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A REVIEW ON DEVELOPMENT IN LC-MS FOR PHARMACEUTICALS

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ABSTRACT

The new development in Liquid chromatography- Mass spectrometery (LC- MS) is reviewed. This review includes a short history of LC-MS method, and explanation about different techniques like atmospheric pressure ionization (API), here ionization of the specified sample got ionized with respect to vacuum created and pressure inside the source after that the ionized ions pass to dector and quantities based by mass spectrometer, Microspray and Nanospray, Matrix effect, and microfluidics. For the purpose of qualitative and quantitative liquid chromatography-tandem mass spectrometery (LC-MS/MS) analysis, commonly using Atmospheric pressure ionization sources are Electro spray Ionizations (ESI) and atmospheric pressure chemical ionizations (APCI). Reduction in ESI gas load offers a few likely advantages in ion formation and ensuing mass analysis, and this review discuss about how micro/nano spray helps in the formation of stable spray.

Keywords: Liquid Chromatography-Mass spectrometery (LC-MS), Electrospray ionization (ESI), Microspray, Nano spray, Matrix effect

INTRODUCTION

History:

The pairing of chromatography with mass spectrometery is a very much evolved analytical system from 1950. Gas Chromatography–Mass spectrometery initially presented in 1952, in that time James

and Martin; they were attempting to make a development in the field of Tandem-Mass investigation technique **[1]**. Advancement in the LC-MS frameworks took more time than Gas chromatography-mass spectrometery and was straightforwardly identified with the finding of exact interfaces. Tal'roze and colleagues began advancement of Liquid chromatography-Mass spectrometery in the mid 1970s, to associate liquid chromatography columns with mass spectrometery source they initially utilize capillary **[2]**. Another comparative procedure examined by McLafferty and associates in 1973. The initial and accurate method for pairing liquid chromatography and mass spectrometery was this and it is called as the Capillary Inlet Interface. Here, this developed interface for Liquid chromatography-mass spectrometery had similar analytical capacities of Gas chromatography-mass spectrometery but it was restricted to analytes they are volatile in nature and mixtures of non polarcompounds with low molecular mass (under four hundred Da).Principle problem of this interface was the vaporization of solvent in the capillaries **[3]**.

Barber (1981)conducts a simple analysis at the start of the period of development of Fast Atom Bombardment for the molecule they are non volatile in nature and they are not stable thermally. It Mays contended that this method gone about as an impetus for the advancement of other ionization method like MALDI and ESI **[4]**.

Atmospheric pressure ionization

LC-MS/MS used for both qualitative and quantitative analysis and for this, an atmospheric pressure ionization source are essential, and usually ESI and APCI are utilized for this purpose **[5]**. During past decade, Electro spray ionization and APCIturned into the prevailing methods displacing thermo spray and so on **[4]**.

Electrospray has the ability to produce ion at very high mass when it used for mass spectrometery. Although, analysis of mass and determination of particles which is formed from polystyrene molecules having an average molecular weight of fifty one thousand is almost an inconceivable assignment using new instrumentation. Other than the demands in patents there is no other document of experimental data on small molecular compounds. Two research studies exhibited that electro spray is practical as a Liquid chromatography/Mass spectrometery interface for microbore liquid chromatography with five to ten FL/minute flow rate.Electrospray does not put any type of heat in the spray-ionization step, because

of this, even labile and sample having polar nature are also get ionized without heat degradation **[6]**.

One of the major issues in the atmospheric pressure ion sources while the free jet expansion is the sampling of ions into the vacuum area of the MS without much gathering of aqueous and molecules having polar nature. By placing curtain which is filled with nitrogen gas in the orifice of sample ion can prevent the entering of solvent vapors to orifice **[7]**. Electric field which draws the ions towards the orifice. Due to this, in the ionization region clusters of neutrals with many samples, solvents and other ions are formed .The nitrogen curtain gas molecules which carries different ions collide with the cluster ions due to the acceleration of the above cluster ions during free jet expansion step .Even a small collision may lead to the breakage of hydrogen bond, in that sense a vigorous collision must have the strength to cause the fragmentation of the sample ions **[8, 9]**.

The development happened in the field of ESI and APCI leads to the production of ions by the corona discharge at atmospheric pressure. When compared with ESI , Atmospheric pressure chemical ionization method is mostly used for compounds having less polarity, and it usually produce singly charged ions and it also find to have the ability to carry the flow rates of 1-2 ml/minutes, and for evaporate solvents it utilize heated nebulizer. For ion suppression process APCI technique is less suitable when compared with ESI. Even its dynamic concentration range is wider, it is inappropriate for compounds which are thermally unstable **[4]**.

