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GAS CHROMATOGRAPHY

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Abstract:

*Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compound that can be vaporized without decomposition. The principle of separation in GC is "**partition**". There are mainly 2two type of chromatography Gas solid chromatography (GSC), Gas liquid chromatography(GLC).Gas chromatography have pharmaceutical applications like quality control and analysis of drug products such as antibiotics, anti-virals, general anesthetic, sedatives etc.It is also used for determining purity of compound. The present review summarizes the instrumentation, validation methods and applications of gas chromatography.*

Keyword ; Chromatography, Stationary Phase, Mobile Phase, Absorption, Partition

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INTRODUCTION:

Chromatography is a laboratory technique for the separation of mixture in to its components. This was first invented by Michael Tswett, a Russian botanist in 1906.

CHROMATOGRAPHY

Chromatography is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases.

The word chromatography is derived from two Greek words- *chroma* means-color and *graphein- to write*.

In this technique the mixture is dissolve in a fluid solvent (gas or liquid) called the mobile phase, which carry it through a system (a column, a capillary tube, a plate or a sheet) on which a material called the stationary phase is fixed. Because the different constituents of the mixture tend to have different affinities for the stationary phase and are retained for different lengths of time depending on their interactions with its surface sites, the constituents travel at different apparent velocities in mobile fluid, causing them to separate. The separation is based on the differential partitioning between the mobile and stationary phases.

Absorption chromatography-It is a type of chromatography in which a mobile liquid or gaseous phase is adsorbed onto the surface of stationary solid phase.

Partition Chromatography – It is the separation of components between two liquid phases viz original solvent and the film of solvent used in the column.

TYPES OF CHROMATOGRAPHY

Based on modes of chromatography
- ❖ Normal phase mode
- ❖ Reverse phase mode

Based on principle of absorption
- ❖ Adsorption chromatography
- ❖ Ion exchange chromatography
- ❖ Partition chromatography
- ❖ Size exclusion chromatography

Based on analysis
- ❖ Qualitative analysis
- ❖ Quantitative analysis

Based on elution technique
- ❖ Isocreatic separation
- ❖ Gradient separation

TYPES OF CHROMATOGRAPHIC METHOD

- Paper chromatography
- Liquid chromatography
- Gas chromatography
- High performance liquid chromatography

GAS CHROMATOGRAPHY

Definition:

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compound that can be vaporized without decomposition.

Gas chromatography is also known as **vapor-phase chromatography** (**VPC**) or **gas-liquid partition chromatography** (**GLPC**). It involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is absorbed onto the surface of an inert solid.

It is probably most utilized of all the chromatographic techniques. Since the theory was developed and amino acids were separated in the early 1950's literly thousands of applications have been reported in organic, general and biochemistry. It has been applied to a wide variety of theoretical and practical problems in the separation and identification of components of the atmosphere, gases, liquids, drugs and commercial products. The primary limitations that the sample must be capable of being volatilized without under going decomposition. Because of this limitation now being replaced to a large extent by high performance liquid chromatography.

TYPES OF GAS CHROMATOGRAPHY

- ➢ Gas solid chromatography (GSC)
- ➢ Gas liquid chromatography(GLC)

Gas solid chromatography (GSC)

- In this type gas is used as mobile phase and a solid adsorbed as a stationary phase.
- GSC s based up on selective adsorption on a solid.
- It is not widely used because of limited number of stationary phase available.

Gas liquid chromatography (GLC)

- In GLC Stationary phase is a thin layer of non-volatile liquid bound to the solid support. A partition process occurs. .
- GLC is based upon the partition between gas and immobile liquid phase.GSC is not widely used because of limited number of stationary phases available.

Gas chromatography is powerful tools available for separations. The major factor for this are the speed, resolving power and extreme sensitivity of the technique .For example using this technique,it is possible to separate the ten isomers of the heptanes in less than ten seconds. Detectors are available with detection limit as low as 10^{-12} to 10^{-14}g.

