Review Article

Liquid Chromatography in conjunction with Mass Spectrometry (LC-MS)

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ABSTRACT

This review describes brief introduction to the methods of quantitative analysis which are one of the most valuable course in scientific training. An introduction to HPLC such as stationary phase used, applications of HPLC is briefly overviewed. The basic principle of mass spectrometry is shortly discussed. The main objective of this review is to discuss well known and most widely used hyphenation technique, liquid chromatography in conjunction with mass spectrometry [LC-MS]. Interface plays an important role in the hyphenation technique; as eluent from liquid chromatography is transferred to mass spectrometer with the help of interface. The development and type of interfaces used in this hyphenation technique are thoroughly discussed. The mass analyzers such as Quadrupole, TOF used in mass spectrometry with principle and working are also discussed.

Keywords: Liquid Chromatography, Mass Spectrometry, LC-MS.

1. INTRODUCTION

Modern physical methods of analysis are extremely sensitive, providing precise and detailed information from small samples of material. These are, for the most part, rapidly applied, and, in general, are readily amenable to automation. For these reasons, these are now in widespread use in product development, in the control of manufacture and formulation, as a check on the stability during storage, and in monitoring the use of drugs and medicines[1]

The study of analytical chemistry provides ideal training for nearly all scientists. A course in quantitative analysis equips one with ability to plan and exercise the experimental work; it develops the ability to record and to interpret such experimental work, and it trains to understand and to communicate what has been done. The course in quantitative analysis is a very important link in the chain of studies that develops the scientific ability in the chemist. With its multiple emphases on theory, laboratory work and high accuracy in the analysis of unknown sample, quantitative analysis is one of the most valuable courses in scientific training.

There are various methods used in Quantitative Analysis which are broadly classified as-

1.1 Chemical /classical methods

These methods depend upon quantitative performance of a suitable chemical reaction and either measuring the amount of reagents needed to complete the reaction or ascertaining the amount of reaction product obtained, e.g. Titrimetric (acid base titration, Oxidation – reduction titration, non-aqueous titration, complex formation) gravimetric and volumetric methods, etc.[2]

1.2 Instrumental methods

These methods are based upon the measurement of physical properties of a substance such as electrical or optical and to correlate them for determination of concentration of analyte. These properties are being exploited for developments of analytical methods such as spectrophotometry, HPLC, GLC, Polarography, etc.

Now a day's instrumental methods of analysis are widely accepted over the classical methods. These methods are

extremely sensitive, providing precise and detailed information from small sample materials. Depending upon the nature and type of material, either single or in combination, an appropriate method of analysis is adopted. Instrumental methods are usually much faster than chemical methods and are applicable at concentration far too small to be amenable to determination by chemical methods and find wide application in industry.

2. LC-MS

High performance liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50µm) packing contain in column with a small bore (2-5 mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase)[3]. HPLC is one of the most widely used analytical techniques today among different chromatographic procedures due to significant evolution in Liquid chromatographic instruments providing superior qualitative and quantitative results, reproducibility, high detection sensitivity and unsurpassed reliability[4].

The basic principle of the mass spectrometry (MS) is to generate ions from either inorganic or organic compounds by any suitable method to separate these ions by their mass to charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundances. The analyte may be ionized thermally by electric field or by impacting energetic electrons, ions, photons. The ions may be single ionized atoms, molecules or their fragments or associates[5].

Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection. This includes high performance liquid chromatography (HPLC)-MS, capillary electrophoresis (CE)-MS and more recently capillary electro chromatography (CEC)-MS. The technique is still fast developing, particularly in the mass spectrometry area, with vastly improved sensitivity and resolution. It is probably the most powerful technique currently available for pharmaceutical analysis[6].

