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REVIEW ARTICLE **OPEN ACCESS**

Applications of LC-MS/MS in Human Biological Fluid Samples for Small Molecules

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ABSTRACT
The solicitation of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) describes one of the most exciting chances for developments in mortal healthiness through the organization of reference measurement procedure capabilities, extensive chemical treatment, and a rich history in support of drug development since the 1990s onwards. Clinical solicitation of these technologies has activated to gather pace in numerous laboratories, with different applications reaching from extended newborn screening to documentation of emerging toxicants. The potential of these skills is vast and the need is deep; however, the journey can be challenging. Perhaps to some degree unique among analytical techniques. This review article provides a stepwise roadmap for systematically developing and validating anew liquid chromatographic (LC) techniques and mass spectrometric (MS) instruments have been reported assay for small molecule analytes. In addition, efforts have also been made in this review to address several Novel approaches to bioanalytical concepts.
Keywords: *Clinical, Liquid Chromatography and Mass Spectrometry, Validation, Application, Biological Matrix*

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INTRODUCTION

Bioanalytical techniques stand solutions for truthful toxicokinetic and pharmacokinetic calculation of drug applicants in support of provincial or global health authority submission. With globalization of medicine development, bioanalytical techniques are constantly made over between spots within a company and/ or from laboratories in pharmaceutical companies to contract exploration associations (CROs), in particular, the CROs in the arising request (e.g., India and China).[1-2]

Allocation of bioanalytical techniques presents some tasks. The experiment can be specific to the method itself, process, communications, or cultural differences between the laboratories, and so on. If not well prepared, method transfer may end up with delay of the proposed study. Then requests are: what should be prepared and what procedures should be in place to ensure a smooth method transfer? What are the acceptance criteria in line with the regulatory expectations and how to deal with failure in method transfer, Partial evidence on the above subjects is accessible in the public area and complete process given in Figure 01. [3]

The modern development of liquid chromatography and mass spectrometry instrumentation has led to short analysis run times, upgraded selectivity and increased throughput in drug bio-analysis. Currently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has developed into a energetic technology used to accomplish predictable experimentations in frequent clinical and pharmaceutical laboratories.[4]

It is known that the drug discovery development requires high throughput selection methods and thus LC-MS/MS became precise vital device in pharmaceutical industry. The use of LC-MS/MS surprises in the drug discovery phase and endures until or afterwards drug industrial. Various ionization methods are used on behalf of mass spectrometry. In pharmaceutical industry the atmospheric pressure ionization (ESI and APCI) has been the supreme used practice in combination with tandem mass spectrometry. LC-MS/MS has modernized the approaches and accomplishment of modern drug discovery.[5] In addition LC-MS/MS has

converted the technique-of-choice in quantitative bio-analysis. Figure 02indicationsclassiclife cycle practice of LC-MS/MS in drug bio-analysis operating various drugs with different sample extraction techniques.[6]