Micro/nano spray

Decrease of ESI gas load offers a few likely advantages in the formation of ions and ensuing mass analysis Such as productive desolvation of analyte particles, lowering of charge competition, and decrease mass analyzer pressure, and it results much effective analyte ionization with expanded sensitivity. Using a micro electro spray source is one of the ways to reduce the increasing gas load of the Electrospray ionization **[10]**. In Micro-ESI, the charged droplets originate from the Microbore fused with silica capillary which is used as needle. The name Micro electro spray which stands for the microbore size of the Electrospray ionization needle and it does not have any connection with the flow rate at which it functioned **[11]**.

The main aim was to utilize nano-LC for biological samples concentration **[12]**. Due to the larger bed volume and higher flow rate,

typical microbore liquid chromatography columns are inappropriate for the analysis of sample at ato-molar concentration. Due to this, ESI forced to adapt nano-LC to reduce the internal diameter of the needle which used to spray and which allows the formation the stable spray at sub-mL/minute flow rate. By packing micro-electro spray ionization needle with the trapping medium can cause the elimination of the post column dead volume and thus increase the delicacy. This was the further stage in the formation of nano- liquid chromatography and micro-electro spray ionization **[10]**.These two techniques together known as micro Electro spray ionization/Liquid chromatography.

Micro-Electro spray ionization have lots of other advantages such as: (a) High sampling efficacy **[13]**, (b) Micro- Electrospray ionization have lower flow rate and which can greatly reduce the gas load which present in the analyzer by reducing the solvent delivery, and (c) Micro- Electrospray ionization have the ability to produce very small charged molecules and which result in the great sample desolvation **[14]**. Nanospray **[15]** picospray **[16]** are the miniaturized form of Micro-ESI, and these are greatly accepted for sample having small volume and non sheath Capillary zone electrophoresis interfaces due to their reduced solvent flow **[17, 18]**.

For the working of Microspray or Nanospray required a low i.d capillary outlet, for this the capillary tip are usually coated with metal .But the metal tip coating may not have enough strength and for doing coating a practically skilled operator is required, these are the disadvantage of this technique **[4]**.

Microfluidics

Microfluidic is a technology which is very useful for the processing of samples they are much precious and there for limited in volume. Some of the biological samples show the property of high sample complexity and low in concentration **[19]**.

According to the Kevin Killer and team **[20]**, Chip based HPLC or Chip-Liquid chromatography/Mass spectrometry is a combined form of sample enriched stationary phase, high pressure driven column of liquid Chromatography and also an integrated tip of electro spray ionization. The overall performance of this device can be increased by optimization of the work of Electrospray ionization, by the elimination of biologically retentive compounds, and by reducing the connecting volume.

William M and team **[21]**, with the purpose of conducting ions present in the atmosphere to vacuum an atmospheric inlet were

designed, and they coupled a MS having high pressure with a microchip ionization source. Using a mini-CIT detector which is functionedat pressure more than one Torr using air as a buffer gas, Micro chipinfusion and CE separations were performed to demonstrate the ability of a miniaturized Capillary Electrophoresis-Electrospray ionization-Liquid chromatography system. This study shows that, this type of system has the ability to displace costly LC Analytical chemistry mass spectrometery system for targeted applications.

Microfluidics incorporated on glass chips with etched micro channel from which solutions were electro sprayed. Often with a serpentine configuration, separations like Capillary electrophoresis and Capillary electrochromatography can be functioned on these chips. Diagrammatic representation of an Electrospray chip is shown in **Figure 1**.

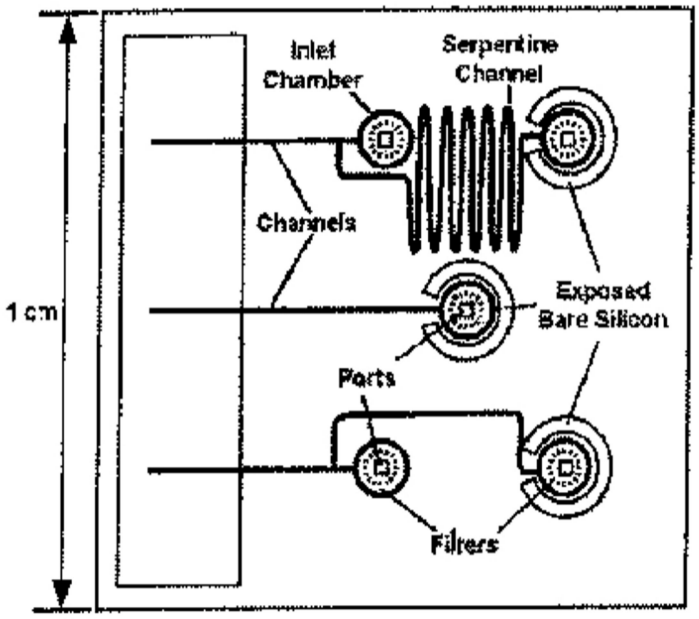

Figure 1: Diagrammatic representation of an electro spray chip

Matrix effect

Analysis of the complex biological matrix is the main function of Liquid chromatography-Electrospray ionization-Mass spectrometery. Analyte response may get suppressed by the components present in the matrix due to the rivalry for Electrospray ionization droplets surface and therefore ionization take place. Due to this effect, the sample in matrix and standard may show different response and which cause struggling in quantitative studies and compound detection. It highlights the significance of chromatography in an

analysis. This effect can be reduced by good separation process **[4]**.

