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HYPHENATED TECHNIQUES: AN OVERVIEW

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ABSTRACT

The hyphenated technique is developed from the coupling of a separation technique and an online spectroscopic detection technology. The remarkable improvements in hyphenated analytical methods over the last two decades have significantly broadened their applications in the analysis of biomaterials, natural products, elemental species, explosives, trace elements and so on. In this article, recent advances in the applications of various hyphenated techniques, e.g., GC-MS, LC-MS, LC-FTIR, LC-NMR etc in the different areas like forensic science, environment, biotechnology, geography, pharmaceutical etc. are discussed with appropriate examples.

KEYWORDS: Hyphenated technique, GC-MS, LC-MS, LC-FTIR, LC-NMR, separation technique.

INTRODUCTION

Advances in both chemistry and technology are making new techniques available and expanding the use of existing ones. The number of existing techniques has been combined to expand the utility of component methods. Separation of mixtures by chromatographic processes is a central part of analytical and preparative chemistry. The direct conjugation of chromatographic technique with spectroscopic examination of separated fraction constitutes several powerful analytical techniques. Chromatography - Produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy – Produces selective information for identification using standards or library spectra. A couple of decades ago, Hirschfield introduced the term "hyphenation" to refer to the on-line combination of a separation technique and one or more spectroscopic detection techniques. This technique, developed from a marriage of a separation technique and a spectroscopic detection technique, is nowadays known as hyphenated technique (Figure 1).

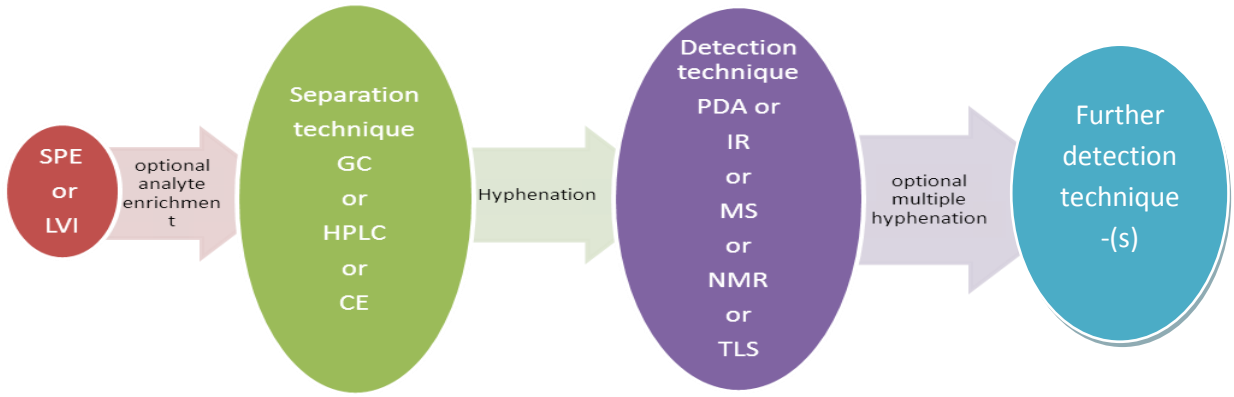

Figure 1: Hyphenated technique.

The hyphenation does not always have to be between two techniques; the coupling of separation or detection techniques, more recently, so called double hybrid e.g. LC-PDA-MS, LC-MS-MS, LC-NMR-MS instruments have become available and have been applied to pharmaceutical problem solving. Where the trace element is vital and the analyte enrichment is essential, online coupling with solid phase extraction (SPF), solid phase micro extraction or large volume injection can be incorporated to build in a more powerful integrated system e.g. SPF-LC-MS, LVI-GC-MS.[1]

This is more clearly represented by Figure 2.[2]

