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REVIEW ARTICLE

A Comprehensive Review on Hyphenated Techniques in Pharmaceutical Analysis

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ABSTRACT
Hyphenated techniques are analytical methods that combine spectroscopic or spectrometric methods with chromatographic methods to analyze various kinds of biological, chemical, and toxicological substances. The stability of the active pharmaceutical ingredient in bulk drugs & pharmaceutical products can be examined using a technique called stability indicating assay. The drug's active ingredient is isolated and resolved from contaminants. A combination of spectroscopic detection techniques and chromatographic separation may be employed in a stability indicating study. The separated components of the mixture from the chromatographic approach will then enter the spectroscopic method through an interphase. The technique introduced are LC-MS, GC-MS, CE-MS, FFF-MS are MS based technique and LC-NMR, CE-NMR, HPLC-NMR are NMR based technique are discussed in this review.
Keywords: *Stability, Impurity, Hyphenated technique, LC-MS, GC-MS, CE-MS, FFF-MS, LC-NMR, CE-NMR, HPLC-NMR*

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INTRODUCTION

The term "hyphenation" which was first used by Hirschfield a few decades ago, describes the online combination of one or more spectroscopic detection & separation methods. Hyphenation is a technique which evolved from the combination of spectroscopic technique and separation methods [figure1]. Recently, the hyphenated methodology has gained increasing attention as the main technique for resolving challenging analytical problems. The methods of HPLC, GC, LC, and CE are connected to spectroscopic detection methods which helps to obtain structural information that facilitates the identification of compounds present in a crude sample. Such as NMR and MS, which led to the development of recent hyphenated methods. Such as LC-MS, GC-MS, CE-MS, FFF-MS & LC-NMR, CE-NMR, HPLC-NMR. The most popular analytical separation method for identifying chemicals in natural product extracts, both qualitatively and quantitatively, is HPLC. The ability to solve structural issues with complex natural products has increased due to the physical connection between HPLC and NMR or MS. Compared to LC-NMR, LC-MS has been used more widely due to its higher sensitivity. [1]

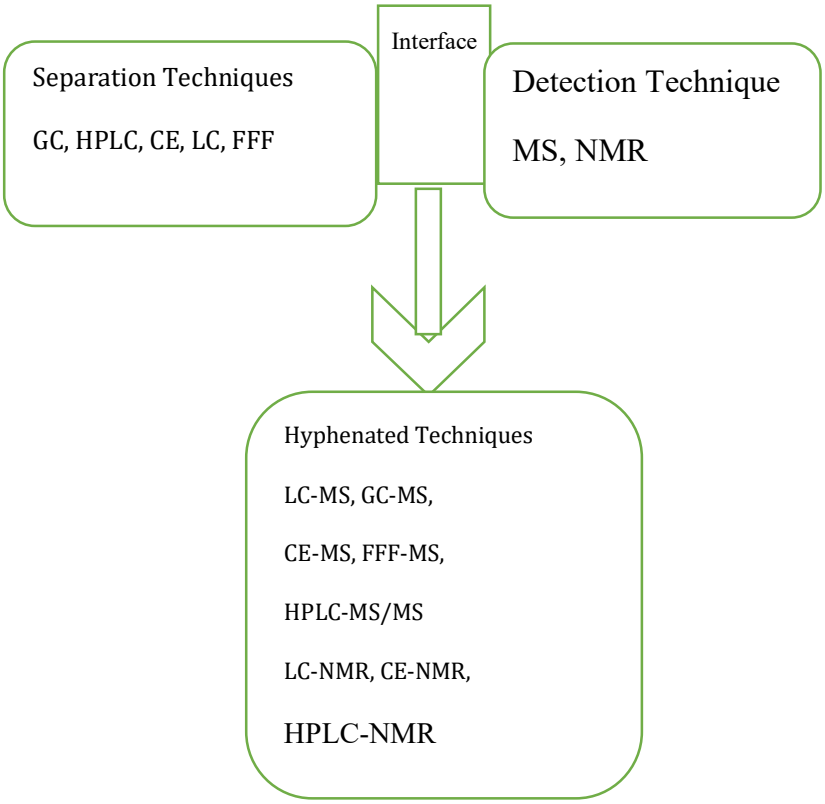

Figure 1: Hyphenation of spectrometric & chromatographic methods

MS Based technique:

Thermal-MS technique

Polymers can be broken down by heat using thermal-MS based techniques. [2] Using a mass spectrometer, decomposed materials are analyzed to learn more about artificial flaws in the chain of polymer & the material's decomposition by products. Several coworkers have investigated pyrolysis products & polymer structure using a variety of techniques. [3] The TA-APGD-MS analysis of the degradation products without sample pretreatment provided details about the polymer's structural properties, which is particularly significant for examination of difficult-to-dissolve polymer materials. In addition to matrix assisted laser desorption/ionization (MALDI)-MS, Barton employed electrospray ionization (ESI)-MS to examine the mechanisms of decomposition of poly (propylene oxide) (PPO) can be obtained via heating the polymer before MS analysis. [4] Thermal techniques have the remarkable benefit of enabling mass spectrometric analysis of insoluble polymers. [5] Thermal-MS methods that can be employed for temperature-dependent degradation products and reveal information about their physical and structural characteristics, assisting in the development of sturdy products.

MS/MS technique

MS/MS procedures involve fragmenting analyte ions in MS to get more precise structural information. A variety of scanning techniques, including product ion scanning, precursor ion scanning, neutral loss scanning, & monitoring of specific reactions able to be applied to conduct an MS/MS analysis. The product ion scan is most often used for structural characterization of different synthetic polymers among all of these modes. A precursor analyte ion is separated during technique of MS/MS analysis, and it is then activated and fragmented inside MS. Finally, a thorough examination of precursor ion structure is conducted using all of the fragmentation results. Numerous activation techniques have been created, such as post-source decay, surface-induced dissociation, electron transfer dissociation, photodissociation, collision activated dissociation, electron capture dissociation. However, there are many fragmentation methods that may be used on synthetic polymers, collision activated dissociation, which is intimately related to post-source decay and in source decay (ISD) methods, is the most popular method. [6] By combining either of these methods with MS/MS, it is possible to gain more precise data about the type of end groups, monomer sequences, isomeric structure existence, and substitution degree.

Liquid chromatography-mass spectroscopy (LC-MS)

All the hyphenated methods for characterizing IMP/DP, LC-MS and its variants are most commonly used since they show the capacity to provide structural data. [7] The entry of a liquid into a mass spectrometer's extreme vacuum at a flow rate of 1 ml/min and its subsequent evaporation would immediately cause the high break down of high vacuum, making the coupling of HPLC with mass spectrometry for a very long time a highly delicate and complex procedure. The moving belt interface (Games et al., 1981), in which the eluent was sprayed onto a circulating polyimide belt and dried, was the first commercially available interface. The analyte was delivered into the vacuum with the use of this belt, desorbed by fast heating, and ionized using conventional methods, electron impact (EI), and chemical ionization (CI). The belt could be tricky to clean, and the analyte needed to be thermostable. [8] LC-MS can separate analytes and provide data on their molecular weight and pattern of fragmentation. It is possible to propose reasonable chemical structures based on the fragmentation pattern. [9] Additives and buffers are used in the mobile phases of LC separation. It is possible to adjust the mobile phases' pH to assure the analyte's ionization. [9] [10] LC-MS has seen the fastest and highest level of advancement & range of equipment's are commercially accessible and these are as follows:

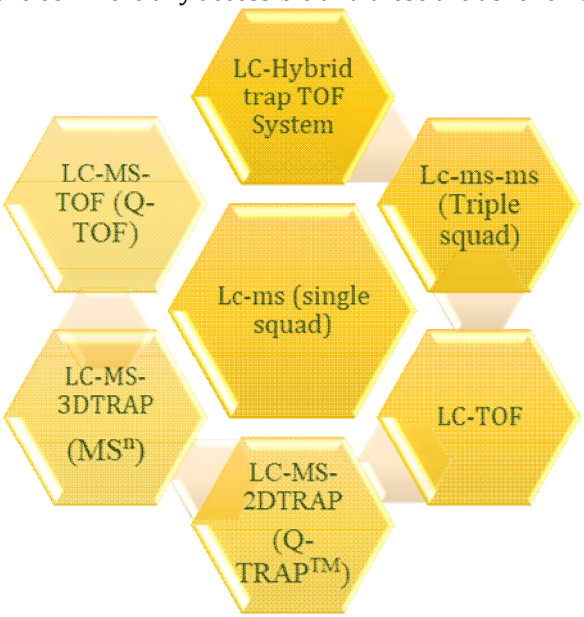

Figure 2: Schematic diagram of an LC-MS system

0For forced degradation impurity profiling, HPLC and LC-MS were chosen because of their excellent resolution, accuracy, specificity, and capacity. [11-13]

