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Liquid Cheomatography Andtandem Mass Spectrometry(Lc-Ms-Ms) As Tool For Characterization And Quantification Of Pharmaceuticals

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ABSTRACT
With the invention of electrospray ionisation (ESI), a simple and robust interface, liquid chromatography-mass spectrometry (LC-MS) has become a common technique. It may be used to analyse a wide range of biological molecules, and the use of tandem mass spectrometry and stable isotope internal standards allows for the development of very sensitive and precise assays, however some method optimization is required to reduce the effects of ion suppression Fast scanning speeds provide for a high degree of multiplexing, allowing for the measurement of several substances in a single analytical run. With the development of more economical and reliable instruments, LC-MS is beginning to compete with traditional liquid chromatography and other techniques such as immunoassay in numerous areas of clinical biochemistry.

KEYWORDS: Liquid Chromatography, Tandem-mass spectrometry

LIQUID CHROMATOGRAPHY

Liquid chromatography is a method used to split a pattern into its character parts. This separation takes place primarily based totally at the interactions of the pattern with the cellular and desk bound stages.(1) Because there are numerous desks bound/cellular segment combos that may be hired while keeping apart an aggregate, there are numerous distinctive varieties of chromatography which can be categorized primarily based totally at the bodily states of these stages. (2) Liquid-strong column chromatography, the maximum famous chromatography method and the only mentioned here, capabilities a liquid cellular segment which slowly filters down thru the strong desk bound segment, bringing the separated additives with it.(3)

General Scheme

Components inside an aggregate are separated in a column primarily based totally on every element's affinity for the cellular segment.(4) So, if the additives are of various polarities and a cellular segment of an awesome polarity is surpassed through the column, one element will migrate thru the column quicker than the opposite. (5) Because molecules of the equal compound will usually flow in companies, the compounds are separated into awesome bands within side the column. If the additives being separated are coloured, their corresponding bands may be visible. Otherwise as in excessive overall performance liquid chromatography (HPLC), the presence of the bands is detected the use of different instrumental evaluation strategies including UV-VIS spectroscopy.(6) The following discern indicates the migration of additives inside an aggregate: In the primary step, the aggregate of additives sits atop the moist column. As the cellular segment passes thru the column, the 2 additives start to separate into bands. In this instance, the purple element has a more potent affinity for the cellular segment even as the blue element stays surprisingly constant within side the desk bound segment. (7) As every element is eluted from the column, every may be accumulated one after the other and analysed with the aid of using something technique is favoured.(8) The relative polarities of those compounds are decided primarily based totally at the polarities of the desk bound and cellular stages. If this test had been carried out as ordinary segment chromatography, the purple element could be much less polar than the blue element.(9) On the opposite hand, this end result yielded from opposite segment chromatography could display that the purple element is extra polar than the blue element.(10)

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History of Liquid Chromatography

The first acknowledged chromatography is historically attributed to Russian botanist Mikhail Tswett who used columns of calcium carbonate to split plant compounds in the course of his studies of chlorophyll. (11)This passed off within side the 20th century (1901). Further improvement of chromatography passed off while the Nobel Prize changed into offered to Archer John Porter Martin and Richard Laurence Millington Synge in 1952. (12) They had been capable of set up the fundamentals of partition chromatography and additionally increase Plate principle. (13)

Column Chromatography

The desk bound segment in column chromatography is maximum usually a great adsorbent strong; a strong this is Capin a position maintains onto fuel line or liquid debris on its outer floor. The column usually utilized in column chromatography appears much like a Pasteur pipette (Pasteur pipettes are used as columns in small scale column chromatography).(14) The slender go out of the column is first plugged with glass wool or a porous plate that allows you to aid the column packing fabric and hold it from escaping the tube. Then the adsorbent strong (typically silica) is tightly packed into the glass tube to make the keeping apart column. (15)The packing of the desk bound segment into the glass column need to be carried out cautiously to create a uniform distribution of fabric. A uniform distribution of adsorbent is critical to decrease the presence of air bubbles and/or channels within side the column.(16) To end making ready the column, the solvent for use because the cellular segment is surpassed thru the dry column. Then the column is stated to be "wetted" and the column needs to continue to be moist in the course of the complete test. Once the column is effectively organized, the pattern to be separated is located on the pinnacle of the moist column. A photograph of a packed keeping apart column may be determined within side the hyperlinks. (17)

Components

Chromatography is powerful due to the fact distinctive additives inside an aggregate are drawn to the adsorbent floor of the desk bound segment with various tiers relying on every additive's polarity and its particular structural characteristics, and additionally its interplay with the cellular segment.(18) The separation this is completed the use of column chromatography is primarily based totally on elements which can be related to the pattern.(19) So, an element this is extra drawn to the desk bound segment will migrate down the keeping apart column at a slower charge than an element that has a better affinity for the cellular segment. Also, the efficacy of the separation is depending on the character of the adsorbent strong used and the polarity of the cellular segment solvent. (20)

- **Stationary Phase** The form of adsorbent fabric used because the desk bound segment is crucial for green separation of additives in aggregate. Several distinctive strong can be hired. Adsorbent fabric may be selected primarily based totally on particle length and interest of the strong. (21) The interest of the adsorbent is represented with the aid of using its interest grade, that's a degree of an adsorbent's enchantment for solutes within side the pattern answer. The solids with the very best interest grading are the ones which can be absolutely anhydrous. Silica gel and alumina are some of the maximum famous adsorbents used. (22) Alumina caters properly to samples that that require precise situations to safely separate. However, the usage of non-impartial desk bound stages ought to be carried out with remarkable caution; a growth or lower of pH within side the alumina desk bound segment might also additionally permit chemical reactions within side the additives of the aggregate. Silica gel, however, is much less likely than alumina and may usually be used as an all-round adsorbent for maximum additives in answer.(23) Silica is likewise favoured due to its excessive pattern capacity, making it one of the maximum famous adsorbent materials.