2.1 Components of an LC–MS system

The various components of LC-MS system are shown in Fig.1

(a) Autosampler (loads the samples onto the HPLC);
(b) HPLC;
(c) Ionization source (interface for LC to MS);
(d) Mass spectrometer

There are various types of ionization sources that can be used as the interface between the HPLC eluent and the mass spectrometer. The two most common sources are

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI); both of these source types are now standard equipment on mass spectrometers that are used for LC–MS applications. For both ESI and APCI, the ionization occurs at atmospheric pressure, so these sources are often referred to as atmospheric pressure ionization (API) sources. For both ESI and APCI, some combination of high voltage and heat is used to provide the ionization that is needed to produce the ions that are assayed by the MS system[7].

2.2 LC/MS Interfaces

In this part of discussion developments in the LC/MS interfaces are briefly overviewed.

2.2.1 Moving Belt Coupling

Scott et al[8] developed moving wire interface and McFadden et al[9] developed moving belt interface. The technique involves the deposition of eluent onto a stainless-steel wire or a plate usually made of polyimide and later on removal of solvent by applying vacuum. The residual solid analyte in vapor form is then introduced into an ion source. In order to remove the solvent by heating and for volatilization of solid analyte electric current is passed through the wire[10].

The ionization of solid analyte is achieved either by electron ionization (EI) or chemical ionization (CI) which is one of the advantage of continuous moving belt interface. Apart from EI or CI, the direct ionization of solid analyte from the transporting surface has been reported using FAB[11]. The surface ionization technique such as laser desorption (LD) together with moving belt interface has also been successfully reported[12,13].

2.2.2 Direct Liquid Introduction (DLI)

The technique involves the introduction of liquid eluent into the ion source through capillary or a pinhole diaphragm[14,15]. In DLI-LC/MS, the analyte in solution form is introduced into the MS ion source[16]. When sufficient energy is given to the solution, the preformed ions can be desorbed into a mass spectrometer and bulk of the solvent is vaporized and eliminated by the vacuum system.

2.2.3 Thermospray (TSP)

It consists of a heated vaporizer, a desolvation chamber and an extraction skimmer. The pumping of solution into a heated stainless-steel capillary causes rapid evaporation of solvent from liquid surface resulting in an ultrasonic spray of vapor and charged droplets. Repetitive disintegration of charged droplets occurs due to continuous evaporation of solvent and development of coulombic repulsion takes place between like charges and causes ions as well as neutral molecules to be released from the surface of the microdroplets. The extraction and acceleration of ions towards the mass analyzer is achieved by electrostatic system voltage. As TSP acts as an interface as well as ion source there is no need of separate ionization source.

The ionization in TSP can also be possible in a two-step manner similar to chemical ionization (CI) [17]. As in first step of CI, ionization of reagent gas followed by second step in which reaction of reagent ions with analyte molecules in the gas phase occurs to generate positive analyte ions. In a similar manner, negative analyte ions can also be generated.

2.2.4 Continuous-Flow Fast Atom Bombardment (FAB)

The principle of FAB is very similar to secondary ion mass spectrometry [SIMS], however in FAB there is a introduction of liquid matrix such as glycerol in which sample is dissolved. The matrix enhances the sensitivity and ion current stability.

FAB was introduced by Barber et al[11] in1981 in which liquid target surface is bombarded by a beam of fast atoms such as xenon or argon causing desorption of ions. In FAB spectrum, analyte ions formed usually is protonated ions in positive FAB mode and deprotonated ions in negative FAB mode.

2.2.5 Particle Beam Interface

The interface has been developed based on the original work of Browner and co-workers [18] being able to separate solvent from solute with minimization of solute loss.

As eluent passes from LC it is subjected to nebulizer converting it into a spray of fine liquid droplets with high velocity. Solvent evaporation takes place and solute starts concentrating. The liquid droplets exit through the heated chamber as a fast moving particle beam and the beam entering into the ion chamber is subjected to ESI or CI ionization.

2.2.6 Electrospray Ionization (ESI)

It is most commonly used interface for LC-MS. Fenn and co-workers[19] recognized ESI as an important interface for LC/MS. It is used to analyze polar molecules that make preformed ions in solution by transforming ions in solution to ions in the gas phase. The ionization of heat –labile and high molecular weight compounds such as proteins and peptides can be done by this technique. ESI can be effectively used as an interface for HPLC. It is soft-ionization technique which is used for the molecular weight determination of wide variety of analytes. ESI resembles to LC/MS interface such as TSP[20,21] and ion evaporation[22].