Gas chromatography-mass spectroscopy (GC-MS)

Shortly after the introduction of gas-liquid chromatography and organic mass spectrometry, Holmes and Morrell presented the first coupling of gas chromatography with mass spectrometry. [14] In forced degradation studies, gas chromatography-mass spectroscopy (GC-MS) is a quick, and accurate technique for drug and impurity separation, quantification, and identification. [15] One of the examples used in GC-MS in solo to identify IMPs in ecstasy tablets sold illegally that contained derivatives of amphetamine. [16-18] GC-MS has been utilized in combination with a range of methods for the purpose of characterizing pharmaceutical IMPs. The method has been used, in particular, to characterize substances that exhibit molecular ion mass and fragmentation in electron impact (EI) and chemical ionization (CI) sources of GC-MS but resist ionizing of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources of LC-MS. An intriguing study used an unknown DP of TCH346 (an investigative chemical) could not be ionizable in LC-MS ESI or APCI positive/negative modes; hence, the molecular weight of this compound was determined using CI-GC-MS. Its pattern of fragmentation was identified through ion trap mass spectrometry (CI-MS/MS). [19] The GC column is used to separate the samples for GC-MS analysis, where volatization of the analytes occurs. After passing through the MS ion source, analytes will be bombarded by ionizing electrons, which will cause cation radicals to develop. These radicals will subsequently breakdown into molecule ions. A carrier gas, such as argon, helium, nitrogen, or hydrogen,

carries the sample down the column. While a mixture's components are gradually separated by the GC, each component's structural identity can be assisted by the information provided by the MS detector. This approach exhibits satisfactory analytical performance (i.e. LoD, LoQ, linearity, robustness) and is quick, specific, selective, accurate, and exact method. [20]

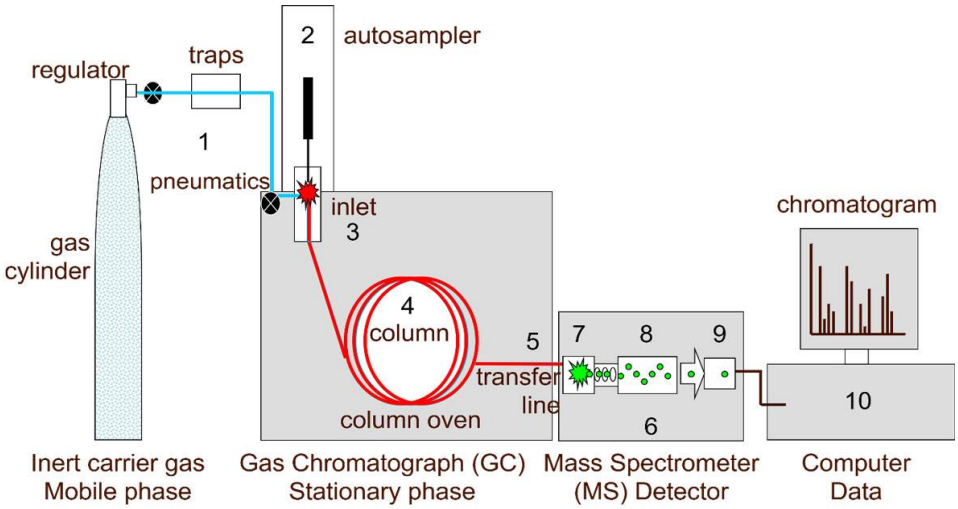

Figure 3: Diagram of GC-MS system

Capillary electrophoresis-mass spectroscopy (CE-MS)

An electric field drives CE analysis, which is carried out in small tubes and has the potential to quickly separate hundreds of distinct substances. It is a technique for isolation in which molecules are identified by differences in their electrophoresis motilities and structural details. [21] When voltage is supplied, it is usually employed to divide ions that move at different rates depending on their charge & size. Through buffer-filled capillaries, voltage is applied to distinguished the species. The solutes show as peaks as they pass through the detector, and because the area of each peak corresponds to the solute's concentration, quantitative measurements are made possible. When an MS detector is coupled to a CE system to receive online MS data of the separated component, the combination is referred to as a CE-MS combination. [22] [figure 4].