- **Mobile Phase**

The right cellular segment needs to additionally be selected for the exceptional separation of the additives in an unknown aggregate.(24) This eluent can be selected primarily based totally on its polarity relative to the pattern and the desk bound segment. With a sturdy polar adsorbent desk bound segment like alumina, a polar solvent used because the cellular segment can be adsorbed with the aid of using the desk bound segment, which might also additionally displace molecules of pattern within side the aggregate and can motive the pattern additives to elute range quickly. This will offer little separation of the pattern, so its miles exceptional to begin

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elution with a solvent of decrease polarity to elute the additives which can be weakly adsorbed to the desk bound segment first. (25) The solvent can also be modified in the course of separation that allows you to alternate the polarity and consequently elute the numerous additives one after the other in an extra well-timed manner. This technique may be very much like the gradient technique of separation utilized in High Performance Liquid Chromatography (HPLC). (26)

Types of Chromatography

- **Normal Phase Chromatography:**

The additives in an aggregate will elute at distinctive costs relying on every one's polarity relative to the next. When the column for use for the separation is extra polar than the cellular segment, the test is stated to be an ordinary segment technique. In ordinary segment (27) chromatography, the desk bound segment is polar, and so the extra polar solutes being separated will adhere extra to the desk bound adsorbent segment. (28) When the solvent or gradient of solvents is surpassed thru the column, the much less polar additives can be eluted quicker than the extra polar ones. The additives can then be accumulated one after the other, assuming good enough separation changed into completed, so as of growing polarity. This technique of chromatography isn't particular to liquid-strong column chromatography and is regularly used while appearing High Performance Liquid Chromatography (HPLC). Although HPLC is an instance of liquid-liquid chromatography, wherein each the desk bound and cellular stages are liquid, ordinary segment elution is completed with the aid of using coating the strong adsorbent column with a polar liquid. (29)

- **Reverse Phase Chromatography:**

In opposite segment chromatography, the polarities of the cellular and desk bound stages are contrary to what they had been while appearing ordinary segment chromatography. Instead of selecting a non-polar cellular segment solvent, a polar solvent will be selected. Or, if the test calls for a solvent polarity gradient, the gradient need to be executed with the maximum polar solvent first and the least polar solvent last (opposite order of ordinary segment chromatography). Common polar solvents combos of solvents encompass water, methanol, and acetonitrile. (30) It is barely extra tough and pricey to reap a column wherein the desk bound segment is nonpolar, as all strong adsorbents are polar with the aid of using nature. The nonpolar desk bound segment may be organized with the aid of using coating salinized silica gel with a nonpolar liquid. Salinizing the silica gel reduces the silica gel's cap potential to adsorb polar molecules. (31) Common non polar liquid stages encompass silicone and numerous hydrocarbons. An opportunity to this form of column is utilized in HPLC, wherein a bonded liquid segment is used because the desk bound segment. The much less polar liquid is chemically bonded to the polar silica gel within side the column. So, the use of opposite segment, the maximum polar compounds with inside the pattern answer can be eluted first, with the additives following having reducing polarities. (32)

- **Flash Chromatography:**

Because the elution charge of the cellular segment in normal column chromatography as defined above is managed in the main with the aid of using gravity, chromatographic runs can probably take a completely long term to complete. (33) Flash chromatography is a changed technique of column chromatography wherein the cellular segment movements quicker thru the column with the assist both pressurized air or a vacuum. A vacuum line is connected to the lowest of the keeping apart column this pulls the cellular segment solvent, and the additives within side the cellular segment, thru the column at a quicker charge than gravity does. A discern of this set-up may be visible within side the hyperlinks section.(34) Flash chromatography is powered with the aid of using compressed air or air pumps works with the aid of using pushing the cellular segment thru the column and achieves quicker glide costs of the cellular segment simply as vacuum facilitated flash chromatography does. For this technique, a pressurized airline is connected to the pinnacle of the keeping apart column. (35)

It is because of this that flash chromatography is likewise known as medium stress chromatography. An inert fueloline is used as to now no longer engage with the cellular or desk bound segment or the element aggregate. Nitrogen fueloline is usually used for this technique of chromatography. Many gadgets are to be had to carry out flash chromatography as effectively as viable: pricey columns, pumps, and glide controllers. This keeps a consistent and specific air stress or vacuum to the column that allows you to reap regular glide charge of the cellular segment and favourable separation of the samples in answer. However, much less pricey options are to be had, as glide controllers may be made in order that pressurized air may be used to facilitate flash

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chromatography. By the use of the above apparatus, shopping pricey air pumps may be avoided. This technique is beneficial to an extent. (36)

Since the glide charge of the pressurized fuel line is managed manually with the aid of using the glide charge controller, its miles extra tough to quantify the glide charge and hold that glide charge consistent. Instruments to be had for flash chromatography are capable of set glide costs digitally and hold glide charge consistent. Flash chromatography is much like HPLC in that the cellular segment is moved thru the column with the aid of using making use of stress to the solvent that allows you to reap a faster end result. However, in flash chromatography, handiest medium stress is carried out to the device within side the answer. In HPLC, pressures as excessive as 5000 psi may be carried out within side the column with the aid of using excessive overall performance pumps. (37)

Other Varieties of Liquid Chromatography

- **Partition Chromatography:**

In this technique, each the desk bound segment and the cellular segment are liquid. The desk bound segment liquid could be an immiscible liquid with the cellular segment. Liquid-Solid Chromatography (38) this technique is much like partition chromatography handiest that the desk bound segment has been changed with in bonded inflexible silica or silica primarily based totally element onto the inner of the column. Sometimes the desk bound segment can be alumina. The analytes which can be within side the cellular segment which have an affinity for the desk bound segment can be adsorbed onto it and people that don't will by skip thru having shorter retention times. (39)Both ordinary and opposite stages of this technique are relevant.

