A REVIEW ON LC-MS METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT: *The development of bio analytical techniques brought a progressive discipline with in the pharmaceutical trade is to get a quantitation life of a drug and its metabolites .They introduced some combined instrument in assessing the bioanalysis of the medicine. LC-MS is one of the bio analytical techniques that combine the physical separation capabilities of liquid chromatography with the mass analysis of mass spectrometry. This combination was first reported in 1967 and the first LC-MS system was introduced in 1980's. The main objective of this is to over view the principle, method development and validation.*

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 is briefly overviewed. The mass analyzers such as Quadra pole, TOF used in mass spectrometry with principle are also discussed.

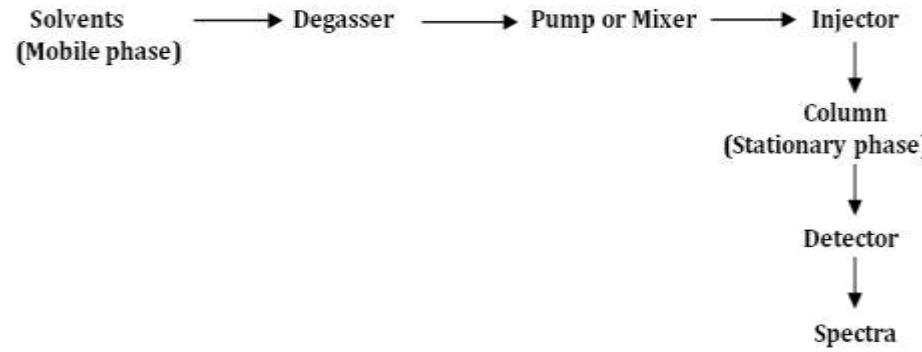
Key Words: *Bio analytical, liquid chromatography, Mass spectrometry, Method development and Validation.*

INTRODUCTION:

Liquid chromatography:

- Liquid chromatography is a separation process used to isolate the individual components of a mixture. This process involves the mass transfer between a sample and a polar mobile phase as well as non-polar stationary phase.
- LC separates the molecules in a liquid mobile phase using solid stationary phase.
- This separation occurs based on the interaction of the sample with the mobile and stationary phases. It can be used for the analytical or preparative application.
- This technology requires sophisticated instrument operating at high pressures, hence using abbreviation of LC and HPLC interchangeable.
- The reasons for the popularity the method are its sensitivity, its ready adaptability to accurate quantitative determination, its ease of automation, its suitability for separating non-volatile species or thermally fragile once its wide spread applicability to substances that are important to industry and many fields of science .[1]
- The columns were packed with 50 to 500 cm length of solid particles coated with an adsorbed liquid that formed the stationary phase.
- To ensure reasonable flow rates through this type of stationary phase, the particle size of the solid kept larger than 150 to 200 um.

INSTRUMENTATION OF LC:



Mass spectrometry:

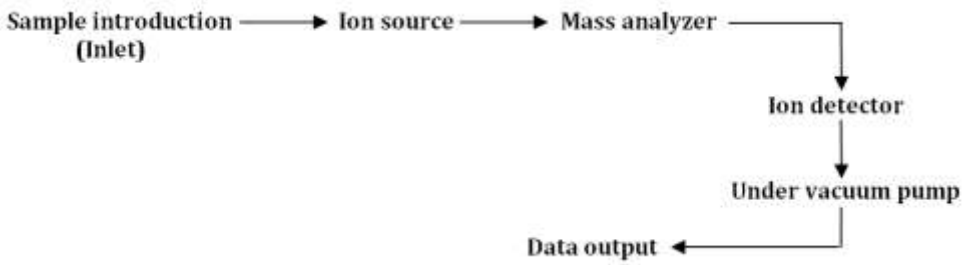
A mass spectrometer is an instrument that produces ions and separates them according to their mass to charge ration (m/z).

Mass spectrometric analysis involves the following steps:

- Atomization
- Conversion of a substances fraction of the atoms formed in step1 to a stream of ions.
- Separating the ions formed in step 2 on the basis of their mass to charge ratio [m/z].

M=mass of the number of ion in atomic mass unit.