The Accuracy and Precision of the bio analytical process may get affected by the matrix effect and thereby it decreases the quality of the result. The matrix effect largely works on the protein precipitation sample preparation along with LC-MS/MS, one of the most commonly used method in bio analysis. Matrix effect can be eliminated by considering some of the points like, using more sensitive instruments, cleaner sample preparations, inject fewer materials, using separate chromatographic methods and so on **[22]**.

OTHER METHODS FOR IONIZATION

If one of the method, which can ionizes all analytes with similar effectiveness, then it can called as the best ionization supply. Nonetheless, suppression effects are the significant problem in accomplishing this optimal situation.

Atmospheric pressure photoionization is one of the new methods of ionization for LC-MS. In non polar compounds, a charged molecular ion is formed by the absorption of proton and ejection of electron from a molecule; this is the mechanism of APPI **[23, 24]**.

Developments keep goingin pairing liquid chromatography and other techniques for ionization. Cappiello **[25]** and his team explain two techniques for pairing EI, with capillary high performance liquid chromatography.

Major tasks for Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) is the clear- cut identity of analyte. Because of the changeability in situ time-of-flight (TOF) m/z measurement, it is struggle to pair the current MALDI-TOF/TOF MS units using of external calibration this variability is currently denoted, which may decrease the attainable accuracy of mass for matrix assisted laser desorption ionization imaging mass spectrometryand made it challenging to pair those information to downstream LC-MS/MS result **[26]**.

Capillary electrophoresis (CE) and capillary electrochromatography (CEC)

Capillary electrophoresis interfacing, currently by means of Electrospray ionization and nanospray is great, because it works at rates of flow ordinary of these delivered in capillary electrophoresis. Traditional Electrospray ionization works at high flow rates, requiring "make-up" float to around five ml/minute and this is exceptional given through a co-axial device, as to initially depicted by way of Smith's and his crew **[27]**. Regularly an organic solvent like methanol is

used as a make-up liquid and which working with much effective sprays formation when blended on the tip of the capillary with the aqueous capillary electrophoresis eluent. Improvements in Capillary electrophoresis/Mass Spectrometry reviewed by Von Bocke and team.

CEC makes use of similar interfaces as CE, as might be normal, being fundamentally same as strategies. CEC utilizes capillaries, normally fifty to hundred mm i.d., loaded with the stationary phase of high performance liquid chromatography. Drift of Eluent is accomplished by electro osmotic flow, instead of hydrodynamic pumping as in high performance liquid chromatography and detachment might be viewed as a combination of Capillary electrophoresis and high performance liquid chromatography partition mechanism. Main pairing of Capillary electro chromatography with Mass spectrometery becomes a way of Verheij **[29]**, who utilized pressure assistant for eluent stream in the method referred to as "pseudo electro chromatography" and Continuous flow-Fast atom bombardment-Mass spectrometry. Genuine Capillary electro chromatogram with electro osmotic flow was moreover initially paired to Continuous flow-Fast atom bombardment-Mass spectrometry **[30]**, yet practically all of the resulting reviews have applied Electrospray ionization. A review **[31]** and text **[32]** give additional data.

Mass Analyzers

Advancements on Time of-flight analyzers have been fast in the course of the most recent couple of years, especially on account of improvements in computerized electronics. The revival of interest in Time of flight analyzers most likely emerged from their utilization in matrix assisted laser desorption/ionization. At first, TOF was a little goal procedure; however is currently fit for 20000 or more, resolution, to a great extent in light of the utilization of reflection. This resolution grants“exact mass” calculation based on small or medium molecules, from which, empirical formula is decided. Analyzers include fast spectral acquisition, accuracy in mass calculation springing up from stability over the m/z range and no theoretical restrict to m/z are the other advantages of time of flight **[33]**. The utilization of TOF instruments is probably going to keep on expanding for years to come, overriding the utilization of magnetic sector instruments in numerous spaces.

CONCLUSION

LC-MS has ended up being an incredibly sensitive method for the analysis of drugs. It

assumes significant parts within the investigations of drugs metabolisms, disclosure of latest drug and analysis, reorganization and classification of impurities present in the drug products. Pairing high-throughput sample planning methods with multiplexed LC-MS-MS will allow much fast analysis and the ability of interfacing liquid chromatography-nuclear magnetic resonance with mass spectrometery to give a Liquid chromatography-Nuclear magnetic resonance-mass spectrometry method can permit the clear identification of drug. There are troubles in the technical side to connecting micro and capillary technique with nanospray mass spectrometery, progress can be expected in this area. Utilization of microfluidic system provides possibilities for miniaturized chip separation and surprisingly chance of miniaturized mass spectrometers in the fairly extra some distance off destiny.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest among us.

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