- **Ion Exchange or Ion Chromatography:**

This is a form of chromatography this is carried out to split and decide ions on columns which have a low ion trade capacity. This is primarily based totally at the equilibrium of ion trade among the ions in answer and the counter ions to pair with the oppositely charged ions which can be constant to the desk bound segment. (40) This desk bound segment could both have high quality of bad purposeful companies affixed to it, typically sulfonate (-SO3-) or a quaternary amine (-N (CH3)3+), being a cation and anion exchanger respectively.

- **Size Exclusion Chromatography:**

Size exclusion chromatography separates molecules with the aid of using their length. This is carried out with the aid of using having the desk bound segment be full of small debris of silica or polymer to shape uniform pores. The smaller molecules get trapped within side the silica debris and could elude from the column at a charge this is extra than that of large molecules. Thus, the retention time relies upon on the dimensions of the molecules. Larger molecules can be swept away within side the cellular segment, consequently having a smaller retention time. Also be aware that during this form of chromatography there isn't any interplay, being bodily or chemical, among the analyte and the desk bound segment. (41)

- **Affinity Chromatography:**

This form of chromatography includes binding a reagent to the analyte molecules in a pattern. After the binding, handiest the molecules which have this ligand are retained within side the column, the unbound analyte is surpassed thru within side the cellular segment.(42) The desk bound segment is typically agarose or a porous glass bead this is capable of immobilize the bonded molecule. It is viable to alternate the elution situations with the aid of using manipulating the pH or the ionic power of the binding ligand. This technique is regularly utilized in biochemistry with inside the purification of proteins. The ligand tag is bonded and after separation the tag is then eliminated and the natural protein is obtained.

- **Chiral Chromatography:**

Chiral chromatography allows the usage of liquid chromatography to split a racemic aggregate into its enantiomeric parts. A chiral additive may be delivered to the cellular segment, or a desk bound segment that has chiral residences may be used. A chiral desk bound segment is the maximum famous option. The desk bound segment must be chiral that allows you to apprehend the chirality of the analyte, this could create appealing forces among the bonds and additionally shape inclusion complexes.(43)

- **Plate Theory and Rate Theory**

Plate principle and Rate principle are theories which can be relevant to chromatography. Plate principle describes a chromatography device as being in equilibrium among the desk bound and cellular stages. These perspectives the column as divided into some of imaginary theoretical plates.(44) This is extensive due to the fact because the wide variety of plates in a column will increase or the peak equal theoretical plates or HETP will

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increase, so does the separation of additives. It additionally offers an equation that describes the elution curve or the chromatogram of a solute it is able to additionally be used to locate the quantity and the column efficiency.

HETP=LN (1) (1) HETP=LN
Where in L= column duration and N= wide variety of theoretical plates

The Rate principle however describes the migration of molecules in a column. This covered band shape, broadening, and the diffusion of a solute. Rate principle follows the Van Deemter equation, that's the maximum suitable for prediction of dispersion in liquid chromatography columns. It does this with the aid of using thinking of the numerous pathways that a pattern need to journey thru a column. Using the Van Deemter equation, its miles viable to locate the premier pace and a minimal plate height.

H=A+Bu=Cu (2) (2) H=A+Bu=Cu

Where in AA = Eddy-Diffusion, BB = Longitudinal Diffusion, CC = mass switch, uu = linear velocity

Instrumentation

This schematic is of the simple instrumentation of a liquid-solid chromatograph. The solvent inlet brings within side the cellular segment that's then pumped thru the inline solvent clear out and surpassed thru the injection valve. This is wherein the cellular segment will blend with the injected pattern. It then receives surpassed thru some other clear out after which surpassed thru the column wherein the pattern can be separated into its additives. The detector detects the separation of the analytes and the recorder, or typically a pc will file this information. The pattern then is going through a backpressure clear out and into wastes. (45)

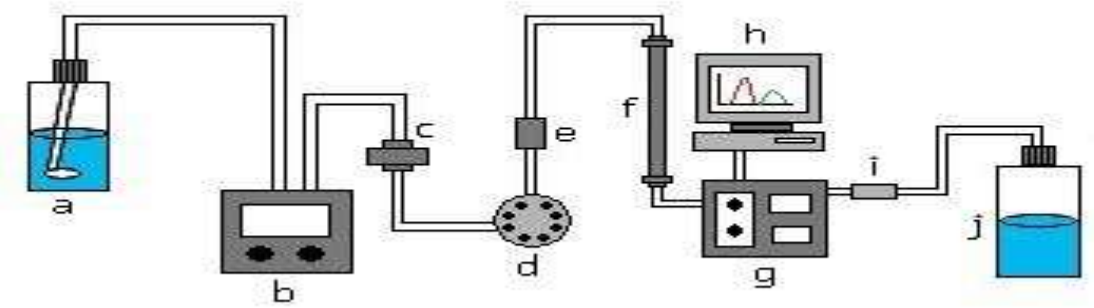

Fig: -1 liquid-solid chromatography

A simple LC device is composed of (a) a solvent inlet filter (b) pump, (c) inline solvent filter (d) injection valve, (e) precolumn clear out (f) column, (g) detector, (h) recorder, (i) backpressure regulator, and a (j) waste reservoir.