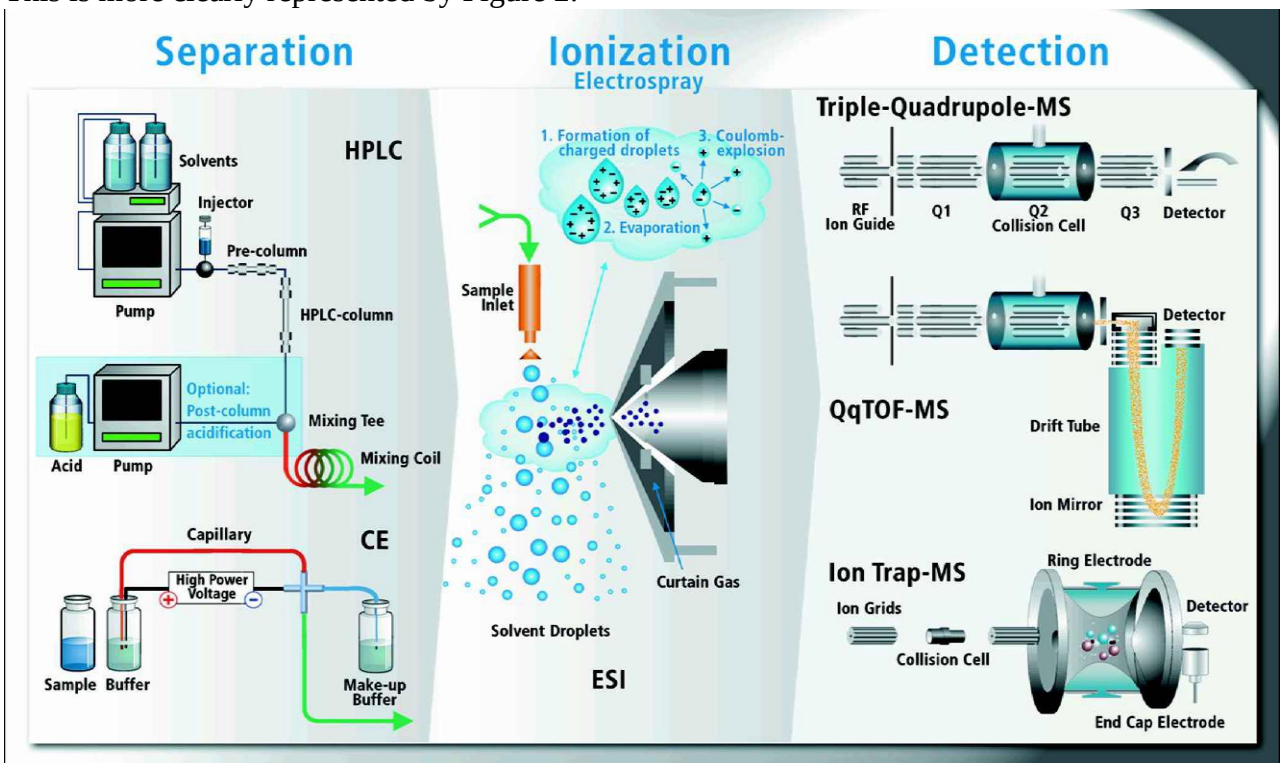

Figure 2: Hyphenated techniques (LC-ESI-MS, CE-ESI-MS).

Advantages

These hyphenated techniques offer

1. Shorter analysis time
2. Higher degree of automation
3. Higher sample throughput
4. Better reproducibility
5. Reduction of contamination because it is a closed system
6. Enhanced combined selectivity and therefore higher degree of information

Hyphenated Techniques[3, 4]

1. GC-MS

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952.[5]

GC/MS instruments using quadrupole technology had become both essential to chemical research and one of the foremost instruments used for organic analysis. Today computerized GC/MS instruments are widely used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine.

The GC-MS is composed of two major building blocks: the gas_chromatograph and the mass spectrometer (Figure 3).