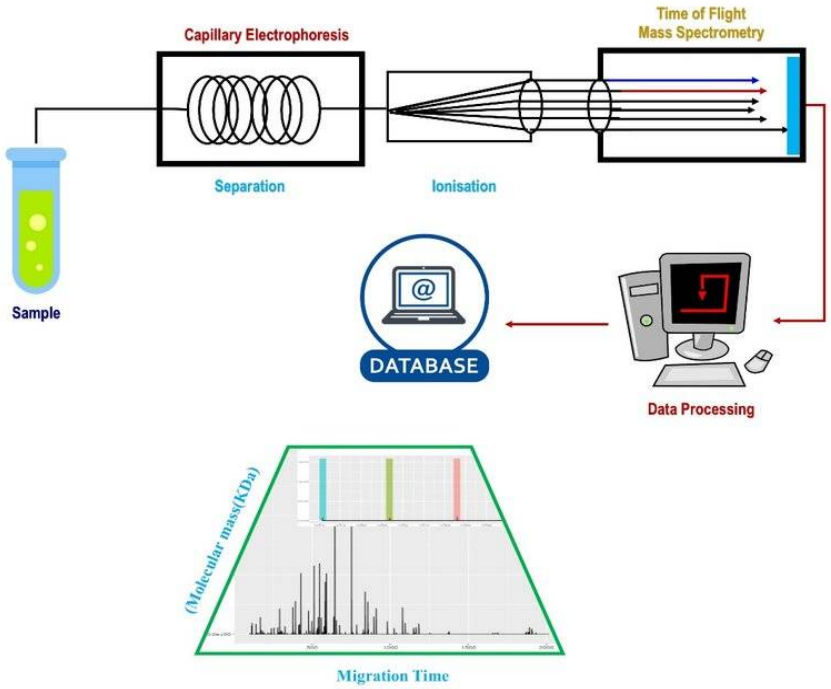

Figure 4: Schematic diagram of CE-MS system

High performance liquid chromatography- tandem mass spectroscopy (HPLC-MS/MS)

Alongside advancements in medicinal chemistry, pharmacology, and clinical chemistry natural products bioanalysis in model organisms and humans has generally shifted away from HPLC-UV platforms and move towards HPLC-MS/MS technology. [23-27] Tandem quadrupole (QqQ) based HPLC-MS/MS assays are frequently used when analyzing herbal preparations [28] or crude drug batches quantitatively. [29-31]. Six distinct firms now make HPLC MS/MS systems with the ability to conduct related experiments and perform selective reaction monitoring (sometimes referred to as multiple reaction monitoring): Agilent, MDS Sciex, GSG, Thermo-Fischer, Varian, and Waters. Among them, Waters, Thermo-Fisher, and MDS Sciex have been manufacturing tandem mass spectrometer instruments for over a decade, similarly Agilent and Varian have included HPLC–MS/MS systems. GSG is a relatively new rival that produces HPLC-MS/MS equipment that are extremely equivalent to commonly used instruments. The manufacturers provide a variety of tandem mass spectrometry systems with various ion sources and technological features. Although HPLC-MS/MS instruments themselves have a relatively small footprint (starting at roughly 2 m^2 for compact device), it requires a significant amount of additional space to deploy these devices [32-33] [figure 5].