Advantages / Disadvantages

Liquid-strong column chromatography is a powerful separation method while all suitable parameters and device are used. s. Key advantages in this context are the high analytical specifi city, the wide range of potential target analytes and the fl exible development of sensitive analytical methods for large numbers of drug candidates.

This technique is especially powerful while the compounds with inside the aggregate are coloured, as this offers the scientist the cap potential to look the separation of the bands for the additives within side the pattern answer. Even if the bands aren't seen, positive additives may be found with the aid of using different visualization techniques.

One technique that may fit for a few compounds is irradiation with ultraviolet light. This makes it surprisingly smooth to accumulate samples one after some other. (46)However, if the additives within side the answer aren't seen with the aid of using any of those techniques, it is able to be tough to decide the efficacy of the separation that changed into performed. In this case, separate collections from the column are taken at certain time intervals. Since the human eye is the number one detector for this manner, it's miles simplest

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while the bands of the awesome compounds are seen.

Liquid-solid column chromatography is likewise a much less pricey manner than different techniques of separation (HPLC, GC, etc.). This is due to the fact the maximum simple kinds of column chromatography do now no longer require the assist of pricey equipment like excessive stress solvent pumps utilized in HPLC. In techniques except flash chromatography, the glide of the cellular segment, the detection of every separation band, and the gathering of every element, are all carried out manually with the aid of using the scientist. (47) Although this introduces many caps potential times of experimental error, this technique of separation may be very powerful while carried out effectively. Also, the glass put on used for liquid-strong column chromatography is surprisingly less expensive and with ease to be had in lots of laboratories. Burettes are usually used because the keeping apart column, which in lots of instances will paintings simply in addition to a pricey pre-organized column. For smaller scale chromatography, Pasteur pipettes are regularly used. (48)

Flash chromatography has the cap potential to be extra high priced than the preceding techniques of separation, especially while sophisticated air pumps and vacuum pumps are needed. When those portions of equipment aren't needed, however, a vacuum line may be alternatively related to an aspirator2 on a water faucet. Also, home-made pressurized air glide controllers may be made as proven previously.

LC-MS/MS Hyphenation: The term "**hyphenation**" was first adapted by Hirsch Feld in 1980 to describe a possible combination of **two or more instrumental analytical methods in a single run** (Hirschfeld, 1980)**.** The aim of the coupling is to obtain an information-rich detection for both identification and quantification compared to that with a single analytical technique.

LC-MS is a chemistry technique that combines the physical separation of liquid chromatography (or HPLC) with the mass spectroscopy. A typical automated LC-MS system (Figure 3) consists of double three-way diverter inline with an autos ampler, LC system, the Mass spectrometer. The diverter generally operates as an automatic switching valve to divert undesired portions of eluting from the LC system to waste before the sample enters the MS. The ionization techniques used in LC-MS are generally soft techniques that mainly display the molecular ion species with only a few fragment ions. The information obtained from a single **LC-MS run is not sufficient for confirmation of the identity of the compound. Nevertheless, the problem has now been solved by the introduction of tandem mass spectrometry (MS-MS), which provides fragments through collision-induced dissociation of the molecular ions produced. Use of LC-MS-MS is increasing speedily day by day**. Hyphenated techniques such as HPLC coupled to UV and mass spectrometry (LC-UV-MS) have been proved to be extremely useful in combination with biological screening for a rapid survey of natural products. Nowadays, various types of LC-MS systems incorporating different types of interfaces are available commercially. The interfaces are designed in such a way that they offer adequate nebulization and vaporization of the liquid, ionization of the sample, removal of the excess solvent vapour, and extraction of the ions into the mass analyser. The two most widely used interfaces, especially in relation to natural product analysis, are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)Mass spectrometry allows the identifi cation, characterization, and quantifi cation of chemical compounds as target analytes based on their respective molecular masses and fragmentation patterns; the molecular mass of an entire molecule and / or the mass fragmentation pattern of thermodynamically favoured and stable fragments of the molecule are used to describe a specifi c compound. (49)

The three basic processes of mass spectrometry are:

1. Generation of ions (Fig.2)
2. Transport and selection of ions in space in an environment of high vacuum, typically according to their mass-to-charge (m / z) values
3.Ion detection. (Fig.3)

The principle of MS/MS is that a sample is ionized and analyzed in one stage of mass spectrometry. A particular m/z value is selected from the mass spectrum and directed into a collision cell containing a neutral gas (in general argon, helium or nitrogen). The collision with the gas excites the ion vibrationally, a process known as collision induced dissociation (CID). (50) Other post-source induced fragmentation approaches in the reaction chamber include electron capture and electron transfer dissociation methods (ECD and ETD), surface-induced-dissociation (SID) and photodissociation. The ions (fragments) generated

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by these processes are separated and recorded in a second stage of mass spectrometry. Mass spectrometers that perform the three steps of the MS/MS process (precursor ion selection, induced dissociation and mass analysis of the product ion(s)) in spatially separated devices, are called "tandem-in-space mass spectrometers", whereas devices that execute the three steps sequentially in the same device are called "tandem-in-time mass spectrometers". Examples of the first type are QQQ and hybrid MS instruments (Q-TOF, IT-TOF, etc), while the second category includes IT, Orbitrap, and FTICR. (51)