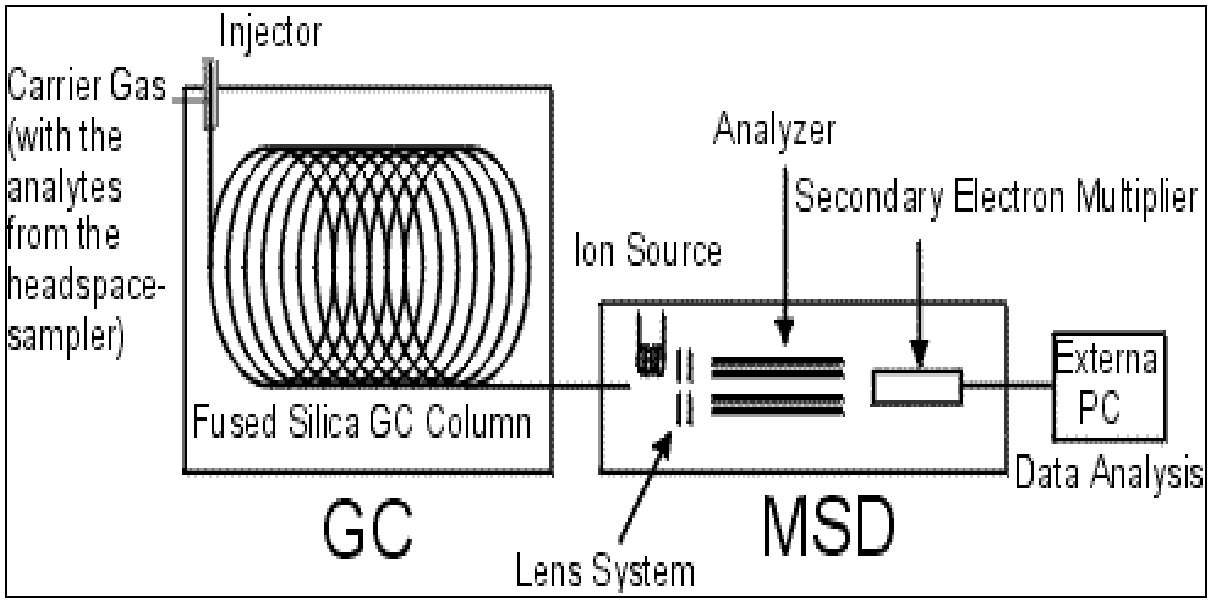

Figure 3: Gas Chromatography- Mass Spectroscopy.

In GC-MS, a sample is injected into injection port of GC device, vaporized & separated in the GC column. The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off of) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.

The equipment used for GC-MS generally consists of an injection port at one end of a metal column (often packed with a sand-like material to promote maximum separation) a detector (MS) at the other end of the column, a carrier gas (argon, helium, nitrogen, hydrogen, to name a few) propels the sample down the column. The GC separates the components of a mixture in time and MS detector provides information that aids in the structural identification of each component.

LC-MS

LC-MS is a chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry.

A typical automated LC-MS system (Figure 4) consists of double three-way diverter in-line with an Auto sampler, LC system, the Mass spectrometer. The diverter generally operates as an automatic switching valve to divert undesired portions of elute from the LC system to waste before the sample enters the MS. The ionization techniques used in LC-MS are generally soft ionization techniques that mainly display the molecular ion species with only a few fragment ions.[6] The information obtained from a single LC-MS run, is not sufficient for confirmation of identity of compound.[7] Nevertheless, this problem has now been resolved by the introduction of tandem mass spectrometry (MS-MS), which provides fragments through collision-induced dissociation of the molecular ions produced. Use of LC-MS-MS is increasing speedily day by day.[8]

Hyphenated techniques such as HPLC coupled to UV and mass spectrometry (LC-UV-MS) have been proved to be extremely useful in combination with biological screening for a rapid survey of natural products.

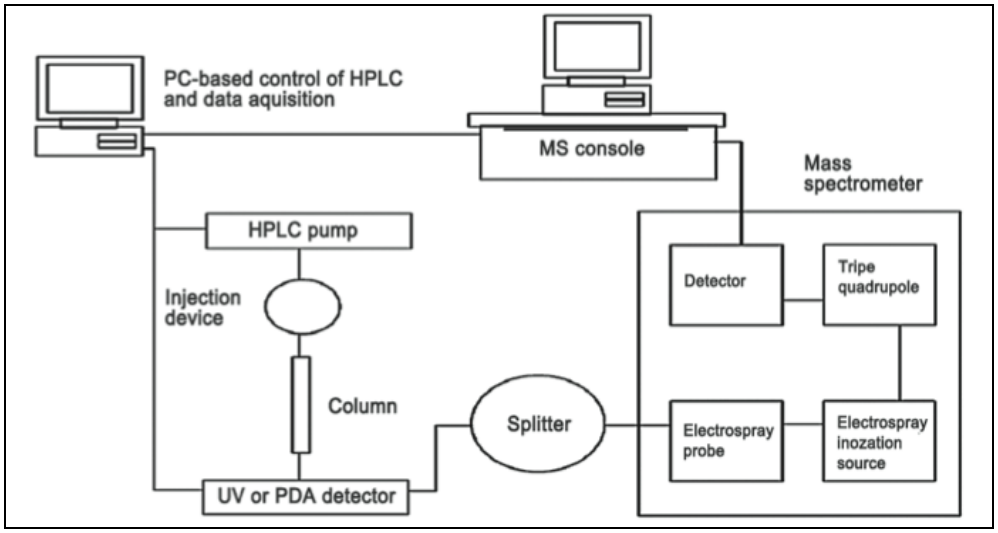

Figure 4: Liquid chromatography- Mass spectroscopy.