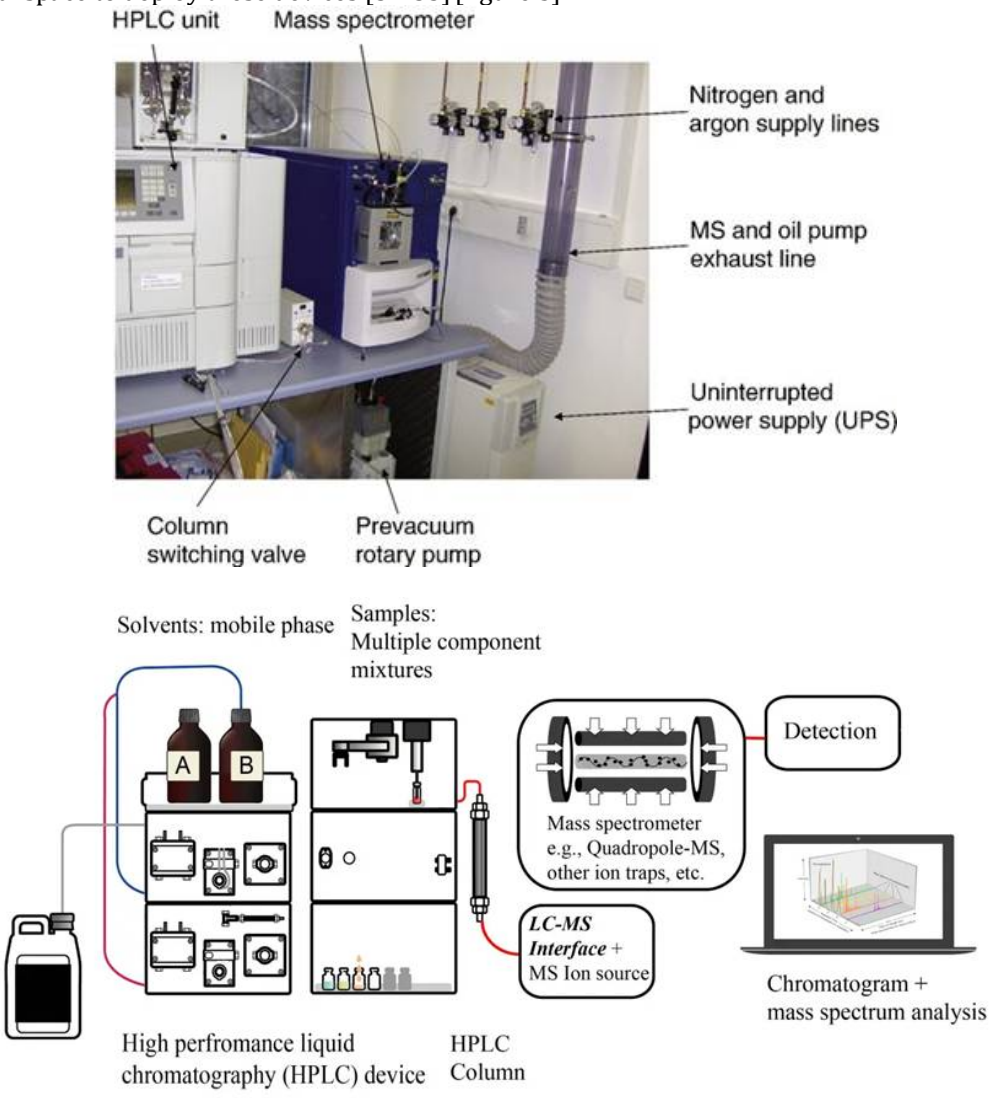

Figure 5: SPE–HPLC–MS/MS System

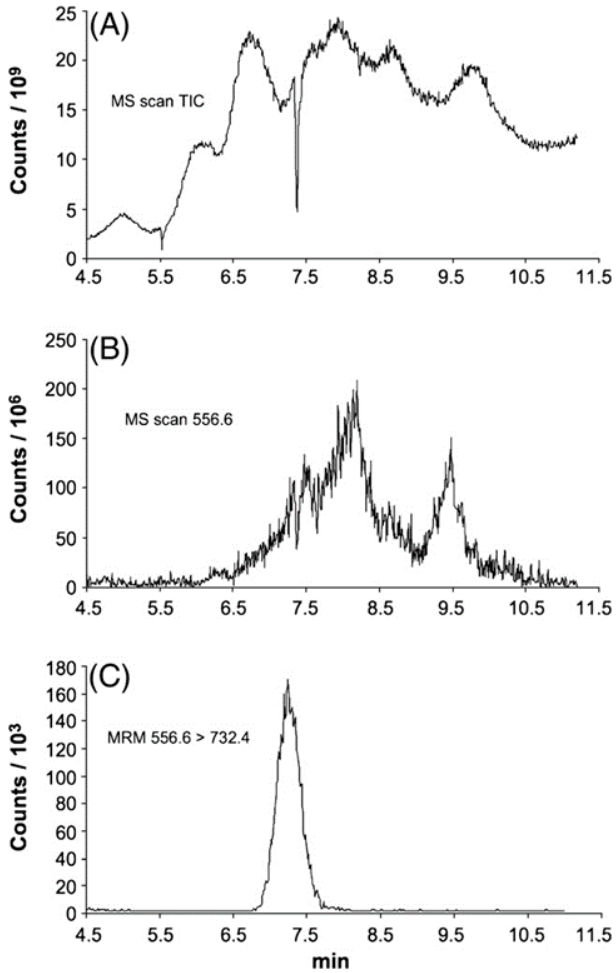

Figure 6: Three distinct HPLC–MS analyses of a plasma sample demonstrate the effectiveness of multiple reaction monitoring (MRM) and selected reaction monitoring (SRM) studies. [34]

Field flow fractionation-mass spectroscopy (FFF-MS)

Field-flow fractionation is method of splitting that depends on varying velocities in a field and is typically used for water-based solutions of dispersed and charged or neutral species. [35] Although it is less frequently employed in conjunction with the MS approach, field-flow fractionation is utilized to create synthetic polymers. Because of high molar masses of the compounds under investigation, coupling this approach with a mass spectrometer is difficult. FFF was used to separate the low molar mass polymers and then their composition was determined by ESI MS. Connecting a thermal field-flow fractionation (ThFFF) system to MALDI-ToF mass spectrometry for examination of PS standards with high molar mass is an illustration of the integration of FFF and MS. [36]

NMR Based hyphenated technique:

Liquid chromatography-Nuclear magnetic resonance (LC-NMR)

The linkage of NMR and LC effluent was first reported in 1978. [37] Cryoprobe, microprobes and strong field magnets technologies are only a few of the technological breakthroughs that are connected to contemporary LC-NMR systems. [38-40] The first Superconducting magnet-based LC-NMR experiment was reported late in the 1970s (Bayer et al., 1979; Haw et