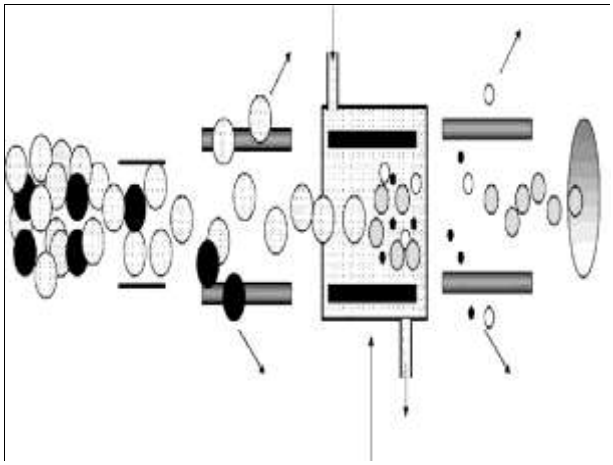

Fig.2 Generation of ions (ESI , APCi, APPI)

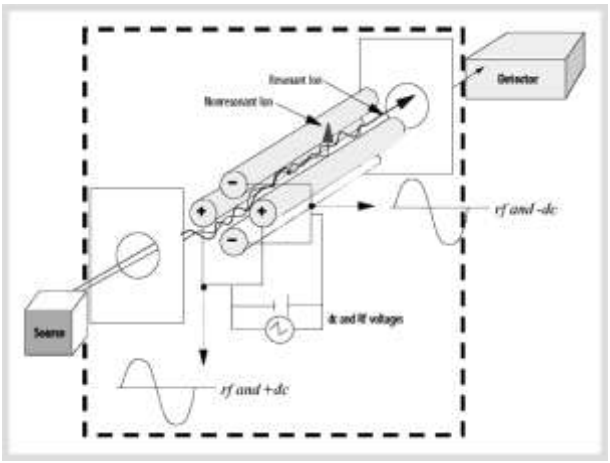

Fig.3Scheme of a quadrupol mass fi lter (ion Detection)

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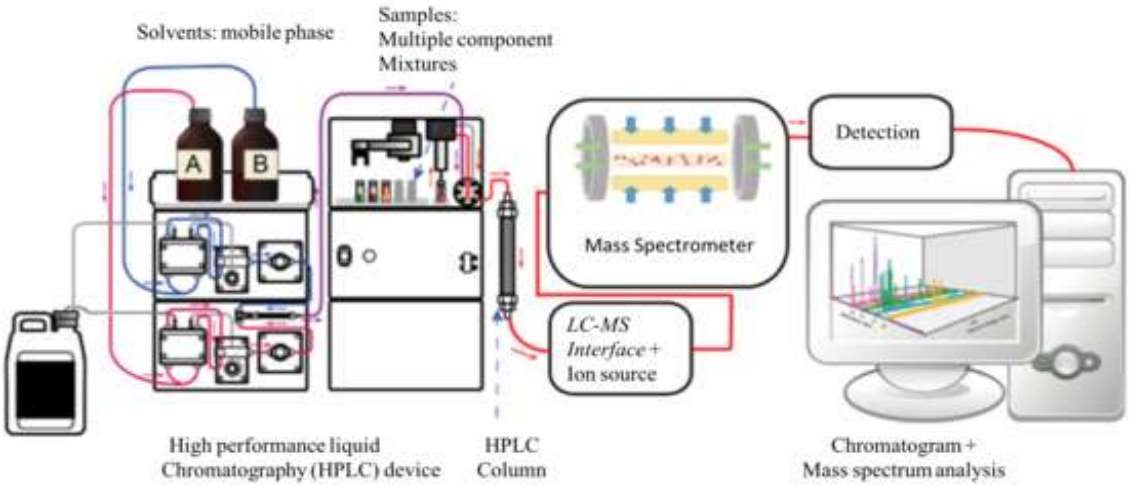
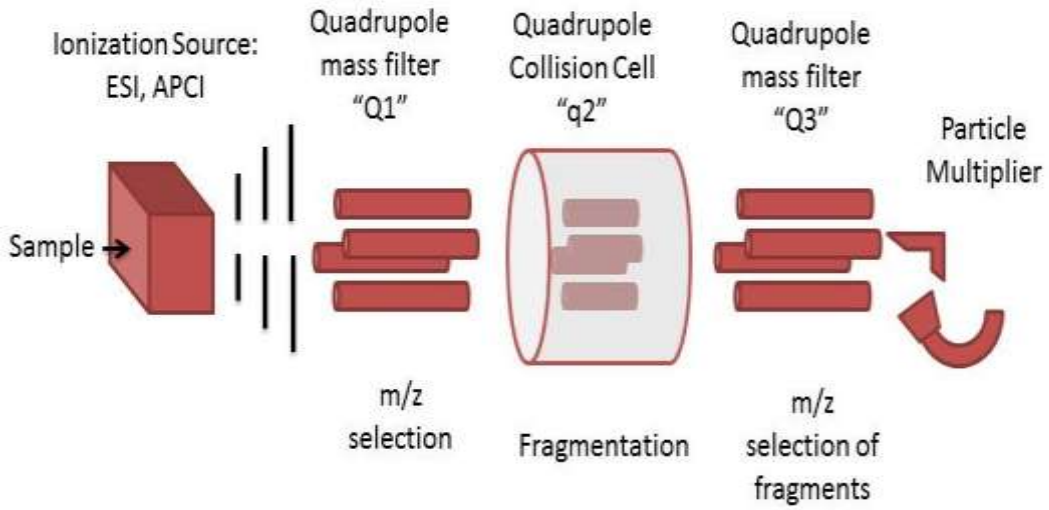