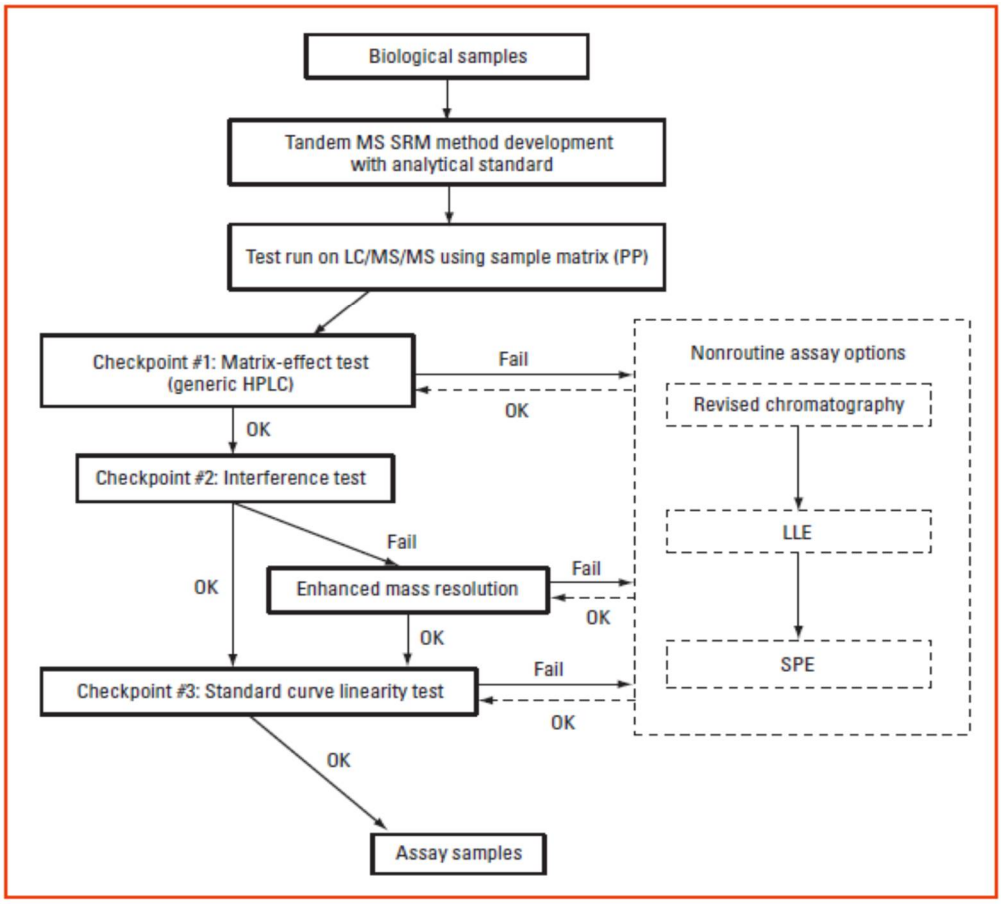

Figure 01: Bioanalytical Method Development Process

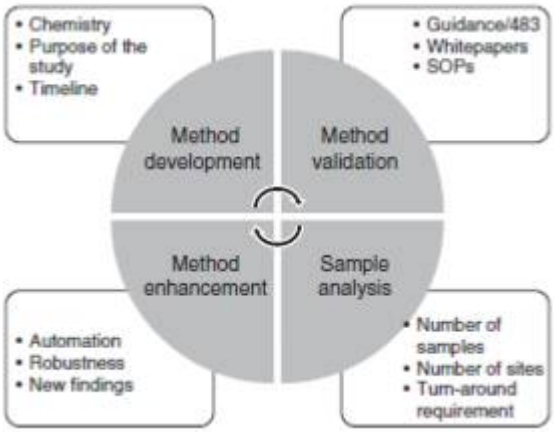

Figure 02: Life cycle process of Bioanalytical method

Liquid Chromatography-Mass Spectrometry

Liquid Chromatography-Mass Spectrometry (LC-MS) or High Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS) is an analytical method that coupled high resolve chromatographic separation with specific and sensitive mass spectrum identification. This contains High Performance Liquid Chromatography (HPLC)-MS, Capillary Electrophoresis (CE)-MS and Capillary Electrochromatography (CEC)-MS. The fusion of Gas Chromatography(GC) and Mass Spectrometry (MS) was head stated in 1958 and prepared accessible commercially in 1967.[7]Fusion of LC with MS is a major progress in the record of chromatography (1980's). Mass spectrometry in LC-MS helps to determine the essential composition and structural explication of a sample.[8]