al., 1980; Albert et al., 1985). A LC-NMR system comprises of an HPLC system equipped with loop collector or valve to stop flow studies coupled to a high-resolution NMR instrument (400–800 MHz) with a specific LC–NMR flow probe. For the synchronization of the various activities, the NMR's data acquisition system is linked to the HPLC control unit. The radio frequency (RF) coil encircles a non-rotating glass tube, which is connected to HPLC tubing at both ends to form the flow cell (Albert, 1995). [41] Volumes required for 1 H-NMR employing iron magnets have decreased from 200 to 500 mL to 40 to 200 mL for super-conducting magnets over the years, reflecting a general trend toward smaller LC-NMR detection cells (Behnke et al., 1996). [42] Reversed-phase columns with isocratic or gradient elution and a binary or tertiary solvent mixture are used in the majority of LC-NMR operations. The protons in the mobile phase's solvents make it extremely difficult to get a good NMR

spectrum. The powerful solvent signals & faint substance signals cannot be processed by the NMR spectrometer's receiver simultaneously. Three major ways are presaturation, soft-pulse repeated irradiation, water suppression enhancement from that presaturation utilize a z-gradient, which can be used to induce solvent signal suppression through T1 effects (WET). However, in order to successfully achieve the LC-NMR coupling, several critical problems had to be solved. [43] Wann et al. examined over twenty LC-NMR pharmaceutical research applications, including IMP and DP characterization.

