- Identification and separation of chiral compounds: The CH3CN:D2O ratio of 7:13 is used to identify and characterize the photo isomer of azadirachtin, a powerful pesticide extracted from neem tree seeds.
- Using LC-NMR-MS, vitamin E analogs present in palm oil extract have been identified.
- By analyzing crude extracts of natural compounds and chemicals derived from plants, the method is intended to rapidly discover potential drug candidates in plant products.
- The data from LC-NMR/MS, which offers thorough characterization of all impurities present in the medicine, makes it feasible to identify bulk drug contaminants during drug stability testing.
- Analysis of chemicals that are unstable or produced on-site but cannot be identified or isolated using current techniques.
- Examining biofluids, such as plasma or urine, to learn more about the metabolism of medications. NMR can easily detect drug residues like 19F, which are linked to prescription drugs.
- For ring-fused and heterocyclic compounds with few protons, the LC-2D-NMR method provides carbon shift and bonding information, which is very useful for structural analysis.
- Composition profiling, which looks at the arrangement and makeup of the constituents in a chemical combination and provides valuable data for the development of a production process in the chemical sector.
- The great resolution of LC-NMR enables the analysis of the microstructures of biopolymers, including proteins, and synthetic polymers.
- Researching the complex, unknown, non-living natural organic matter (NOM) found in soil, sediments, the seas, and the atmosphere. 25 LC-NMR and LC-SPE-NMR have been used to investigate the dissolved NOM from freshwater and alkaline soil extracts to separate and characterize the components in the complex combination. the identification of medical disorders via the use of metabolomics and bodily fluid analysis.

3. Conclusion

Advancements in hyphenated technologies have markedly improved analytical precision and efficiency in several disciplines, including medicines, environmental monitoring, and biochemistry. These approaches offer exceptional precision and adaptability in assessing complex mixtures by integrating separation and detection methodologies, facilitating advancements in drug discovery, environmental safety, and material characterisation. This study emphasizes the transformational influence of hyphenated procedures and their increasing significance in tackling complex analytical issues. As these approaches advance, they are poised to enhance social development by promoting innovation, assuring safety, and facilitating sustainability. Future endeavors should concentrate on enhancing these technologies and expanding their applicability in developing scientific and industrial fields.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Patel KN, Patel JK, Patel MP, Rajput GC, Patel HA. Introduction to hyphenated techniques and their applications in pharmacy. Pharm Methods. 2010, 1(1):2-13. Available from: https://doi.org/10.4103/2229-4708.72222.

[2] Wilson ID, Brinkman UA. Hyphenation and hypernation: The practice and prospects of multiple hyphenation. J Chromatogr A. 2003, 1000:325-56.

[3] Szpunar J, Lobinski R, Prange A. Hyphenated techniques for elemental speciation in biological systems. Appl Spectrosc. 2003, 57(3):102A-12A.

[4] Joshi RR, Gupta KR, Patil SS. Hyphenated technique—a boon to the analytical world. IJPSR. 2012, 3(11):4184-91.

[5] Patel KN, Patel JK, Patel MP, Rajput GC, Patel HA. Introduction to hyphenated techniques and their applications in pharmacy. Pharm Methods. 2010,

[6] James AT, Martin AJP. Gas-liquid partition chromatography: The separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. Biochem J. 1952, 50:670-80.

[7] Herderich M, Richling E, Roscher R, Schneider C, Schwab W, Humpf HU. Application of atmospheric pressure ionisation HPLC-MS-MS for the analysis of natural products. Chromatographia. 1997, 45:127-32.

[8] Jeyaprakash. (2024). Recent Advances and Developments in Hyphenated Techniques and their Applications.

[9] Sweedler JV. The continued evolution of hyphenated instruments. Anal Bioanal Chem. 2002, 373:321-2.

[10] Albert K. Hyphenated techniques. Anal Bioanal Chem. 2002, 372:25-6.

[11] Bruins AP, Covey TR, Henion JD. Ion spray interface for combined liquid chromatography-atmospheric pressure ionization mass spectrometry. Anal Chem. 1987, 59:2642-6.

[12] Chassaigne H, Lobinski R. Hyphenated techniques in speciation analysis. Analusis. 1997, 25:M37-M40.

[13] Agnes GR, Horlick G. Developments in plasma source mass spectrometry. Appl Spectrosc. 1994, 48:649-54.

[14] Agnes GR, Stewart II, Horlick G. Advances in elemental speciation analysis. Appl Spectrosc. 1994, 48:1347-59.

[15] Kajiwara H. Developments in chromatography. J Chromatogr. 1991, 559:345-56.

[16] Richards MP. Biomedical applications of chromatography. J Chromatogr B. 1994, 657:345-55.

[17] Sasaki TA, Wilkins CL. Gas chromatography with Fourier transform infrared and mass spectral detection. J Chromatogr A. 1999, 842:341-9.