PRINCIPLE:

The principle of separation in GC is "**partition**". The mixture of components to be separated is converted to vapour and mixed with gaseous mobile phase. The component which is less soluble in stationary phase travels faster and eluted out first .The component which is more soluble in stationary phase travel slower and eluted later. No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient. Partition coefficient is the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.

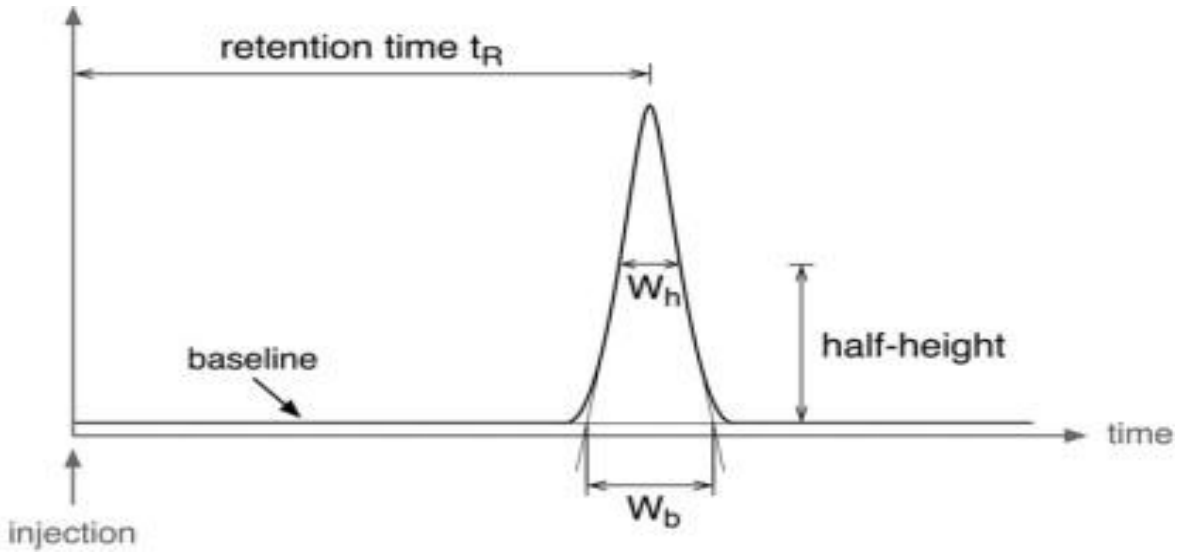
To obtain optimal separations, sharp, symmetrical chromatographic peak must be obtained. This means that band broadening must be limited.

THEORIES

1. **Plate Theory** (Old theory developed by Martin and Synge in 1941)
2. **Rate Theory** (Current theory developed by Van Deemter in 1956)

PLATE THEORY

- The plate theory concept was adapted to chromatography by Martin and Synge in 1941.
- According to plate theory, a chromatographic column consists of a series of discrete yet continuous horizontal layers which are termed as theoretical plates.
- Solute molecules get equilibrated between stationary and mobile phase at each of these plate. Migration of solute is then assumed to occur by a series of stepwise transfer between one plate to other immediately below.
- Separate equilibrations of sample between the stationary and mobile phase occure in these plate.
- Efficiency of separation column increases and H decreases.
- These plates are imaginary plates. They are considered to understand to the process occurring in the column.
- When the compound is passing through the column, it is getting distributed between the stationary phase and mobile phase.
- After equilibrium ,the solute is carried by the mobile phase from one plate to another and the process is continued till the solute elute out of the column with its characteristic retention time and peak width.
- As the number f theoretical plates is increased in a column, the solute peak becomes narrower and this result in a much better resolution between different different sample components in a run.



Efficiency of column is expressed by the number of theoretical plates. It can be determined by using the formula;

$$n = 16\ Rt^2/w^2$$

Where,

n = number of theoretical plates
Rt=Retention time
W =Peak width at base

RATE THEORY OF CHROMATOGRAPHY

- The rate theory of chromatography is important for chromatographic analysis. The theories help understand how the analyates move in the stationary phase as the mobile phase flow through it.