Nowadays, various types of LC-MS systems incorporating different types of interfaces are available commercially. The interfaces are designed in such a way that they offer adequate nebulization and vaporization of the liquid, ionization of the sample, removal of the excess solvent vapor, and extraction of the ions into the mass analyzer. The two most widely used interfaces, especially in relation to natural product analysis, are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The latter is considered as "the chromatographer's LC-MS interface" because of its high solvent flow rate capability, sensitivity, response linearity, and fields of applicability. With these interfaces, various types of analyzers, e.g., quadrupole, ion trap, or TOF, can be used. Each of these analyzers, however, offers varying degree of mass accuracy and resolution. In the LC-UV-MS mode, thermospray (LC-TSP-MS) and continuous-flow FAB (LC-CF-FAB) interfaces can also be applied. For phytochemical analysis, the TSP has been found to be the most suitable interface as it allows introduction of aqueous phase into MS system at a flow rate (1-2 ml/min) compatible with that usually used in phytochemical analysis.

LC-NMR

Among the spectroscopic techniques available till date, NMR is probably the least sensitive and yet it provides the most useful structural information. The online combination of HPLC and NMR offers the potential for rapid collection of detailed structural information from the samples in a way no other hyphenated technique can. Direct coupling of liquid chromatography (LC) to NMR (Figure 5) using stop flow method was reported in 1978.[9, 10]

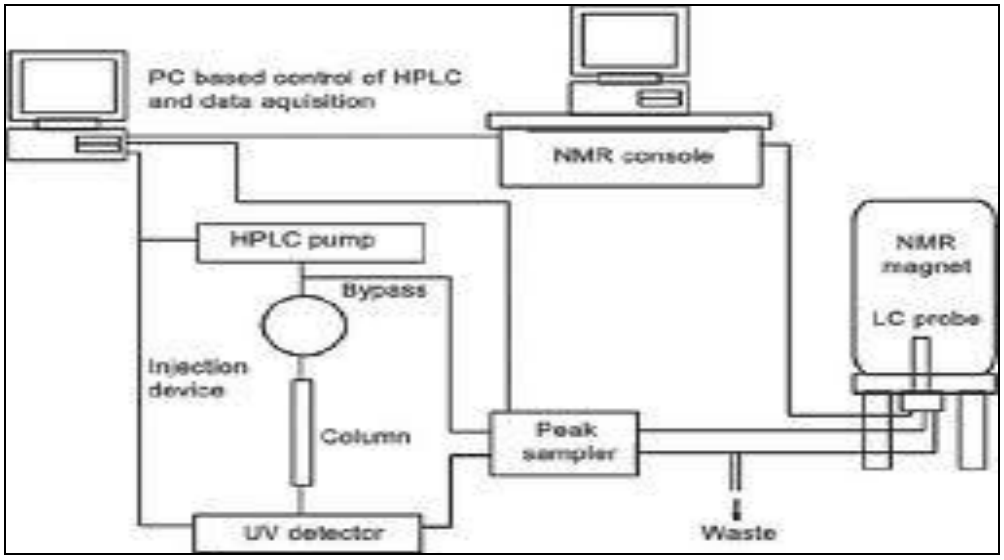

Figure 5: Liquid chromatography- Nuclear Magnetic Resonance.

LC-NMR experiments can be performed in both continuous-flow and stop-flow modes. A wide range of bioanalytical problems can be addressed using 500, 600, and 800 MHz systems with 1H, 13C, 2H, 19F, and 31P probes. The main prerequisites for on-line LC-NMR, in addition to the NMR and HPLC instrumentation, are the continuous-flow probe and a valve installed before the probe for recording either continuous-flow or stopped-flow NMR spectra.[11] A UV-VIS detector is also used as a primary detector for LC operation. Magnetic field strengths higher than 9.4 T are recommended, i.e., 1H resonance frequency of 400 MHz for a standard HPLC-NMR coupling.