The sample in solution form is sprayed through the capillary needle which is maintained at approximately 5kv electric potential. Applied voltage across the needle causes liquid spray to be charged due to nebulization. As droplets passed through a stream of dry gas and heat gets evaporated. As the droplet decreases in size, the charge density on the droplet surface increases. When the Coulombic repulsion between like charges on the surface overcomes the forces of surface tension (the Rayleigh limit [23], the droplet disintegrates explosively to form second-generation liquid droplets. The process occurs repeatedly and ions leave the droplet which are directed into the mass analyzer. A schematic presentation of the ESI process is illustrated in Fig. 2.

2.2.7 Atmospheric Pressure Chemical Ionization (APCI)

In APCI ions are produced at atmospheric pressure by reaction between analyte molecule and reagent gas. Ionization of solvent molecules is initiated by a corona

discharge at the tip of the corona needle. The LC eluent is introduced into a heated pneumatic nebulizer [24,25] where the liquid is nebulized pneumatically into a heated tube, allowing the droplets to collide with the hot walls.

MS analysis of samples pyrolyzed under controlled conditions makes use of chemical ionization at atmospheric pressure. On-line LC-MS employing heated pneumatic nebulizer interface together with APCI is suitable for analysis of moderately polar samples that are not too labile. Highly polar, thermolabile, and ionic samples and biopolymers require electrospray ionization at atmospheric pressure combined with on-line separation by LC or CE. The determination of the molecular mass of proteins, nucleic acids and other polymers by electrospray ionization has accelerated the formerly slow development of atmospheric pressure ionization[26].

2.3 Mass Analyzers

After ions are formed in the source region they are accelerated into the mass analyzer by an electric field. The mass analyzer separates these ions according to their *m/z* value. The selection of a mass analyzer depends upon the resolution[27], mass range, scan rate and detection limits required for an application. Each analyzer has very different operating characteristics and the selection of an instrument involves important tradeoffs.

2.3.1 Quadrupole[28]

Quadrupole is most commonly used mass analyzer. Its fast scan rate, high transmission efficiency, compact size and modest vacuum requirements are ideal for inexpensive instruments. Most quadrupole instruments are limited to unit *m/z* resolution and have a mass range of *m/z* 1000. Many benchtop instruments available with mass range of *m/z* 500 but research instruments are available with mass range up to *m/z* 4000.

In the mass spectrometer, an electric field accelerates ions out of the source region and into the quadrupole analyzer. The analyzer consists of four rods or electrodes arranged across from each other as shown in fig.3. As the ions travel through the quadrupole they are filtered according to their *m/z* value so that only a single *m/z* value ion can strike the detector. The *m/z* value transmitted by the quadrupole is determined by the Radio Frequency (RF) and Direct Current (DC) voltages applied to the electrodes. These voltages produce an oscillating electric field that functions as a band pass filter to transmit the selected *m/z* value.

2.3.2 Time-of-Flight[29]

The time-of-flight (TOF) mass analyzer separates ions in time as they travel down a flight tube shown in fig.4. It is a very simple mass spectrometer that uses fixed voltages and magnetic field is not required. The major drawback of TOF instruments is its poor resolution, usually less than 500. These kinds of instruments have high transmission efficiency, very low detection limits, fast scan rates and no upper *m/z* limit. Recent developments in pulsed ionization technique and new instrument designs with improved resolution regained interest in TOF-MS.

2.3.3 Quadrupole Ion Trap[30-32]

The Quadrupole ion storage trap mass spectrometer (QUISTOR) is a recently developed mass analyzer with some special capabilities which are very sensitive, relatively inexpensive, and scan fast enough for GC/MS experiments. The sensitivity of the QUISTOR results from trapping and then analyzing all the ions produced in the source. Since all the ions are detected, the S/N is high.