Z=number of fundamental charges. [2]

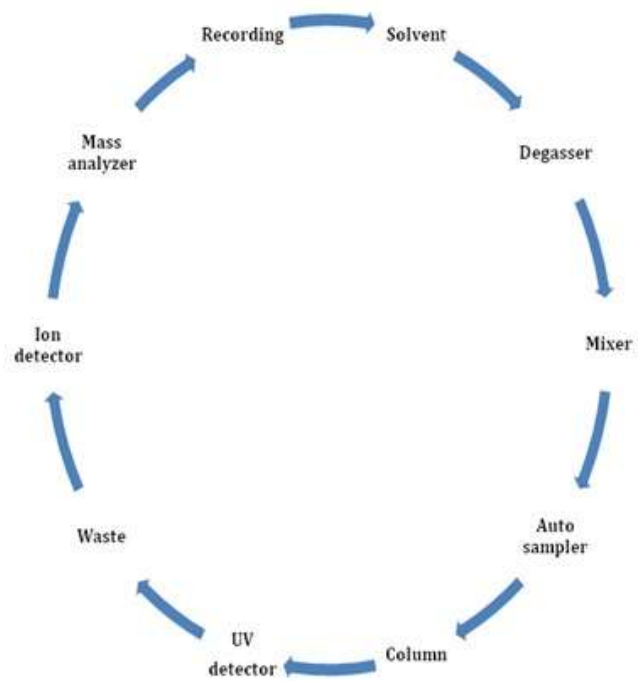


Normally mass spectroscopy is used in three principal ways:

- To measure relative molecular masses [molecular weights] with very high accuracy.
- To detect with in a molecule the places at which it prefers to fragment.
- As a method for identifying analytes by comparison of their other mass spectra.

LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY:

- LC-MS is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrum detection.
- Combination of LC-MS is an important development in the history of chromatography.
- Mass spectroscopy in LC-MS helps to determine the elemental composition and structural elucidation of a sample.
- It is powerful technique used for many applications which has very high sensitivity and selectivity.
- It is commonly used in the pharmacokinetic studies of pharmaceuticals and it is the most frequently in field of bioanalysis.
- LC-MS also play a role in pharmacognosy especially in the field of molecular pharmacognosy when it comes to the ingredients in the different aspects of phenotypic cloning. [3]



ADVANTAGES OF LC-MS COMBINATION:-

Diabelmashni et.al outlines the advantages of liquid chromatography coupled with mass spectrometry detection.

It is one of the most powerful analytical tools for organic compound analysis.

The key advantages of LC/MS Method over HPLC Methods includes:-

1. Selectivity – co-eluting peaks can be isolated by mass selectivity and are not constrained by chromatographic resolution.
2. Peak assignment – A chemical finger print for the compound of interest generated ensuring correct peak assignment in the presence of complex matrices.
3. Molecular weight information – confirmation and identification of known and unknown compounds.
4. Structural information – controlled fragmentation enables structural elucidation.
5. Rapid method development – provides easy identification of elucidated analytes without retention time validation.
6. Sample matrix adaptability - decreases sample preparation time.
7. Quantitation – quantitative and qualitative data can be obtained easily with limited instrument optimisation. [4]

DISADVANTAGES:

1. Expensive
2. Not portable
3. Requires an experienced technician
4. Moderate through put.

E.g. : If you want to analyse, for example 1,00,000 samples by LC-MS , it would probably take about a year depending on how fast the run time were through put does not matter if you are only analysing 5-10 samples , but in drug screening where the analysis of thousands of samples are in routine , it can be a major limitation. [5]

BIO ANALYTICAL TECHNIQUES:

Some techniques normally utilised in bio analytical studies:-

1. Hyphenated techniques
   LC-MS (Liquid chromatography –mass spectrometry)
   GC –MS (Gas chromatography – mass spectrometry)
   CE –MS (Capillary electrophoresis – mass spectrometry)
2. Chromatographic strategies
   HPLC (High performance liquid chromatography)
   Gas activity. [6]

