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REVIEW ARTICLE

A CONCISE SYSTEMATIC REVIEW ON LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY WITH SPECIAL EMPHASIS ON ITS APPLICATIONS

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Abstract: **Background:** Mass spectrometry (MS) is a wide-ranging analytical tool, which involves the production and subsequent separation and identification of charged species. MS provides valuable information of molecular mass, molecular structural information and quantitative data all at high sensitivity. However, it is best separation techniques applied to complex mixtures before mass spectrometry is undertaken. Liquid chromatography (LC) is excellent for separating mixtures but generally poor at identification of compounds. It is an arrangement of two or more mass spectrometers tandem-in-space and time placed one behind the other. Literature reveals many analytical approaches with detection systems, automation tools for an effective separation, enhanced selectivity and sensitivity for quantitation of many analytes. The bioanalytical method (s) development plays an important role during the process of drug discovery and development culminating in a marketing approval. The intention of this review is to cover various key areas where LC/MS/MS shows its applicability in bioanalytical areas.

Keywords: LC-MS/MS, Separation, Bioanalytical, Review.

INTRODUCTION:

Liquid chromatography is a fundamental separation technique in the life sciences, pharmaceuticals and related fields of chemistry [1-3]. Unlike gas chromatography, which is unsuitable for nonvolatile and thermally fragile molecules, liquid chromatography can safely separate a very wide range of organic compounds from small-molecule drug metabolites to peptides and proteins. Mass spectrometers work by ionizing molecules and then sorting and identifying the ions according to their mass-to-charge (m/z) ratios [3-7]. Two key components in this process are the ion source, which generates the ions and the mass analyzer which sorts the ions. Several different types of ion sources are commonly used for LC/MS. Each is suitable for different classes of compounds. Each has advantages and disadvantages depending on the type of information needed [7-11].

INSTRUMENTATION

ll mass spectrometers have three basic components:
a) Ionization source
b) Mass analyzer & c) Detector

a) Ionization source:

Much of the advancement in LC/MS over the last ten years has been in the development of ion sources and techniques that ionize the analyte molecules and separate the resulting ions from the mobile phase. Earlier LC/MS systems used interfaces that neither did not separate the mobile phase molecules from the analyte molecules (direct liquid inlet, thermospray) nor did so before ionization (particle beam). The analyte molecules were then ionized in the mass spectrometer under vacuum, often by traditional electron ionization. These approaches were successful only for a very limited number of compounds [11-15]. Various types of ion sources are available in mass spectrometry, which are listed and compared in Table 1. The applications of various ionization techniques used in mass spectrometry are explained in Fig. 1.

b) Mass analyzer

It is defined as a separation filter which can separate ions by mass or more precisely by momentum or energy in space or in time. The separation can be achieved by the use of combination of electric and magnetic fields and sometimes radio frequeny (RF) fields [16-20]. The commonly use mass analyzers are following types:

- Magnetic Sector Mass Analyser

- Time-of-Flight (TOF) Mass Analyser
- Quadruple Mass Analysers
- Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Analyser

Each mass analyzer is having its own characteristic and specificity. There are various types of mass analyzer are available which are shown in Fig. 2. Apart from above discussed four types of mass analyzers, there are also a large number of other variations which include hybrid systems. Hybrid ones are composed of two of the basic types of mass spectrometer to make the system more specific and accurate [21-23].

c) Detector

The use of detector is the measurement of the ions which are leaving the mass analyzer [24-26]. Various types of mass detectors used in LC-MS/MS are as follows:

1. Electron Multipliers Detector

It emits electrons from first dynode caused by incident ion beam are accelerated to next dynode where each of the electrons causes generation of two more electrons. The same thing happens with third dynode and so on.

2. Scintillator Detector or ('Daly' detector)

Here electrons are emitted due to fast ions and those are accelerated toward a second dynode which is made of a scintillator (which emits light or photons) and that emission is detected converted into an electric current by a photomultiplier tube. Good amplification even due to a single ion appearance may be achieved using this detector due to its high sensitivity. It is also helpful for metastable ion studies.

3. Faraday Cup Detector

Here secondary electron ejection is caused due to the strike of the high speed travelling ions inside of a metal built cup also known as Faraday cup. A temporary flow of current is initiated due to the production of electrons until they are recaptured. Advantages of Faraday cup detector are its simplicity and robustness but it is less sensitive.