[18] Wolfender JL, Ndjoko K, Hostettmann K. The potential of LC-NMR in phytochemical analysis. Phytochem Anal. 2001, 12(1):2-22.

[19] Lim CK, Lord G. Current developments in LC-MS for pharmaceutical analysis. Biol Pharm Bull. 2002, 25(5):547-57.

[20] Corcoran O, Spraul M. LC-NMR-MS in drug discovery. Drug Discov Today. 2003, 8(22):1021-2.

[21] Pan C, Liu F, Ji Q, Wang W, Drinkwater D, Vivilecchia R. The use of LC/MS, GC/MS, and LC/NMR hyphenated techniques to identify a drug degradation product in pharmaceutical development. J Pharm Biomed Anal. 2006, 40(3):581-90.

[22] Lianghai H, Mingliang Y, Xiaogang J, Shun F, Hanfa Z. Advances in hyphenated analytical techniques for shotgun proteome and peptidome analysis—a review. Anal Chim Acta. 2007, 598:193-204.

[23] Szpunar J, Lobinski R, Prange A. Hyphenated techniques for elemental speciation in biological systems. Appl Spectrosc. 2003, 57(3):102A-12A.

[24] Huffman SW, Lukasiewicz K, Geldart S, Elliott S, Sperry JF, Brown CW. Analysis of microbial components using LC-IR. Anal Chem. 2003, 75(17):4606-11.

[25] Lee DY, Fiehn O. High-quality metabolomic data for Chlamydomonas reinhardtii. Plant Methods. 2008, 4:7.

[26] Fiehn O. Extending the breadth of metabolite profiling by gas chromatography coupled to mass spectrometry. Trends Analyt Chem. 2008, 27(3):261-9.

[27] Potter D, McCurdy E. ICP-MS. 2009.

[28] Wilschefski SC, Baxter MR. Inductively coupled plasma mass spectrometry: Introduction to analytical aspects. Clin Biochem Rev. 2019, 40(3):115.

[29] Thermo Fisher Scientific. Inductively coupled plasma mass spectrometry (ICP-MS) information. Thermo Fisher Scientific, 2020.

[30] Sakai K. Benefits of the Agilent 8900 ICP QQQ with MS/MS operation for routine food analysis. 2016, 12.

[31] Agilent Technologies. Introduction of accurate mass Q-TOF LC/MS system. Technology Networks, 2020. Available from: https://www.technologynetworks.com.

[32] Agilent Technologies. 6545 Quadrupole time-of-flight LC/MS system. Agilent, 2020.

[33] Quadrupole spectrometry: Time-of-flight mass—a paradigm shift in toxicology screening applications. Clin Biochem Rev. 2019.

[34] Brief A, Testing H. Why LC/MS/MS and GC/MS/MS are required for the analysis of certain pesticides. n.d., 6–9.

[35] Thermo Fisher Scientific launches world's first Tribrid LC-MS. n.d. Available from: http://www.ionsource.com.

[36] Thermo Fisher Scientific. Orbitrap LC-MS. Available from: https://www.thermofisher.com.

[37] Thermo Fisher Scientific. Orbitrap Fusion™ Tribrid™ mass spectrometer—Product specifications. Thermo Fisher Scientific, 2015.

[38] Jiang X, Blank M, Drew D, Delanghe B. Optimized search strategy to maximize PTM characterization and protein coverage in proteome discoverer software. Thermo Fisher Scientific, 2016.

[39] Finlayson B, Obeid M. 5D-ULTRA e. 2016. Available from: https://doi.org/10.1080/205818312016.1156347.

[40] Shimadzu Corporation. 5D Ultra-e: Features. Available from: https://www.shimadzu.com.

[41] Shimadzu Corporation. Shimadzu releases comprehensive 2D chromatography e-Series: The Nexera-e and the Ultra-e. Available from: https://www.shimadzu.com.

[42] Jones O. Two-dimensional liquid chromatography: Principles and practical applications. Germany, Singapore: Springer, 2020.

[43] Shimadzu Corporation. Nexera-e: Applications. Available from: https://www.shimadzu.com.

[44] Szpunar J, Lobinski R, Prange A. Hyphenated techniques for elemental speciation in biological systems. Appl Spectrosc. 2003, 57(3):102A-12A.

[45] Huffman SW, Lukasiewicz K, Geldart S, Elliott S, Sperry JF, Brown CW. Analysis of microbial components using LC-IR. Anal Chem. 2003, 75(17):4606-11.

[46] Corcoran O, Spraul M. LC-NMR-MS in drug discovery. Drug Discov Today. 2003, 8(22):1021-2.

[47] Pan C, Liu F, Ji Q, Wang W, Drinkwater D, Vivilecchia R. The use of LC/MS, GC/MS, and LC/NMR hyphenated techniques to identify a drug degradation product in pharmaceutical development. J Pharm Biomed Anal. 2006, 40(3):581-90.