PRINCIPLE INVOLVED IN LC-MS:-

- Typical LC-MS system is the combination of HPLC with MS using Interface (Ionisation source). The sample is separated by LC and the separated sample species are sprayed into atmospheric pressure ion source, where they ate converted into ions in the gas phase.
- The mass analyser is then used to sort ions according to their mass to charge ratio and detector counts the ions emerging from the mass analyser and may also amplify the signal generated from each ion.
- As a result mass spectrum (a plot of the ion signal as a function of mass to charge ratio) is created, which is used to determine the elemental nature of a sample, the masses of the particle and also molecules, and to elucidate the chemical structures of molecules.
- Two key components in this process are the ion source, which generates the ion and mass analyser, which sorts the ions.
- Several different types of ions sources are commonly used for LC/MS. [7]

REQUIREMENTS/COMPONENTS OF LC-MS:-

- A major difference between traditional HPLC and Chromatography used in the LC-MS is that the scales is much smaller , with respect to the internal diameter of the column and with respect to flow rate since it scales as the square of diameter.

- For a long time, 1mm columns were typical for LC-MS work.
- 300µm and even 75µm capillary columns have become more prevalent.
- At the low end of these column diameters the flow rates approach 100 ml/min and are generally used with nanospray sources.

MOBILE PHASE:-

- The mobile phase is the solvent that moves the solutes throughout the column.

General requirements:-

1. Low cost, UV transparency, high purity.
2. Low viscosity, low toxicity, non flammability.
3. Non – Corrosive to LC-MS system components.

Column type: - Specialised mode.

- The di –functional (or) Tri functional silanes creates bonded groups with two (or) three attachment points leading to the stationary phase with higher stability in low (or) higher P^H.
- Most widely used columns for LC-MS are:-
  1. Fast LC column
     The use of short column (15-50 mm)
  2. Micro LC column
     The use of large column (20 -150mm)

SAMPLE PREPARATION:-

- Sample preparation generally consists of concentrating the analyte and removing the compounds that can cause back ground ion (or) suppress ionisation.
- Examples of sample preparation include :-
  1. On – column concentration to increase the analytes concentration.
  2. Desalting to reduce the sodium and potassium adduct formation that commonly occurs in electroscopy.
  3. Filtration to separate a low molecular weight drug from proteins in plasma, milk (or) tissues.

SOLVENT PUMPING AND SOLVENT INJECTION IN HPLC:-

- HPLC requires a pump to inject the solvent. The common types of pumping are :-
  1. **Direct gas – pressure system** :-
     Which is inexpensive and reliable. However, changing solvent is difficult.
  2. **Syringe pumps :-**
     Which is inexpensive and reliable, very accurate and precise and can have a large capacity.
  3. **Pneumatic intensifier:-**
     Which operates under constant pressure, any blockage can cause a pressure drop and consequently pulses.
  4. **Reciprocating pumps:-**
     Which are economical solutions that provide a constant flow and high pressure, but can cause pulsing.

The sample injector should work with in very small volumes and withstand high pressure of solvent. Most devices use sample injection works valves instead of direct injection valves because of the former have superior characteristics. It is possible to inject the samples into the valves loop automatically with an auto – sampler (or) manually using a micro syringe. [8-9]

ION SOURCES:-

Electro spray ionisation (ESI)
Atmospheric pressure chemical ionisation (APCI)
Atmospheric pressure photo ionisation (APPI)

- **Electro spray ionisation (ESI):**
  It is a process of ionisation followed by evaporation which occurs in three basic steps:-
  1. Nebulisation and charging :-
     The HPLC effluent is pumped through a nebulising needle which is at ground potential. The

potential difference between the needle and electrode produces a strong electric field. This field charges the surface of liquid and forms spray of charged droplets. There is a concentric flow of gas which assists in nebulisation process.

2. Desolvation :-
   The charged droplets are attracted towards the capillary sampling orifice. There is counter flow of heated nitrogen drying gas which shrinks the droplets and carries away the uncharged material.
3. Ionisation :-
   As the droplets shrinks, they approach to a point where the electrostatic forces exceeds the cohesive forces. This process continues until the analyte ions pass through the capillary sampling orifice into the low pressure region of ion source and the mass analyser.

- **ATMOSPHERIC PRESSURE CHEMICAL IONISATION (APCI):-**

APCI, A process of evaporation followed by ionisation, is complementary to electro spray ionisation.