Principle of LC-MS

Classic LC-MS scheme is fusion of HPLC with MS by using line that ionization source (Figure 03). The sample is separated by liquid chromatography, and the separated sample species are scattered into atmospheric pressure ion source, where they are changed into ions in the gas segment. The mass analyzer is then exercised to associate ions cohering to their mass to charge rate and sensor counts the ions arising from the mass analyzer and may also amplify the signal generated from each ion. As a conclusion, mass scale (a plot of the ion signal as a function of the mass-to- charge ratio) is created, which is applied to identify the essential or isotopic character of a sampler, the masses of particles and of molecules, and to interpret the chemical structures of molecules.[9-11]

Requirement of LC

Typically LC used in liquid chromatography- mass spectrometry is HPLC. The principle of separation in HPLC, is reverse phase mode or normal phase mode of adsorption. Reverse phase constricts with non-polar stationary phase with polar solvent/mobile phase and normal phase constricts with polar stationary phase with non-polar solvent/mobile phase. Reverse phase method have extensive choice of pharmaceutical solicitation. Examples for reverse phase method columns are C18/octa decylsilane (ODS), C8, C4, etc.[12] Normal phase method not extensively used in bioanalytical examination and not sensible for pharmaceutical solicitations subsequently maximum of the drugs are polar in environment and takes longer run time to be separate and detected.[13]

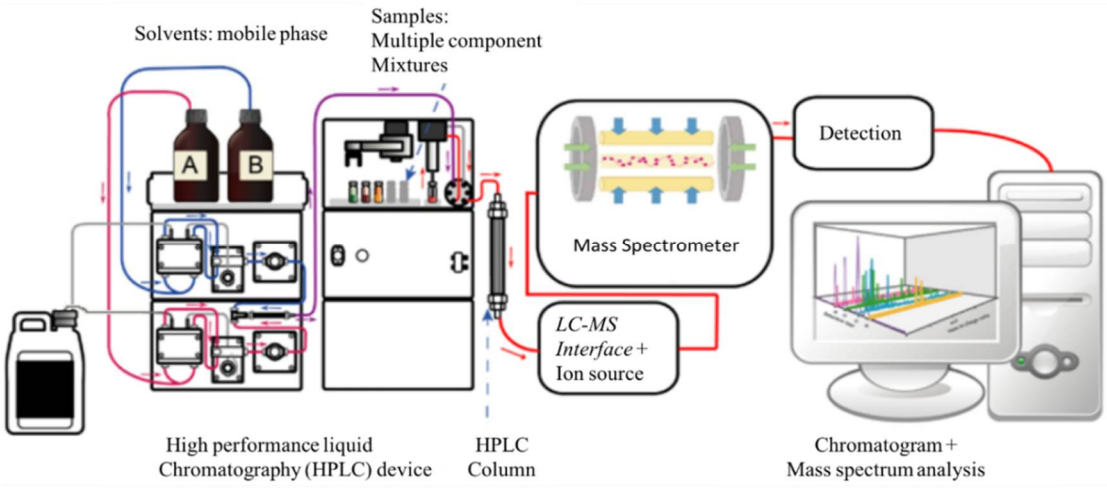

Figure 03: Instrumentation of LC-MS

HPLC Instrumentation

Primarily high pressure liquid chromatography instrument comprises flow pump, mobile phase mixing part (solvent degassing system), sample injector (manual/auto), guard column (if need), analytical columns, detector, plotter and integrators.[14]

Detector in HPLC

i. UV detector
Single wavelength (filter)
Variable wavelength (monochromator)
Multiple wavelengths (Photodiode array detector [PDA])
ii. Florescence detector
iii. Electrochemical detector
iv. Mass spectrometric

Requirements of LC-MS instrumentation

Substantially the LC-MS contains liquid chromatography association, ion generation unit/ ionization source, mass analyzer and mass spectrometric data acquirement.