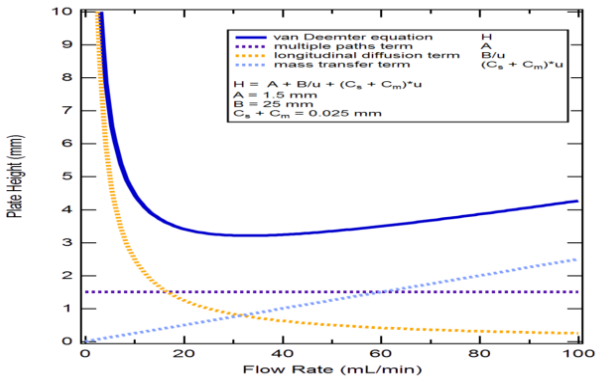
- The rate theory of chromatography defines the activity in a chromatographic column. It shows that when solute elutes out of the column, it impacts the band shape and is affected by the elution rate. Band width increases as solute molecules migrates down the column. Band width is directly related to retention time and inversely proportional to mobile phase velocity.

- It provide information about the shape and breadth of the elution bands as the mobile phase migrate and flow through the column ,it helps understand the process of peak dispersion and factors impacting band broadening.

- It gives a realistic explanation of the process occurring in a chromatographic column. It considers and measures the time taken by the solute to equilibrate between the stationary and mobile phase.

- It considers the rate of elution on the resulting band shape or the chromatographic peak.

- The rate theory of chromatographic is expressed mathematically by the **Van Deemter equation** .The equation helps calculate the variance per unit length of a column in terms of mobile phase velocity and analyte properties. The relationship between a column's efficiency and the mechanism behind band broadening is described by the Van Deemter equation.



- It is represented by

HETP = A +B/U (C_S+C_m).u

Where,

A = Eddy diffusion parameter

B =Diffusion co-efficient of eluting particles in the longitudinal direction

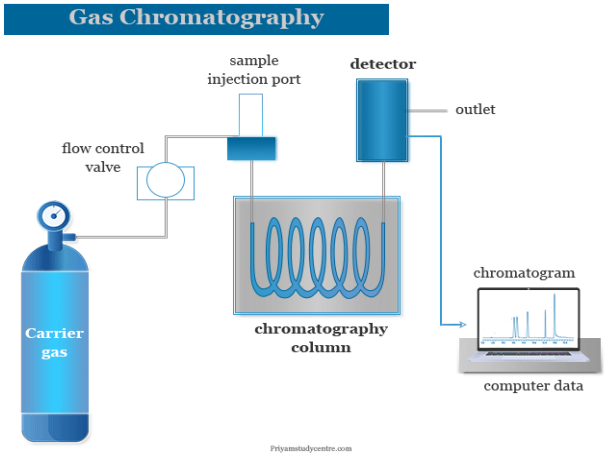
C =Resistance to mass transfer coefficient of the analyte between stationary and mobile phase

u =Speed

C_m =Dispersive convection in the mobile phase.

C_s = sorption and desorption of the solute from the stationary phase.

INSTRUMENTATION :



- Carrier gas
- Pressure regulator
- Flow regulators and flow meters
- Sampling unit
- Column
- Temperature control devices
- Detectors
- Recorders and integrators

1 .CARRIER GAS

The choice of carrier gas is determines the efficiency of chromatographic separation. Most widely used carrier gases are Hydrogen, Helium, Nitrogen and Argon.

Hydrogen:-It has better conductivity, low density. It is used in case of thermal conductivity detectors and flame ionization detector .The disadvantage is that it reacts with unsaturated compounds and it is inflammable.

Helium :-It also has excellent thermal conductivity, but it is expensive. It is a good carrier gas when used with thermal conductivity detector.