1. Nebulisation and desolvation :-
   APCI nebulisation is similar to that in API- ES. However, APCI nebulisation occurs in a hot (typically 250º-400ºc) vaporizer chamber. This heat rapidly evaporates helium spray droplets, resulting in gas – phase HPLC solvent and analyte molecules.
2. Ionisation :-
   The gas – phase solvent molecules are ionised by the discharge from a coronora needle. In APCI, there is a charge transfer from the ionized solvent reagent ions to analyte molecules in the way that is similar to chemical ionisation in GC-MS. These analyte ions then are transported through the ion optics to the filter and detector.

- **ATMOSPHERIC PRESSURE PHOTO IONISATION (APPI) :-**

APPI for LC-MS is a relatively new techniques. As in APCI, a vaporiser converts the LC eluent to the gas phase. A discharge lamp generates photons in a narrow range of ionisation energies. The range of energies is carefully chosen to ionize as many analytes molecules as possible while minimising the ionisation of solvent molecules. The resulting ion passes through a capillary sampling orifice into the mass analyser. [10-11]

MASS ANALYSERS:-

- The mass analyser used for LC-MS are of four types:
  1. Quadra pole
  2. Time of flight
  3. Ion trap
  4. Fourier transform – ion-cyclotron resonance (FT-ICR/FT-MS)

1. QUADRAPOLE :-

A Quadra pole mass analyser consists of four parallel rods arranged in a square. The analyte ions are directed down the centre of the square. Voltages applied to the rods generated electromagnetic fields. These fields determine which mass to charge ratio of ions can pass through a filter at a given time. Quadra poles tend to be the simplest and least expensive mass analyzers.

Quadra pole mass analysers can operates in two modes :-

Scanning (scan) mode
Selected ion monitoring (SIM) mode

- In scan mode, the mass analyzer monitors a range of mass to charge ratios. In SIM mode, the mass analyser monitors only a few mass – to-charge ratios.
- SIM mode significantly more expensive than scan mode but provides information about fewer ions. Scan mode is typically used for qualitative analyses (or) for quantitation when all the analytes masses are not known in advance. SIM mode is used for quantitation and monitoring of target compounds.

2. TIME OF FLIGHT :- (TOF)

In a TOF mass analyser, a uniform electromagnetic force is applied at the same time, causing them to accelerate down a flight tube. Lighter ions travel faster and arrive at the detector first, so the mass to charge ratios of ions are determined by arrival times. TOF analyser has a wide mass range and can be very accurate in their mass measurements.

3. **ION TRAP** :-
   An ion trap mass analyser consists of circular ring electrodes plus two ends caps that together form a chamber. Ions entering the chamber are trapped there by EMF. Another field can be applied to selectively eject ions from the trap. Ions traps have the advantages of being able to perform multiple stages of mass spectrometry without additional analysers.

4. **FOURIER-TRANSFORM CYCLOTRON RESONANCE (FT-ICR):**
   An FT-ICR mass analyser also called as (FT-MS) is another type of circular orbits by powerful electrical and magnetic fields. The ions generate a time dependent current. This current is converted by Fourier transform in to orbital frequencies of the ions which correspond to their mass to charge ratios. [12]

METHOD DEVELOPMENT:-
Method of unit study area being habitually developed, improved, validated, collaboratively studied and applied. Activity separations area unit chiefly needed that rely upon the general approach to LC-MS technique development. In most cases as desired separations achieved simply with solely some experiments in alternative cases a substantial quantity of experimentation could also be released.

PROCEDURE FOR TECHNIQUE DEVELOPMENT:-
- Collect the chemical properties of the drug molecule from the literature.
- Confirm the solubility profile.
- MS scanning and optimisation.
- Mobile phase selection choice.
- Choice of extraction technique and optimisation.
- Choice of activity technique(based on solubility study retention of compound)

1. REVERSED SELECTION CHROMATOGRAHY:-
Reversed selection of packing's like C_{18}, C8 area unit the foremost and wide spread most generally used for reversed selection. Additionally to those C4, C2 and phenyl are on the market. Reversed selection sorbents usually involves learning with associate in nursing organic solvent (e.g.: methanol) followed by associate binary compound solvent (e.g.: water)

2. STEPS IN LC-MS TECHNIQUE DEVELOPMENT:-
Proper information regarding the sample is critical for an efficient technique development. Some data relating to the analyte is like:-
- Variety of compounds.
- Molecular weight of compounds.
- Sample solubility.
- Data stability.
- Concentration of variety of compounds in samples of interest.