Fig.4 LC-MS Hyphenation

Fig. 4.1 LC-MS/MS Hyphenation



Tandem mass spectrometry

Tandem MS is explained in the diagram below. After samples are ionised (by ESI, MALDI, EI, and other methods) to produce a mixture of ions, precursor ions with a certain mass-to-charge ratio (m/z) are selected (MS1) and then fragmented (MS2) to produce product ions for detection. The sequence of selection-fragmentation-detection can also be applied to first-generation product ions. Select product ions obtained in MS2 can, for example, be fragmented further to form another group of product ions (MS3), and so on. (52)

PRINCIPLE OF TANDEM MASS SPECTROMETRY: Application of the principle of tandem mass spectrometry represented a further breakthrough in LC-MS since this technique compensates for the rather poor chromatographic capacity of LC by application of a highly specific mass spectrometric detection. In tandem MS (syn. MS / MS) two quadruple mass fi lters arecombined and target analyte molecules from the fi rst quadrupole are submitted to a controlled fragmentation in a collision cell (Fig. 5). The entirety of ions formed by ESI is transferred into the fi rst quadrupole mass fi lter. Here, the m / z of the intact ionized target analyte is selected, all other ion species are fi ltered out. The selected ions sharing identical mass-to-charge ratio (m / z) are continuously transferred into the collision cell. In

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this part of the MS analyzer, a collision gas – in most cases argon or nitrogen – is present in trace amounts (10 − 3 mbar). Ions selected by the fi rst quadrupole collide with these gas molecules and fragment into characteristic product ions. For most analytes several characteristic, thermodynamically favored product ions are generated. The entirety of these fragment ions are guided to the second quadrupole.(53) The radiofrequency settings of the second analytical quadrupole is adjusted in a way that only one selected fragment ion will pass, while all other fragment ion species are fi ltered out. Thus, one defi Ned"daughter ion" from one defi ned "parention" fi nally reaches the ion detector. The selection of masses of the fi st and the second quadrupoles, defi ning the respective "masstransition" , can be changed within fractions of a second. Thus, a large number of different mass transitions can be monitored in parallel in an analytical run allowing multi-analyte quantifi cation.

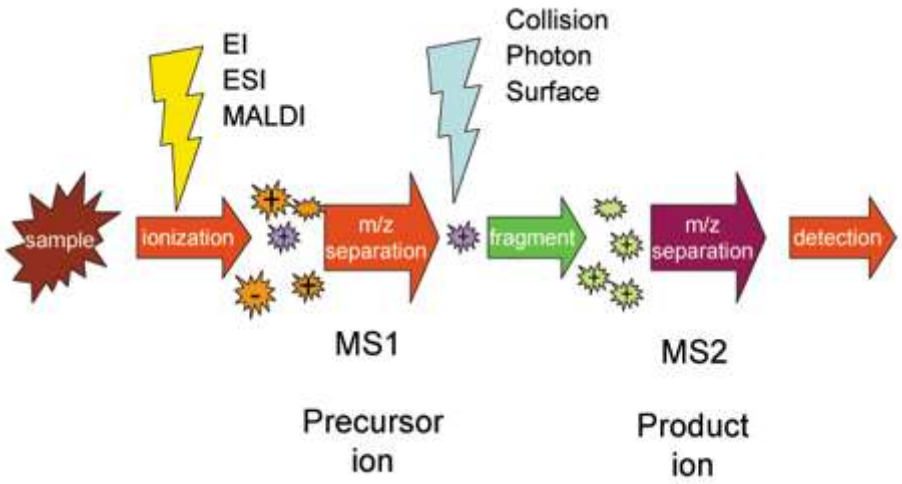

Fig: 5 Tandem-mass-spectrometry

Tandem MS instrumentation

Since Tandem MS includes 3 awesome steps of selection-fragmentation-detection, the separation of those 3 steps may be found out in space or in time.

- **Tandem MS in space**

Typical Tandem MS in area gadgets encompass QqQ, QTOF, and hybrid ion lure/FTMS, etc.

- **QqQ (Triple Quadrupole)**

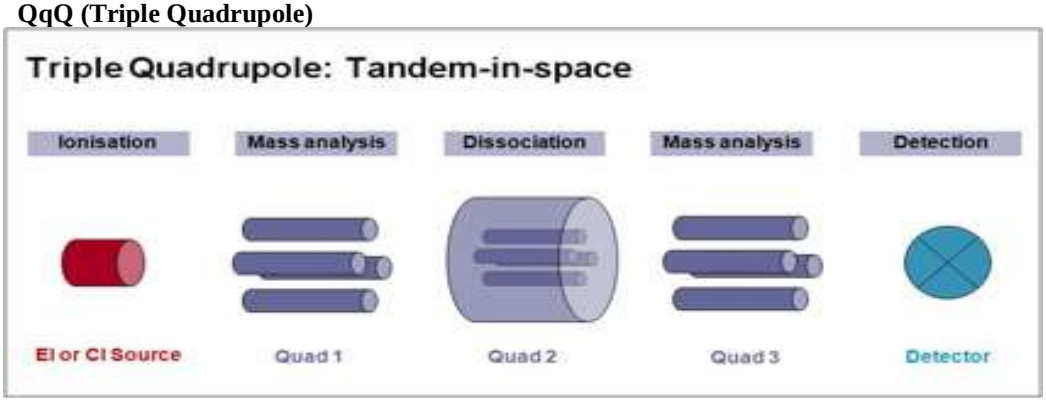

Fig: - 6 Triple Quadrupole: tandem-in-space

Three Quadrupoles (Quad 1, Quad 2, and Quad three) are covered up in a row. Precursor ions are decided on in Quad 1 and despatched to Quad 2 for dissociation (fragmentation). The generated product ions are despatched to Quad three for mass scanning.(54)