Nitrogen: - It is inexpensive but reduced sensitivity.

- The helium gas is mostly preferred because of its efficiency and safety.
- The carrier gas is filled in reservoir tank and regulator controls the flow of gas.
- The carrier gas must be pure
- It is equipped with molecular sieve for filtering and removal of moisture and other impurities if present.
- As carrier gas is compressible, gases are stored under high pressure in cylinder and used when required.

Requirements of Carrier Gas

- ✓ Inertness
- ✓ Suitable to the detector used
- ✓ High purity
- ✓ Easily available
- ✓ Less risk of explosion or fire hazards
- ✓ Should give best column performance consistent with the required speed of analysis.

2. PRESSURE REGULATOR

- Pressure is adjusted within the limits of 1 to 4 atmosphere while the flow control valve measures 1 to 1000 liters per minute of gas.
- Flow valves are by a needle valve mounted on the base.

3. FLOW REGULATORS AND FLOW METERS

- As the carrier gases are stored under high pressure, flow regulators are used to deliver the gas with uniform pressure or flow rate.
- Flow meters are used to measure the flow rate of carrier gas. They have Rotameter and soap bubble flow meter.

❖ **Rotameter**: It is placed conveniently before the column inlet .It has an ordinary glass tube (like burette) with a float held on to a spring. The level of the float is determined by the flow rate of carrier gas and precalibrated.

❖ **Soap bubble:** It is similar to rotameter and instead of a float, soap bubble formed indicate the flow rate .It has a glass tube with a inlet tube at the bottom through when the gas come in. A rubber bulb is used to store soap solution. When the bulb is gently pressed a drop of soap solution is converted into a bubble by the pressure of carrier gas and travels up. The distance travelled upwards is a measure of flow rate of carrier gas .The graduations is also precalibrated.

❖

4. SAMPLING UNIT

- Sampling unit or injection port is attached to the colum head.
- Since the sample should be in vapourised state the injection port is provided with an oven that help its temperature maintain at about 20-50^0 c above the boiling point of the sample.
- Gaseous Sample may be introduced by use of a gas tight hypodermic needle of 0.5 -10 ml capacity.
- For liquid samples, micro syringes may be used.

Injections of sample into the capillary column

✓ **Split injection**: It split the volume of sample stream into two unequal flows by means of a needle valve and allow the smaller flow to pass on to the column. And bigger part allowed to be vented to the atmosphere. This technique is not suitable when highest sensitivity is required.

✓ **Splitless injection**: They allow all of the sample to pass through the column for loading. Sample should be very diluted to avoid over loading of the column and a high capacity column such as SCOT or heavily coated WCOT column should be used.

✓ **On column injector** :A syringe with a very fine quartz needle is used. Air cooled to -20^{0}c below the b.p of the sample .After then the warmer air is circulated to vaporize the sample.

✓ **Automatic injectors** :The solid samples are introduced as a solution or in a sealed glass ampoule, crushed in the gas stream with the help of a gas tight plunger, and flows into column under the influence of carrier gas.

✓

5 .COLUMNS

- Column is one of the important parts of GC which decides the separation efficiency.

Column can be made up of glass or stainless steel.

- Stainless steel have the advantage of long life and can be easily handled without the fear of fragility .But some samples react with them .Hence in such cases glass columns are used (eg: steroids).
- Glass column has the advantage that they are inert and do not react with any kind of sample. The great disadvantage is that they are highly fragile and difficult to handle.

Column can be classified according to the nature as well as its use

A) Depending on its use

- **Analytical column** : Analytical column have a length of 1-15 meters and an outer diameter of 3-6 mm. They are packed column and made up of glass or stainless steel. Only one quantity of sample can be loaded on to the column.
- **Preparative column** Preparative columns are larger when compared to analytical columns since large amount of sample has to be loaded. They have a length of 3-6meter and outside diameter of 6-9mm.