3. METHOD OPTIMISATION:-
During the optimisation stage, the initial sets of the conditions that were evolved throughout the strategy development area unit improved and maximised in terms of resolution and peak from, plate counts, imbalance, capacity, extraction time, detection limits, limits of quantization, and overall ability to quantify the particular analyte.
Optimization of a way will follow either of (2) general approaches like manual (or) laptop driven. The manual approach includes one independent variable at a time, whereas holding all others constant, and records the changes in the response. The variables may contain flow rates, mobile (or) stationary phase selection, composition and temperature etc.

4. MODE OF SEPARATION TECHNIQUE:-
Since most of pharmaceutical compounds are polar in nature thus reverse activity is generally tried initially with in which a non polar stationary phase selection is employed. The mobile phase consists of water (or) buffer and organic solvents (acetonitrile (or) methanol). Thus polar compounds get eluted initial and non-polar compounds maintained for an extended time. The stationary phase in reverse activity is n-octaldecyl (RP-18), n-octyl (RP-8), ethyl group (RP-2), phenyl, cyano, alcohol and hydrophobic polymers.

5. SELECTION OF STATIONARY PHASE/COLUMN:-

Prior to the selection of column, it is necessary to known about the column packing. Oxide tends to dissolve higher than hydrogen ion concentration and cross linked compound particles like phenyl ethylene (or) poly methacrylates are used for the separation of bases. Oxide particles have surface silanol teams, -SIOH that are used for chemical bonding of stationary phases by silanization reactions with chlorosilanes . The normally used non-polar columns like $C8_{,\ and}$ C_{18}, being the fore most wide spread (known as ODS) for octaldecylsilane, C_8 is intermediate in property where ever C_{18} is non polar.

6. SELECTION OF MOBILE PHASE:-

The main criteria in choice and optimisation of mobile selection are to attain optimum separation of all the individual impurities and degradants from one another and form the analyte peak. The parameter for selecting the mobile phase of buffer, hydrogen ion concentration of the buffer.

7. MASS CHEMICAL ANALYSIS DETECTION AND INFORMATION SYSTEM:-

It is the powerful analytical technique that mixes the resolution of liquid activity with detection speficity of mass spectrometric analysis. Mass spectroscopic analysis creates and detects charged ions. The LC-MS information could be also be want to give information about molecular weight, structure, identification, amount of specific sample parts. Structural data can be generated by victimization sure sort of mass spectrometers typically those that are used with multiple analysers that are called tandem bicycle with multiple analysers that are called tandem bicycle mass spectrometers. This could be achieved by fragmenting the sample within the instrument and analysing the product generated. [13-14]

LC-MS METHOD VALIDATION:

- ✓ The procedure for demonstrating the reliability of analytical methods is called validation.
- ✓ Validation is required for all analytical methods but the amount of work and complexity of the issues encountered increases with increasing sophistication of the technique used.
- ✓ LC-MS is an extremely powerful and at the same time highly sophisticated technique.
- ✓ It offers various operation modes, numerous parameters and several possibilities of ionization (protonation, adduct formation).
- ✓ These systems are complex and are always not robust.

PURPOSE OF VALIDATION:

- ✓ Method validation is a key activity in chemical analysis, indispensable for obtaining reliable results.
- ✓ Methods based on LC-MS are notorious for their complexity, on the one hand because of the instrument itself and on the other hand because LC-MS is often applied to most complex samples.
- ✓ Therefore it is important to demonstrate that methods are working as expected (validation) and the obtained results are reliable.
- ✓ This information is important to both the laboratory as well as the customer.
- ✓ The necessisity of validation is increasing day by day and more regulations are affecting laboratories that recognize the validation as a requirement.
- ✓ There is a general agreement on the various validation parameters to be evaluated, diversity prevails about the details and about the methodology employed for validation and acceptance criteria.