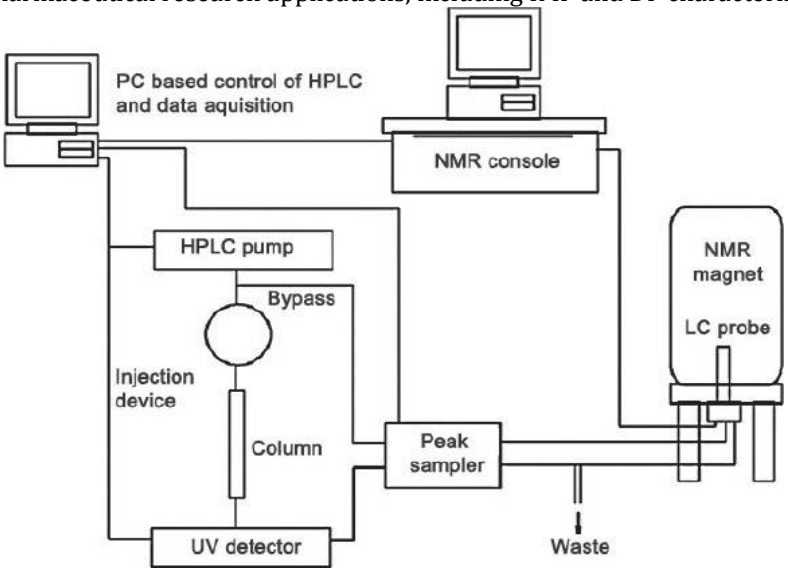

Figure 7: Schematic diagram of an LC-NMR system

Capillary electrophoresis-Nuclear magnetic resonance (CE-NMR)

CE-NMR employs both stopped and continuous flow modes, just like LC-NMR does. The reduced sample volume generated from CE, which causes a reduced NMR residence time and affects detection sensitivity, is the common issue with CE hyphenated NMR, though. [44] This includes the creation of micro-coil probes, which possess a high filling factor and a sample cell with a nanoliter volume and coils with a diameter of no more than 1 mm wrapped directly around the separation capillary. [45-48] Despite the fact that decreasing the coil's diameter generally improves the S/N ratio, microcoils are less sensitive to concentrations than traditional 5 mm probes since they have a smaller sample holding capacity. [49] Only a few studies have described CE-NMR to monitor IMPs and DPs. Online capillary isotachophoresis-NMR (cITP-NMR) has been primarily used in these.

High performance liquid chromatography-Nuclear magnetic resonance (HPLC-NMR)

The HPLC-NMR system uses flow probes with matching analytical HPLC dimensions that are fitted with traditional Helmholtz (saddle) type RF coils. Using a capillary, a flow cell is attached to the LC module takes place of the NMR sample tube typically positioned in the center of these coils in conventional NMR spectrometers. The flow cell is receiving mobile phase that has eluted from the HPLC column, and NMR spectra are being permanently recorded. Numerous NMR experiments (i.e., sixteen scans with a recording time of under a minute) are typically aggregated for one NMR spectra to raise signal to noise ratio. Similar to HPLC-MS, in order to record an NMR spectrum or many for a chromatographic peak, the mean peak widths generated by the chromatographic apparatus must coincide with the NMR spectrum acquisition time. [50,51]

CONCLUSION

Hyphenated technique is the method that resulted from combining online spectroscopic detection technology with separation method. The chemical properties of the drugs and the contaminants determine the technique's applicability and suitability. The utilization of MS systems for detection is significant and possess the potential to independently provide clear structure elucidation of IMPs and DPs. An effective stability-indicating test should be able to quantify variations in API concentration with accuracy, independent of other chemical compounds, pharmaceutical contaminants, degradants, and excipients. It should also be able to identify changes in the stability of medicinal substances and products over time.

ABBREVIATIONS:

Atmospheric pressure chemical ionization (APCI), collision activated dissociation (CAD), Chemical ionization (CI),capillary isotachophoresis (cITP),Degradation products (DPS),electron capture dissociation (ECD),Electron impact (EI),Electrospray ionization (ESI),electron transfer dissociation (ETD), Fourier Transform Ion Cyclotron Resonance (FTICR), High performance liquid chromatography- tandem mass spectroscopy (HPLC-MS/MS), Impurities (IMPS),In source decay (ISD), matrix assisted laser desorption/ionization (MALDI),Multiple reaction monitoring (MRM), Tandem mass spectrometry (MS/MS),poly (propylene oxide) (PPO),post-source decay (PSD),Radio frequency (RF),surface-induced dissociation (SID),Selected reaction monitoring (SRM),Thermal field-flow fractionation (ThFFF),Time-of-flight (ToF),Uninterrupted power supply (UPS).

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