New technical developments are also occurring which, in the foreseeable future, will provide greatly increased NMR sensitivity. These developments include the use of higher magnetic field strengths and hence observation frequencies. In addition, the other development of NMR probes and preamplifiers cooled with cryogenic liquids will provide lower detection limits and higher sensitivities to a degree surpassing any arising from increase in magnetic field.[12-14]

LC-IR

The hyphenated technique developed from the coupling of an LC and the detection method infrared spectrometry (IR) or FTIR is known as LC-IR or HPLC-IR (Figure 6). While HPLC is one of the most powerful separation techniques available today, the IR or FTIR is a useful spectroscopic technique for the identification of organic compounds, because in the mid-IR region the structures of organic compounds have many absorption bands that are

characteristic of particular functionalities, e.g., -OH, -COOH, and so on. However, combination of HPLC and IR is difficult and the progress in this hyphenated technique is extremely slow because the hyphenated technique's 237 absorption bands of the mobile phase solvent are so huge in the mid-IR region that they often obscure the small signal generated by the sample components.[15] Because FT-IR is an absorbance process, the geometry of the sample during the measurement process matters. For a fixed mass or volume of analyte, reducing the diameter by a factor of two creates a deposit with four times the thickness and four times the optical density. Because the IR detector is total light limited, this deposit diameter reduction of two improves the signal-to-noise ratio by four times. Therefore, to achieve a useful instrument that produces full mid-infrared spectra, the LC–IR hyphenation process must.[16]

1. Remove the solvent without thermally damaging the analytes or overloading the vacuum system with diluent gas.
2. Have efficient transmission of analytes to the spectrometer.
3. Present analytes to the FT-IR in a thick deposit.
4. Preserve the chromatographic resolution.

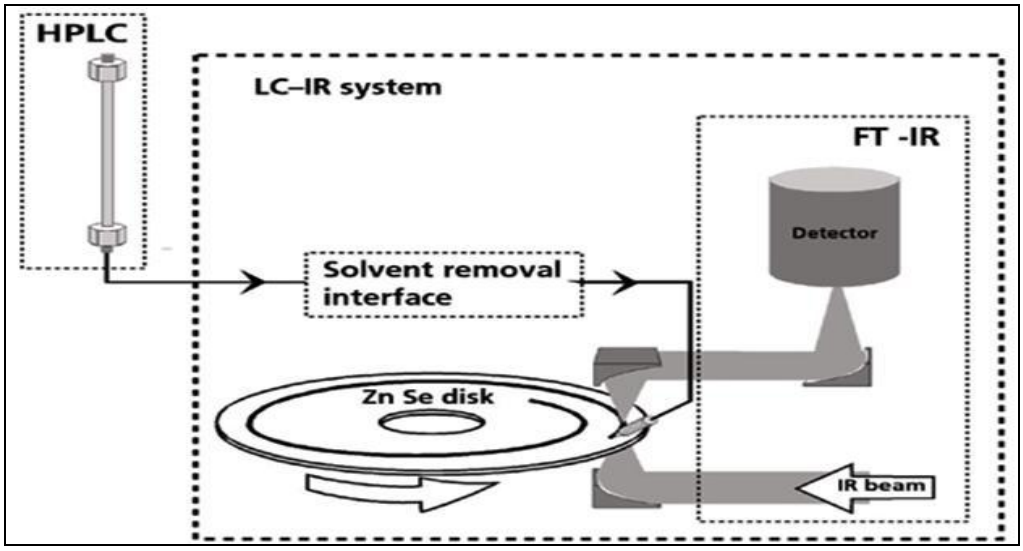

Figure 6: Liquid Chromatography- Infra red Spectroscopy.

Generally, KBr or KCl salts are used for the collection of sample components in the eluent, and heating up the medium before IR detection eliminates the volatile mobile phase solvents. There are two types of interfaces for the solvent-elimination approach: diffuse-reflectance infrared Fourier transform (DRIFT) approach and buffer-memory technique.[17, 18] A unified

interface for GC, HPLC, and SFC hyphenation to FTIR applying IR microscopic technique is also available today.[19]