CARRYING OUT VALIDATION:

- ✓ Before starting a validation, a clear plan is complied, which consists of the reason for validation, planned experiments as well as expected outcomes – requirements that needed to be met by the method.
- ✓ The requirements often result from guidelines or from other regulatory documents.
- ✓ Guidelines generally give recommendations for evaluating separately each performance parameter.
- ✓ At the same time, the guidance on deciding above the whole methods validation is usually very general. Few different cases arise.
    a) When the methods are applied in the scope of standards, laws or directives, then the requirements from those documents, if present, must be followed and decisions on validations should be based on these. When the decision on validity suitability is based

on the guidelines, then for each parameter a separate decision must be given according to the requirements.

b) Sometimes the client can specify the requirements, and then the client's requirements are superior to those in the guidelines.

c) If there are no external requirements, then the analyst can set up the requirements himself/herself based on his/her knowledge of the subject.

- ✓ The validation should start with evaluating the analyte stability and method selectivity as all the other parameters strongly depend on these.
- ✓ For example, if the analyte extensively decomposes in to an auto sampler, no linear relation can be achieved. In that case, non-linear relation can be achieved.
- ✓ For the evaluation of trueness and precision we need to know the linear/ working range of the method.
- ✓ Not only validation but also appropriate documentation of the validation is required for an adequate interpretation as well as on the validity of the obtained results.
- ✓ At the last stage of validation, an assessment of validity of the method should be given, based on the validation results.

SCOPE OF VALIDATION:

- ✓ It is often unnecessary to carry out a determination of all the possible method performance parameters.
- ✓ Validation involving only some of the performance parameters is called partial validation.
- ✓ If a fully validated (eg : using a collaborative trail) method is set up in the laboratory then it is necessary to carry out so called verification: verify that is able to achieve the published values of performance characteristics.
- ✓ Partial validation is justified when a standard method is used; small changes are made to previously validated methods or for the methods with narrow application range.
- ✓ The small changes can include transfer of the method to another laboratory, adding a new matrix, new reagent in sample preparation, etc.

VALIDATION PROCESS:

Successful validation requires co-operative efforts of several departments of organizations includes regulatory affairs, quality control, quality assurance and analytical development.

Steps in method validation include:

- Develop a validation protocol or operating procedure for the validation.
- Define the application, purpose and scope of the method.
- Define performance parameters and acceptance criteria.
- Define validation experiments.
- Verify relevant performance characteristics of the equipment.
- Qualify materials, eg : standards and reagents.
- Perform pre-validation experiments.
- Adjust method parameters and acceptance criteria if necessary.
- Perform full validation experiments.
- Develop SOPs for executing method in routine.
- Define criteria for revalidation.
- Define type and frequency of system suitability tests for routine.
- Document validation experiments and results of validation.

REVALIDATION:

Revalidation is necessary whenever a method is changed and the new parameter is outside the operating range.

The operating parameters need to be specified with ranges clearly defined.

Changes in equipment or chemical quality may also have critical effects on method. So any such change needs revalidation.

VALIDATION GUIDELINES:

- ➢ ICH Q2A text on validation of analytical procedures: definitions and terminology.

- ICH Q2B validation of analytical procedures: methodology.
- FDA (draft) guidance for industry: analytical procedures and method validation.
- Pharmacopoeias USP and European pharmacopoeias.

NEED FOR METHOD VALIDATION:

Method validation is required for the following reasons:

- ✓ For assuring the quality of the product.
- ✓ For achieving the acceptance of the products by the international agencies.
- ✓ It is a mandatory requirement for registration of any pharmaceutical product or pesticide formulation.
- ✓ It is a mandatory requirement for accreditation as per ISO 17025 guidelines.

VALIDATION PARAMETERS:

1. Selectivity and specificity
2. Linearity
3. Range
4. Accuracy
5. Precision
6. Stability
7. Limit of detection (LOD)
8. Limit of quantification (LOQ)
9. Ruggedness and robustness.
10. Sensitivity.

Selectivity and specificity:

- Selectivity of the analytical method is defined as the degree to which a method can quantify the analyte in the presence of interferents.
- The other components which may include impurities, degradants, matrix etc.
- The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other.
- The forced degradation studies are carried out to challenge this method.
- During forced degradation studies, the sample is subjected to the stressed conditions of light, heat, humidity, acid / base hydrolysis and oxidation.
- The selectivity of chromatographic methods may be assessed by examination of peak homogenecity or peak purity.
- Peak purity shows that there is no co-elution of any sample component.