B) Depending on its nature

Packed column : Columns are available in packed manner and hence are called packed columns. Different column ranging from low polar nature to high polar nature are available

Open tubular column or Capillary column or Golay column

- ✓ They are made up of long capillary tubing of 30-90 meters in length and have uniform and narrow internal diameter of 0.025-0.075 cm.
- ✓ These are made up of stainless steel and are in the form of coil.
- ✓ The inner wall of the capillary is coated with the stationary phase liquid in the form of thin film.
- ✓ These columns offer least resistance to the flow of carrier gas and hence they are more efficient than packed columns which offer more resistance to the flow of carrier gas. But the disadvantage is that the sample cannot be loaded.

Support coated open tubular column (SCOT)

- ✓ This is an improved version of Golay columns. As Golay or capillary column have small sample capacity, they can be modified into SCOT columns.
- ✓ These columns are made by depositing a micron size porous layer of support material on the inner wall of the capillary column and then coated with a thin film of liquid phase.
- ✓ These columns also have low resistance to the flow of carrier gas but offer the advantage of more sample load or capacity.

6. TEMPERATURE CONRTOL DEVICES

Preheaters : These are used in gas chromatography to convert the sample into its vapour form and mix them with the mobile phase or carrier gas. The preheaters are present along with the injecting devices soon as the liquid samples are injected they are converted into vapour form.

Thermostatically controlled oven:

- ✓ Partition co efficient as well as solubility of the solute depends upon temperature. Temperature maintenance in the column is highly essential for efficient separation.
- ✓ When a temperature is set for an oven, a controller moniters the actual temperature inside of the oven. If it falls below the set temperature, it sends a signal to activate the heater to rise the temperature back to the set point.
- ✓ In isothermal mode the column oven temperature is maintained at a constant value throughout the analysis run. The temperature is set to around midpoint of the boiling range of the sample component.

7. DETECTORS

- The eluted solute particles along with the carrier gas exit from the column and entre the detector.
- The detector then produces electrical signals proportional to the concentration of the components of the solute.
- The signals amplified and recorded as a peak at intervals on the chromatograph.

PROPERTIES OF A IDEL DETECTORS

- ✓ Detector should have good sensitivity
- ✓ It should have good stability and reproducibility.
- ✓ It should have should short response time.
- ✓ It should be non detective in nature.
- ✓ It should work at higher temperature.(At or above 400^0C)
- ✓ It should produce uniform response to all analytes.
- ✓ It should have wide dynamic range.

DETECTORS USED IN GAS CHROMATOMATOGRAPHY

- ❖ Thermal conductivity detector(TCD)
- ❖ Flame ionization detector (FID)

- ❖ Electron capture detector (ECD)
- ❖ Nitrogen phosphorous detector(NPD)
- ❖ Argon ionization detector (AID)

THERMAL CONDUCTIVITY DERECTOR (TCD OR KATHAROMETER)

Thermal conductivity detector (TCD) is a bulk property detector and a chemical specific detector commonly used in gas liquid chromatography.

- One of the most widely used detectors in gas chromatography is the thermal conductivity detector.
- In these detectors change in thermal conductivity of the carrier gas is measured by the thermistor.
- The carrier gas used is either helium or hydrogen sometimes nitrogen has also been used as the carrier gas. Because helium is costly and hydrogen is dangerous. But the sensitivity is greatly reduced.
- In the detector two pairs of matched thermistors from the wheatstone bridge circuit are arranged.

Principle:- It works on difference in thermal conductivity of pure carrier gas and gaseous solute molecules.

- Carrier gas is passed over one pair (reference) while a mixture of carrier gas and column effluent is passed over the other pair (sample) of thermistors.
- When pure carrier gas passes over both the pairs of thermstors, the bridge is balanced.
- But when the mixture of carrier gas plus effluent gas passes over the other, then the bridge is unbalanced because of unequal cooling of the two pairs of thermistors.
- The extent in imbalance is measure of the concentration of the column effluent in the carrier gas.
- The imbalance is fed to a recorder to give the chromatogram.As can be easily seen this is differential technique based on the difference in thermal conductivities of the reference and sample. Thermal conductivity detectors are comparatively simple, sturdy and reliable and its sensitivity is sufficient for most purposes.