Table 1: Various types of ion sources

Ionization method	Type of ion formed	Analytes	Sample introduction	Mass limit	Method type
Electron impact (EI)	M^+. M^-	small volatiles	GC, liquid or solid probe	10^3	Hard method
Chemical ionization(CI) - Electro-spray ionization (ESI) - Atmospheric pressure chemical ionization (APCI) - Atmospheric pressure photo ionization (APPI)	 $[M+nH]n^+$, $[M- nX]n^-$ $[M +H]^+$, $[M + X]^+$ M + hv _M+	 peptides, proteins nonvolatile small volatiles less polar species small volatiles polar/non polar	 LC or syringe LC or syringe L C or syringe	 2 x10^5 2x10^3 2x10^3	 soft method soft method soft method
Field ionization / desorption (FI/FD)	$[M +H]^+$, $[M + X]^+$	FI: volatiles FD: nonvolatiles	GC, liquid or solid probe	2 x 10^3	soft method

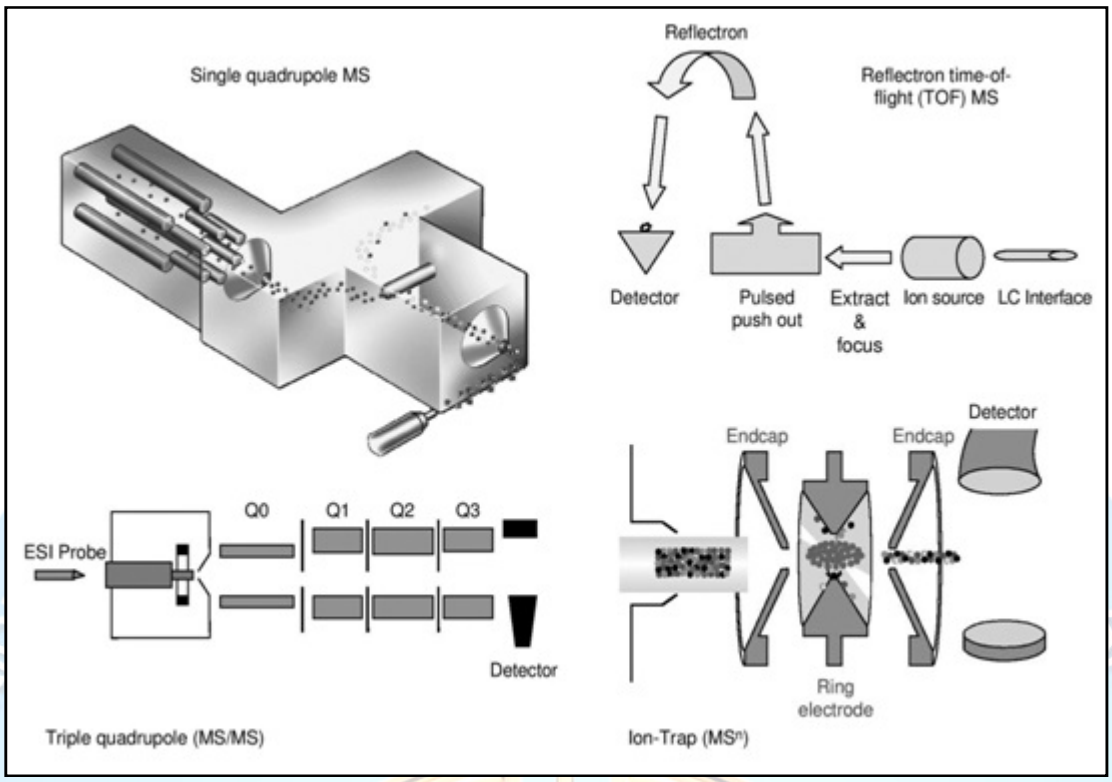

Fig.2: Types of mass analyzers

APPLICATION of LC/MS/MS

The evolution of LC/MS/MS from the early LC/MS instruments has revolutionized vast segments of analytical chemistry that deal with trace level contamination. The initial problems of interferences are reduced significantly by the tandem arrangement. The growth encompasses a wide range of research areas, including: Food Safety, Environmental Protection, and Pharmaceutical Development [27-34]. Much of this is due to the advancements in the instrumentation (primarily in the interface between the LC and the MS and the implementation of atmospheric pressure ionization), which have improved the sensitivity and the selectivity of the technique to that unmatched by most other analysis methods.

1. Environmental Applications: Because of the concern at even part per trillion levels for some of pharmaceutical residues, veterinary medicine residues, and pesticides as well as metabolites of these products, LC/MS/MS has factored heavily in this research area, which is expected by many to grow rapidly in the next decade.
2. Food Safety: The FDA has published several methods using LC/MS/MS for the determination of trace residues of contaminants in food products, many of which have been traced to imported foods. Because of the need to detect very low levels of the compound, LC/MS/MS is the preferred analytical technique.
3. Pharmaceutical: The main use for LC/MS/MS in the pharmaceutical industry has been in biological samples. The determination of target drug and metabolites in plasma and urine are critical for the evaluation of new drugs. The LC/MS/MS is also used for the analysis of impurities in drug substances, and it has become valuable for the detection of extractables and leachables in the packaging industry.

CONCLUSION

Liquid chromatography-tandem mass spectrometry offers analytical specificity superior to that of immunoassays or conventional high performance/pressure liquid chromatography (HPLC) for low molecular weight analytes and has higher throughput than gas chromatography-mass spectrometry (GC-MS). Because scientists are concerned about lower and lower levels of detection for compounds in complex matrices, an expansion in the prevalence of the development of LC/MS/MS methods can be expected as can an increase in the availability of the service throughout the analytical industry.

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