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- **QTOF (Quadrupole Time-of-flight)**

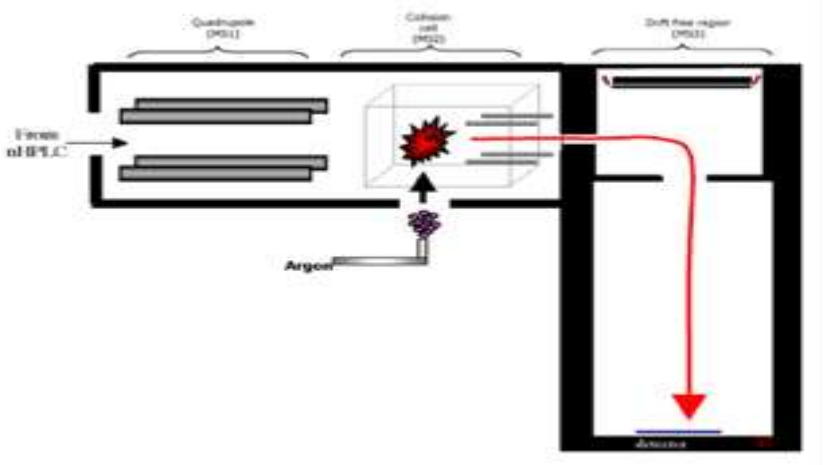

Fig: 7 Quadrupole Time-of-flight

In the QTOF, precursor ions are decided on within side the Quadrupole and despatched to the Collision Cell for fragmentation. The generated product ions are detected with the aid of using time-of-flight (TOF) mass spectrometry. (55)

- **Hybrid Ion Trap/FTMS**

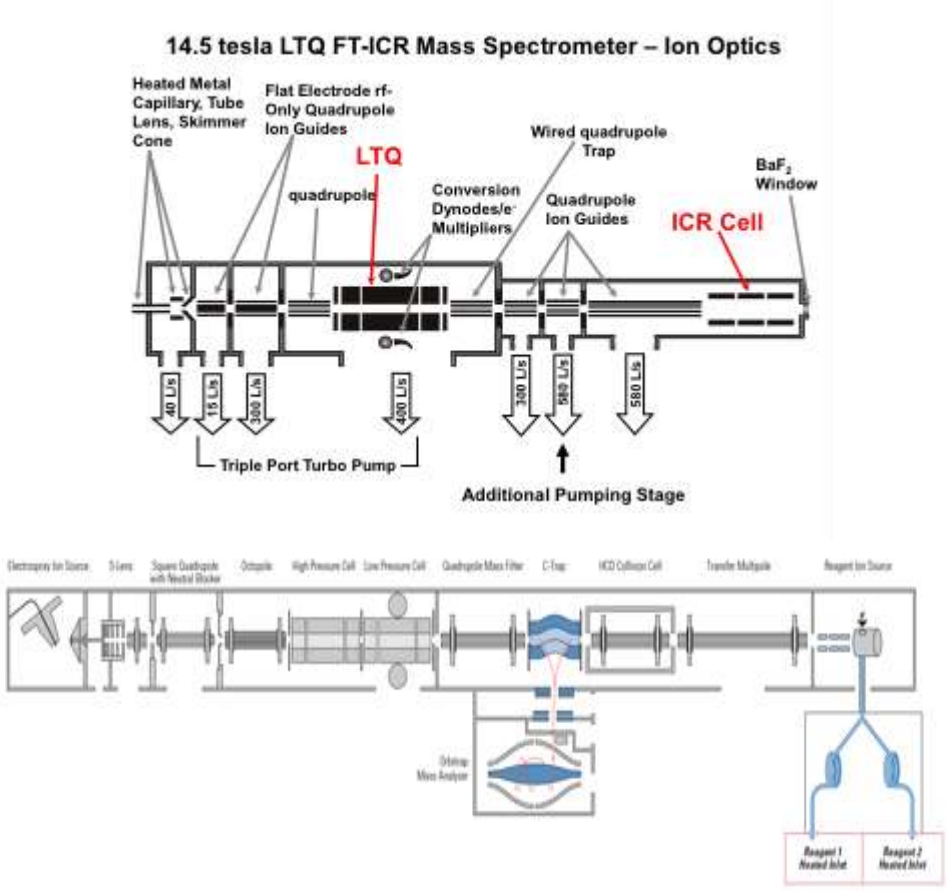

Fig: 8 Hybrid Ion Trap/FTMS

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For the hybrid ion lure/FTMS (FT-ICR or Orbitrap) gadgets, precursor ions decided on and fragmented in an outside ion lure. The generated product ions may be detected both within side the outside lure (decrease mass resolution, however quicker) with the aid of using or with the aid of using FTMS (better mass accuracy and resolution, however slower).

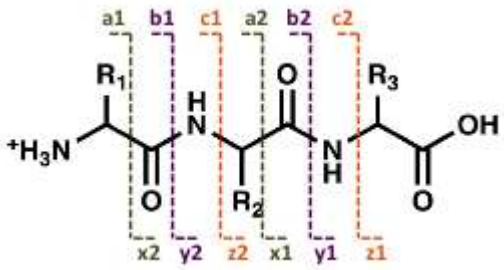
- **Tandem-in-Time MS/MS**

Typical Tandem-in-Time MS/MS gadgets encompass ion lure and FT-ICR MS.