The effluent mobile phase with separated compound from the liquid chromatography is interfaced with the ionization source of the Mass spectrometer.[15]

Ionization source

The following common ionization sources are[16-17],

i) Electrospray ionization (ESI)
ii) Atmospheric pressure chemical ionization (APCI)
iii) Matrix-assisted laser desorption/ionization (MALDI)
iv) Electron impact (EI)

v) Chemical ionization (CI)

Electrospray ionization (ESI): Electrospray is delivered by appertaining a field of strong electric to a liquid transferring through a capillary line with a weak flux. This yields electric large droplets which is then exposed to solvent. It disappearance causes coulombic repulsion to overcome the liquid surface tension, subsequent in discharge of ions from droplets. This is the principle of ion establishment by using this method.

Finding of sensitivity in this method is restricted to 8-10µL, and desires more volume to increase the sensitivity of the detection. By using ESI high mass sample, non-volatile molecules, liquids can be ionized and drawback of this ESI source of ionization is poor sensitivity, low fragmentation and source is instable.[18]

Atmospheric pressure chemical ionization(APCI): The principle of this process involves nebulizing the mobile phase with nitrogen gas (N2) and vaporizing it by heating it to a moderately high temperature (above 400 °C). The resulting vapor is then exposed to a corona discharge electrode to generate ions. APCI is the most commonly used ionization source in LC-MS. APCI are used to analyze clinical, toxicological, pharmaceutical, environmental, and chemicalory/industrial samples.[19-20]

Matrix-assisted laser desorption/ionization (MALDI): MALDI is an ionization technique for large and/or labile molecules such as peptides, proteins, polymers, dendrimers and fullerenes. In this technique, analytes are embedded in a matrix that absorbs energy at the wavelength of the laser. The nitrogen-ultraviolet (UV) laser (337 nm) is applied over the matrix in vacuum to generate analyte ions. The mechanism for the ionization is not clear, but three different models are postulated to explain the desorption of the matrix sample materials from the crystal surface (matrix will be crystalline in vacuum).[21-22]

* Upon laser impact, quasithermal Evaporation occurs resulting from increased molecular motion.

* This causes upper lattice layers of the matrix to be ejected (desorption)

* It is believed that the matrix then transfers protons to the sample molecules, thereby charging the analyte.[23]

The mass spectrometer analyzer

The mass spectrometer analyzer is component that takes ionized molecules and split up them based on charge to mass ratios and productions them to the detector where they are detected and future converted to a digital yield.[24]

Quadrupole Mass analyzer: Triple quadrupole and quadrupole are the most widely used analyzer because they are easy to use and cover a wide mass range (10 to 4000 A.M.U./atomic mass unit). Quadrupole offers good linearity for quantitative work and good resolution (up to 4000), quality of mass spectra, scan speed (5000 A.M.U. per second) and mass accuracy (0.1 to 0.2 A.M.U.). The working principle involves using electric fields to separate ions based on their mass-to-charge ratio as they are guided along the central axis of four parallel, equidistant poles. Ions are separated in a quadrupole based on the stability of their orbits in the oscillating electric fields applied to the rods. The quadrupole (Figure 04) consists of two pairs of metallic rods. Each opposing pair of rods is electrically connected together and a radio frequency (RF) voltage is applied between one pair of rods and the other. A DC voltage is then superimposed on the RF voltage. Ions travel down the quadrupole between the rods. Only ions with a certain mass to charge ratio (m/z) will reach the detector for a given voltage ratio. Other ions have unstable trajectories and collide with the rods. This allows the selection of an ion with a specific m/z and allows the operator to scan for a range of m/z values by continuously varying the applied voltage.[25-26]