The QUISTOR consists of a doughnut shaped ring electrode and two end cap electrodes. A cutaway view of this arrangement is shown in Fig. 5. A combination of RF and DC voltages is applied to the electrodes to create a quadrupole electric field similar to the electric field for the quadrupole mass analyzer. This electric field traps ions in a potential energy well at the center of the analyzer. The mass spectrum is acquired by scanning the RF and DC fields to destabilize low mass to charge ions. These destabilized ions are ejected through a hole in one end cap electrode and strike a detector. The mass spectrum is generated by scanning the fields so that ions of increasing *m/z* value are ejected from the cell and detected. The trap is then refilled with a new batch of ions to acquire the next mass spectrum. The mass resolution of the ion trap is increased by adding a small amount 0.1 Pa (10^{-3} torr)

of Helium as a bath gas. Collisions between analyte ions and the inert bath gas; dampen the motion of ions and increases the trapping efficiency of the analyzer.

CONCLUSION

High Performance Liquid Chromatography is one of the most widely used analytical techniques today among different chromatographic procedures due to significant evolution in Liquid chromatographic instruments providing superior qualitative and quantitative results, reproducibility, high detection sensitivity and unsurpassed reliability. However hyphenations of liquid chromatography with mass spectroscopy increased its detection capability to a greater extent.

Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection. LC-MS is efficient analytical technique for in-vitro determination of drug metabolite, in new drug discovery, screening of plant constituents, analysis and identification of impurities and degradation products in pharmaceuticals. However online LC–NMR–MS has been shown in many studies to be a powerful tool for solving identification and structure related problems that LC–MS or LC–NMR alone cannot handle, without the necessity of laborious fractionation and purification. Thus in fundamental hyphenated techniques which are established further optimization are needed. Advancement in LC-MS i.e. LC-MS/MS (Tandem Mass Spectrometry) can be successfully implemented for separation and identification of analyte ions.

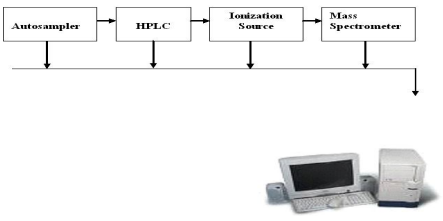

Fig. 1: The components of an LC–MS system

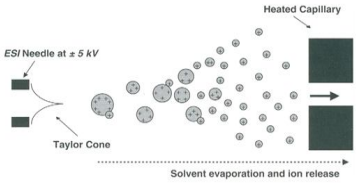

Fig. 2: Schematic presentation of ESI process

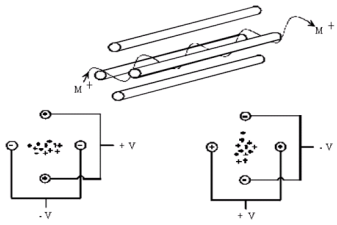

Fig. 3: Quadrupole mass Analyzer

A) Ion trajectory through the quadrupole,
B) Ion focusing during first half of RF cycle,
C) Ion focusing during second half of RF cycle.

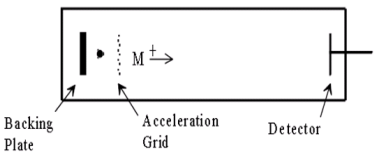

Fig. 4: Time-of-flight

Mass Spectrometer

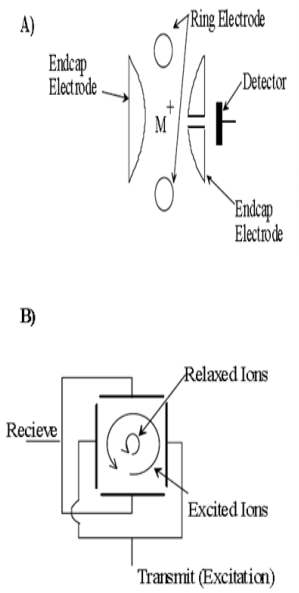

Fig. 5: Ion cyclotron Mass Spectrometer
A) Major components.
B) Ion motion within the trap.

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