Applications in different fields

Study	Rationale	Method used	Refere-nce
Metabolic Profiling	To separate and evaluate in *vivo* metabolites of HIV-1reverse transcriptase inhibitor 5-chloro-1-(2′,3′-dideoxy-3′-fluoro-*erythro*-pentofuranosyl)uracil.	LC-NMR-MS	20
Phytochemical Analysis	To analyze plant metabolites in crude extracts or in enriched fractions are outlined	LC-NMR	21
Metabolic Profiling	To study *in vitro* and *in vivo* drug metabolism, identification and characterization of impurities in pharmaceuticals, analysis of chiral impurities in drug substances.	LC-MS	22
Elemental speciation	To determine organometallic compounds, identification of unknown biomolecules and study of biochemical reaction mechanism.	LC/CE-ICP-MS	23
Microbiological detection	To separate, isolate, and identify the cellular components consisting of proteins, glycoproteins, phospholipids, fatty amides and acids, and genomic materials	LC-IR	24
Drug discovery	To identify novel candidate drugs from diverse complex mixtures within a drug discovery strategy.	LC-NMR-MS	25
Degradation study	To identify a drug degradation product, which will grew over time in the stability study of the drug product and obtains complimentary information for structure elucidation of the unknown.	LC-MS, GC-MS, LC-NMR	26
Protein components	To develop novel Hyphenated analytical techniques to meet the need of large scale analysis as huge number of proteins encoded by the genome.	LC-MS	27
Metabolic Profiling	To study control of metabolism in a photosynthetic organism by using hyphenated method improvement focusing on three parameters: quenching and cell disruption, extract solvent composition and metabolite annotation on The green eukaryote alga *Chlamydomonas reinhardtii.*	GC-TOF	28
Metabolic Profiling	To focus on biological problems rather than on methodological advances.	GC-MS	29
Impurity profiling	To apply LC-MS in conjunction with mechanism-based stress studies in the elucidation of process impurity structure in betamethasone 17-valerate drug substance.	LC-MS	30
Metabolism and Pharmacokinetic profiling	To describe the major hyphenated chromatography-Mass spectrometry techniques to study drug metabolism and pharmacokinetics *in vitro* and *in vivo* and metabolite identification profiles.	LC-MS/MS, GC-MS/MS	31
Environmental analysis	To determine multi-residues of four classes of widely used antibiotics in pig farms, sulfonamides, fluoroquinolone, tetracycline and chloramphenicol from	SPE-LC-MS/MS	32

	samples of swine wastewater and environmental water collected from two pig farms.		
Biomedical Analysis	To analyze quantitatively a small molecules of dried blood spot sample for screening inherited metabolic disorders.	LC-MS/MS	33
Survey	Emphasis on chemometric background correction and other applications of chemometric algorithms used to improve the sensitivity and the resolution.	LC-IR	34
Environmental analysis	To analyze the presence of micropollutants such as pharmaceuticals in seawater and estuarine water samples collected in the Belgian coastal zone.	LC-MS/MS	35
Environmental analysis	To determine perfluorochemicals (PFCs) and fluorotelomer alcohols (FTOHs) in plants from biosolid-amended fields.	LC-MS/MS and GC-MS/MS	36
Applications to different field	To analyze the samples for study in Geochemical research, Forensic (arson, explosives, drugs, unknowns), Environmental analysis, Pesticide analysis and Food safety , Pharmaceutical and drug analysis , Clinical toxicology, Food and fragrance, High end research GC-MS, Service and institution GC-MS analysis	GC-MS	37
Impurity Profiling	To review various approaches for impurity profiling of pharmaceuticals.	LC-MS, GC-MS, ICP-MS, LC-NMR, CE-MS	38
Metal species analysis	To analyze the species as arsenic, antimony, and thallium in different clinical, environmental and food matrices.	ICP-MS, HPLC-ICP-MS,	39
Metabolic Profiling	To study metabolites of Vitamin D from serum by using Solid Phase Extraction.	LC-MS/MS	40

CONCLUSION

The remarkable improvements in hyphenated analytical methods over the last two decades have significantly broadened their applications in the analysis of compounds. In this article, recent advances in the applications of various hyphenated techniques, e.g., GC-MS, LC-MS, LC-FTIR, and LC-NMR along with applications of hyphenated techniques in the different fields like Environment, Forensic, Pharmaceuticals, and Petrochemicals etc. are discussed with appropriate examples.

Hyphenated techniques such as LC-MS, GC-MS, LC-NMR, CE-MS and ICP-MS have been developed to solve various complex analytical problems in different fields. These techniques solve such problems in time efficient manner. Sample requirement for hyphenated technique is less as compare to conventional techniques.

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