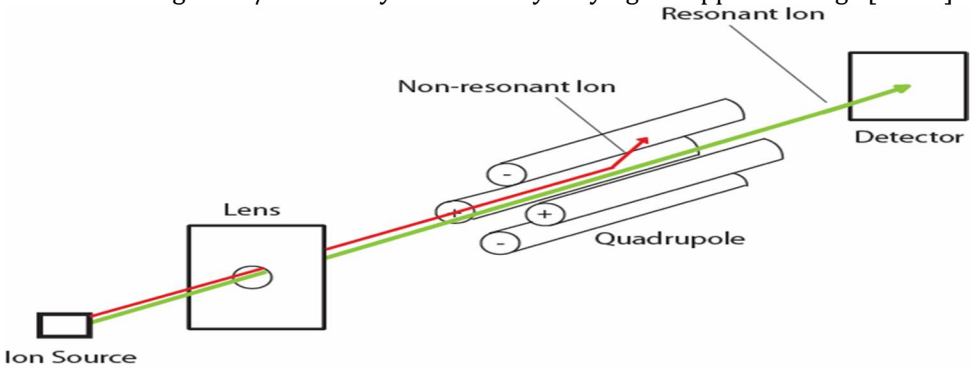

Figure 04: Schematic Diagram of Quadrupole Mass Analyser

Ion trap analyzer: This analyzer is also known as a Quadrupole Ion Trap Analyzer (QIT). Mostly it is used on GC/MS rather than LC/MS. The principle of the trap is to store the ions in a device (ion trap) consisting

of a ring electrode and two end cap electrodes. These ions are manipulated using applied DC and RF fields. The amplitude of the applied voltages allows the analyzer to trap ions with a specific mass-to-charge ratio within the analyzer. Unselected ions are given a trajectory by the electrostatic field that causes them to leave the trap. Fragmentation of selected ions is possible by filling the trap with inert gas. This is useful when structural information is needed.[27]

Time of Flight (TOF) analyzer: This analyzer is commonly referred to as TOF and is used in single MS systems. In the MS/MS configuration, the TOF is associated with a quadrupole (QTof) or another TOF (TOF-TOF) or an ion trap (QIT/TOF). From the ion source, it is accelerated to high velocity by an electric field present in the instrument's drift tube. The accelerated ions are detected by the detector in linear mode or reflection mode.[28]

Magnetic Sector Mass Analyser: In magnetic sector analysers, ions are accelerated by a magnetic field through a flight tube where the ions are separated by charge-to-mass ratios. When moving charges enter a magnetic field, the charge is deflected into a circular motion of a unique radius in a direction perpendicular to the applied magnetic field. Ions experience two equal forces in the magnetic field; Force due to the magnetic field and centripetal force. When similar ions pass through the magnetic field, they are all deflected to the same extent and all follow the same trajectory. Others will collide with either side of the flight tube wall or will not pass through the slit to the detector.[29]

Fourier transform-ioncyclotron resonance (FT-ICR): Ions sintering a chamber are trapped in circular orbits by strong electric and magnetic fields. When excited by a radio frequency (RF) electric field, the ions produce a time-varying current. This current is converted by Fourier transformation into ion orbital frequencies corresponding to their mass-to-charge ratios. FT-ICR mass analyzers can perform multiple stages of mass spectrometry without additional mass analyzers. They also have a large mass range and excellent mass resolution.[30]

Detector

The 3varioustypes of detectors are used in Mass Spectrometry, i.e. Micro channel plates, Dynolyte photomultiplier and Electron multipliers. Microchannel Plate (MCP) are frequently working in Time of Flight spectrometers. It would have very down time response and high degree of sensitivity (<1 nanosecond and single ion signal >50 mV independently). The dynode of Dynolyte photomultipliers transforms the electric ions into electrons. These electrons sticks to a phosphor and produces photons, and that photons are prepared to strike the photomultiplier to achieve multiplied signals for recording. Electron multipliers dynode is used to convert either negative, positive ions into electrons, that will be amplified and identified. It would be extensively used in quadrupole and ion trap mass analyzer spectrometric instruments. [31]

Data acquisition of Mass spectrometric system

The data accession network is allowed to digitalize electrical tocsins from the sensor and transmit them to the data system in a united formation. [32]

* Complete mass spectra (library to solve the analytical problem)
* Raw data of a small range (determine of isotope pattern/molecular weight)
* Mass chromatograms of selected ions (quantification)
* MS/MS experiments with Collisionally Activated Decompositions (CAD), such as daughter ion, parent ion and neutral loss scans structural information.