Linearity:

- Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range.
- Linearity should be evaluated by visual inspection of plot of signals as a function of analyte concentration.
- If there is linear relationship, test results should be evaluated by appropriate by statistical methods, for example, by regression analysis.
- Data from the regression line is helpful to provide mathematical estimates of the degree of linearity.
- It is generally expressed in terms of variance.
- The correlation coefficient (R) should be greater than or equal to 0.999.

Range:

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The following specific ranges should be considered:

- Assay method – 80 – 120 % of the test concentration
- Content uniformity – 70 – 130 % of the test concentration, based on the nature of the dosage form.

- Dissolution study - +/- 20 % over the specified range.
- Impurity determination- from reporting level of impurity to 120% of the specification.
- Transmission efficacy which is the ratio of number of ions that are finally detected and the number of ions that entered the mass analyzer. This is an important measure of sensitivity for mass spectrometers.

Accuracy:

- The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value found.
- Practically no measurement process is ideal, therefore, the true value or actual value cannot be exactly known in any particular measurement.
- The accuracy studies are usually carried out by determining the recovery of spiked sample of the analyte in to the matrix of the sample (a placebo) or by comparing the result to the results of a certified reference material of known purity.
- If the placebo of the sample is not available, the technique of addition is used.
- Accuracy should be assessed using minimum of nine determinations over a minimum of three concentration levels covering the specified range.

Precision:

- The degree of agreement between replicate analyses of a homogenous sample, usually measured as the relative standard deviation (RSD) as a set of replicates.
- The measured standard deviation can be subdivided in to three categories:
    1. Repeatability
    2. Intermediate precision
    3. Reproducibility
- Repeatability expresses the precision under the same operating conditions over a short interval of time. From the repeatability standard deviation is useful to calculate the repeatability limit (r), which enables the analyst to decide whether the difference between duplicate analyses of a sample.
- Reproducibility expresses the precision between laboratories. From this it is useful to calculate the reproducibility limit (R), which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under reproducibility conditions is significant.

Stability:

- Analyte stability is not universally included in validation guidelines as a validation parameter.
- The reason is that if the analyte is unstable its decomposition influences the trueness and precision of the procedure.
- Analyte stability must be ensured during sample collection, processing, storage, extraction and duration of the analysis to generate reliable bio-analytical data.
- Stability tests can be among the most time-consuming tests in the validation procedure.
- Stability is the lowering of the analyte content in the sample over the period of time.
- If the analyte is stable, then the concentration remains the same in time. Eg : 100% of the analyte degrades with time then its concentration is decreased and also the stability is lower than 100%.

Limit of detection:

- It is the lowest amount of the analyte in the sample which can be detected but not necessarily quantified as an exact value. The detection limit can be measured in different ways:

1. Signal to noise ratio: It is determined by comparing measured signals from samples with known low concentration of analyte with those of blank values. The concentration showing signal to noise ratio between 3:1 or 2:1 is generally considered as acceptable detection limit.
2. Standard deviation of response and slope

Limit of quantification:

- The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy.
- It is the concentration showing signal to noise ratio of 10:1.
- Typical acceptance criteria for LOQ are mean recovery at this level between 50-100% with % RSD of less than or equal to 25%.

Ruggedness and robustness:

- Ruggedness is a measure of how well a method stands up to less than perfect implementation.
- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage.

System suitability:

- System suitability testing (SST) is an integral part of analytical procedure.
- System suitability test provide the added assurance that on a specific occasion the method is giving accurate and precise results.
- Limits for system suitability tests are:

  Resolution (R_S) - > 2.0
  Repeatability (RSD) - <1.0% for five replicates.
  Plate count (N) - >2000
  Tailing factor (T_f) - < 2.0
  Separation factor - >1.0

Sensitivity:

- Sensitivity is the change in the response of a measuring instrument divided by corresponding change in the stimulus.
- The main use of sensitivity parameter is a threefold:
  1. Optimization of the method parameters during method development.
  2. Daily optimization of the instrument parameters.
  3. Monitoring of the instrument performance.[15-19]

CONCLUSION:

Liquid chromatography mass spectrometry (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection. It is an efficient analytical technique for invitro determination of drug metabolites, in new drug discovery, screening of plant constituents, analysis and identification of impurities and degradation products in pharmaceuticals. Advancement in LC-MS technique can be successfully implemented for separation and identification of analyte ions.

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