Fragment ion notation

Peptides and oligosaccharides (which include glycolipids) observe distinctive structures of nomenclature for his or her fragment ions. Other instructions of compounds, i.e., phospholipids, etc., do now no longer but have mounted nomenclature structures. (56)

Peptides

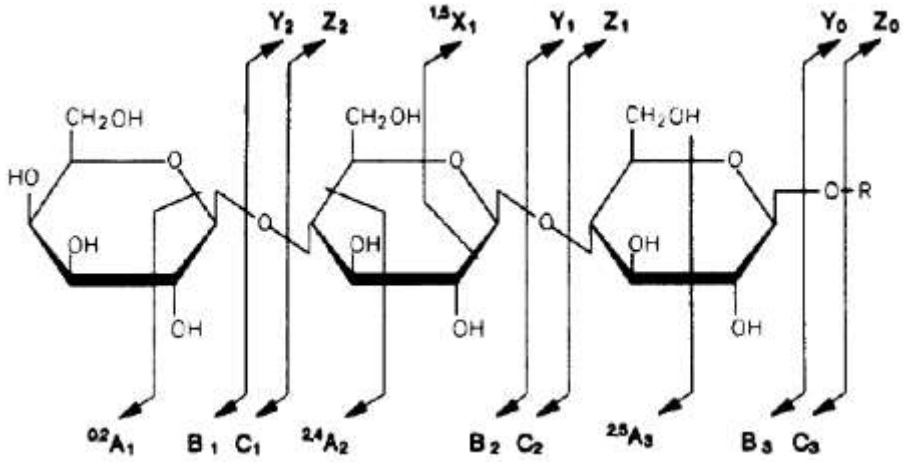


- **Nomenclature for peptide fragments**

Fragments containing the N-terminus are classified a, b, or c, relying at the web website online of the cleavage, while fragments containing the C-terminus are classified x, y, or z. The numbers imply the wide variety of amino acid residues with inside the fragment ion. (57)

Oligosaccharides (which includes glycolipids)

For oligosaccharides, fragments containing the lowering quit (lowering quit is at the right-hand aspect within side the discern) are classified x, y, or z, relying at the web website online of the cleavage, while fragments containing the opposite quit are classified a, b, or c. The numbers imply the web website online of the sugar residue: y, z, b, and c ions are fragments because of glycosidic cleavages (slicing glycosidic bonds protecting adjoining sugar residues), while a and x ions end result from cross-ring cleavage.



Nomenclature for oligosaccharide fragments (which includes glycolipids, while R = ceramide) (Costello, C. E.; Vath, J. E. Methods Enzymol. 1990, 193, 738-768)

- **Fragmentation strategies**

Precursor ions may be activated (with expanded inner power) in lots of distinctive ways.

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Fragmentation styles rely upon how power is transferred to the precursor ion, the quantity of power transferred, and the way the transferred power is internally distributed. Collision-brought on dissociation and infrared multiphoton dissocition are "slow-heating" strategies that growth the Boltzmann temperature of the ion and as a result preferentially cleave the weakest bonds to provide specially b and y ions. (58) These strategies are pretty green for peptides, lipids and different surprisingly small chemical compounds, however may additionally eliminate protein post-translational modifications (e.g., phosphates and sugars).Electron Seize dissociation and electron switch dissociation specially produce c and z ions even as maintaining post-translational modifications (PTMs). Thus, ECD and ETD are extensively carried out to proteins and peptides with labile PTMs. For oligosaccharides (which includes glycolipids), ECD/ETD also can generate cross-ring cleaved A and Z ions, which can be vital for localization of Glycosidic bonds. (59)

Applications of LC-Tandem mass spectrometry

1. In the endocrinological routine laboratory LC-MS / MS competes as a method predominantly with immunoassay tests. This theoretically applies **for thyroid hormones, steroids, and related small molecule hormones as 25-hydroxyvitamin D 3. For quantifi cation of proteo-hormones**, LC-MS / MS is in general not applicable due to the biological variability of these analytes and the typically very low concentrations, while peptide hormones may be potential targets of LC-MS / MS analyses.(60)

2.The analytical performance of the majority of **immunoassays for the quantifi cation of clinically important small molecule hormones is good or acceptable. This applies for example forthyroid hormones, serum cortisol, progesterone, and estradiol**. For these parameters, results are typically required within a short time and for a large number of samples. (61)

3.For some small molecule analytes, available immunoassays suffer from important analytical limitations, particularly with respect to specifi city. Analytes that fall into this group **include 17-hydroxyprogesterone, testosterone, 25-hydroxyvitamin D, 1, 25-dihydroxyvitamin D, cortisol (if assays are applied to urine or saliva), plasma metanephrins, androstendione, and aldosterone**. for some of these analytes, LC-MS / MS may offer a superior alternative even in a routine setting. (62)

4. In drug discovery, major applications are the quantitative analysis of the parent drug and the identification of metabolic biotransformation products. (63)

5. At the discovery stage, it is not only necessary to qualitatively identify metabolites in in vitro and in vivo studies, but it is also important to quantitatively estimate the importance of each metabolite so that metabolic liabilities can be addressed during compound optimization. (64) This means that major pharmacologically active metabolites must be identified and if necessary monitored in discovery and investigational new drug-enabling toxicological studies. Early phase metabolism studies typically rely on the use of LC-MS to analyze samples derived from in vitro incubations (e.g. with liver microsomes or hepatocytes) and/or in vivo studies. LC-MS allows the rapid identification and partial structural characterization of metabolites, with a high sensitivity for most drug candidates and metabolites(65)

CONCLUSION

Helps to academic research, quality control as well as industrial applications. It is automated system gives fast, reproducible and effective results that is a key important role in advancement of Science and Technology. Tandem-mass spectrometry is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production for active pharmaceutical ingredients, bulk drugs and formulations. This versatile analytical technique could be explored for better prospects in future.

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