APPLICATION

Table 1: LC-MS/MS and HPLC application of selected drugs in biological fluids.

S. No	Title	Matrix	Mobile Phase	Flow Rate	Run Time	Stationary Phase	Range	Reference
1	A Rabid Method for Quantification of Selexipag in Human Plasma Using High Performance Liquid Chromatography with Electron Spray Ionization tandem	Human Plasma	5mM Ammonium Acetate: Methanol (75: 25, %v/v)	0.7 mL/min	5.0 min	Zorbax C18 (100×4.6 mm, 3.5 µm)	0.100-50.869 ng/mL	Satheshkumar. S and Muruganantham. V 2021.[33]

	Mass Spectrometry							
2	Bioanalytical Method for Quantification of Torsemide in Human Urine Using LC-MS/MS	Human Urine	5mM Ammonium Acetate: Methanol (70: 30, %v/v)	0. 7 mL/min	3.0 min	Zorbax C18 (100×4.6 mm, 5 µm)	0.443-5000.411 ng/mL	Satheshkumar. S and Muruganantham. V 2021.[34]
3	Bioanalytical Method development and validation of Tacrolimus in human blood using LC-MS/MS	Whole Blood	10mM Ammonium Acetate: Methanol (80: 20, %v/v)	0.6 mL/min	2.0 min	Thermo scientific Hypurity (50 × 4.6mm, 2.6 µm)	0.200-100.176 ng/mL	Muruganantham. V and Satheshkumar. S 2021.[35]
4	A simple and rugged bioanalytical method development and validation of Brivudine in human plasma by using high-performance liquid Chromatography	Human Plasma	0.5% Ortho Phosphoric acid: Methanol (35: 65, %v/v)	0.7 mL/min	10.0 min	Zorbax C18 (250×4.6 mm, 5 µm)	85.205 - 4500.246 ng/mL	Satheshkumar. S and Muruganantham. V 2020.[36]
5	Development of Validated Bioanalytical RP-HPLC Method for Determination of Sofosbuvir In Human Plasma	Human Plasma	1% Ortho Phosphoric acid: Acetonitrile (30: 70, %v/v)	0.7 mL/min	10.0 min	Zorbax C18 (250×4.6 mm, 5 µm)	30.566-2000.381 ng/mL	Satheshkumar. S and Muruganantham. V 2020.[37]

CONCLUSION

In conclusion, good chromatography is a critical element of any bioanalytical system and it can be termed as the capability to constantly accomplish separation conditions all the way through an entire analytical run. A good separation is a critical element of a bioanalytical system and should prop in achieving the needed particularity. Effective separations perform their job in as little time as possible while managing to meet all assay and any nonsupervisory conditions. Separation technology is ever advancing and serves as the foundation for a successful bioanalytical system. Conditions should be defined and tested using a system suitability sample at the starting and end of an analytical run. A duly gutted, tuned, and calibrated MS system benefits to ensure that detector response is sufficient for proper peak identification and integration. Together, these add to an effective analytical run.

A comprehensive understanding of both chromatographic proposition and its practical operation as well as the whole interpretation of sample extraction, chromatography and mass discovery will help the interpreters to develop a robust bioanalytical system. Numerous factors can contribute to the prospective failures of any given bioanalytical system. Some of the most important factors one should consider and mitigate are matrix effects, in-source breakdown of phase II conjugated metabolites, separation of isobaric metabolites, carryover, loss due to nonspecific adsorption, and stability. Besides a good scientific understanding of LC-MS/MS, bioanalytical scientists should be apprehensive of the current health authority[38-41] requirements as a worldwide submission needs arrangement with indigenous conditions.

AUTHORS CONTRIBUTIONS

All author have contributed equally.

CONFLICT OF INTERESTS

Declared none.

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