Advantages of Thermal conductivity detectors

- ➢ Applicable to most compounds
- ➢ Linearity is good
- ➢ The sample is not destroyed & hence used in preparative scale.
- ➢ Simple easy to maintain and inexpensive.

Disadvantages of Thermal conductivity detectors

- ➢ Low sensitivity
- ➢ Affected by fluctuations in temperature and flow rate
- ➢ The response is only relative and not absolute
- ➢ Biological sample cannot be analyse.

FLAME IONIZATION DETECTOR (FID)

The first flame ionization detector was developed in 1957 by scientists working for CSIRP IN Melobourene, Australia. The flame ionization detector is the most sensitive gas chromatographic detector for hydrocarbons such as Butane or Hexane.

Principle : The detection of organic compounds is most effectively done with flame ionization. Biochemical compounds such as proteins, nucleotides and pharmaceuticals can be studied with this detectors due to the presence of nitrogen, phosphorus or sulphur atoms. The compounds may be more easily detected using FID which contain higher carbon concentration.

FID analysis involves the detection of ions. The source of these ions is a small hydrogen flame. Sometimes hydrogen –oxygen flames are used due to an ability to increase detection sensitivity, however for most analysis the use of compressed breathable air is sufficient. The resulting flame burn such a temperature as o pyrolyze most organic compounds, producing positively charged ions and electrons, which are then detected with the help of two electrodynamic fields.

Advantages

- ➢ It is best for detecting Hydrocarbons, and other easily flammable components
- ➢ They are very sensitive to these components and response tends to be linear across a wide range of concentrations.
- ➢ Linearity is excellent
- ➢ Response to most of the organic compounds

Limitations

- ➢ Molecules that contained only carbon and hydrogen respond best in this detector, but the presence ‘ heteroatom’ in a molecule ,such as oxygen decrease the detectors response.
- ➢ The main disadvantage FID is its destructive nature , so it cannot be connected directly to other GC detectors.

ELECTRON CAPTURE DETECTOR

The electron capture detector (ECD) was invented in 1957,by Dr. James,E.Lovelock. It can detect tiny amount of amount of chemical compounds in the atmosphere.It is used in detecting electron absorbing components in the output stream of gas chromatograph. The ECD uses a radio active β-

particles(electron)emitter a typical source containing a metal foil holding 10 millicuries of Nickel -63.The electron formed are attracted to a positively charged anode, generating a steady current.
As the sample is carried into the detector by a stream of nitrogen or a 5% methane,95% argon mixture, analyate molecules capture the electron and reduce the current between collector anode and a cathode. The analyte concentration is thus proportional to the degree of electron capture and this detector is particularly sensitive to Halogens, organometalic compounds ,nitriles,or nitro compounds.

Advantage

- ECD IS 10-1000 times more sensitive than an FID and one million times more sensitive than a TCD.
- Even nanogram quantities can be detected
- Halogenated compounds ,several pesticides can be detected by using this type of method.

Disadvantage

- It can be used only for compound with electron affinity

ARGON IONIZATION DETECTOR (AID)

Argone ionization detector dpends on the excitation of argon atoms to a metastable state, by using radioactive energy.This is achieved by irradiating the carrier gas with either **α**- particle or $_{\beta}$ -particle.**α**-particle can be obtained from radium-D.$_{\beta}$-particles can be obtained from ^{90} Sr or tritum.

These high energy particles ionize the argon atoms and hence they are exited to metastable state.These molecules collide with the effluent molecules and ionizes them.These ions when they reach the detector will cause an increase in current.Thus the compounds can be detected.

Advantages

- Responds to most of the organic compounds.
- Sensitivity is very high.

Disadvantages

- Response is not absolute
- Linearity is poor
- Sensitivty is affected by water and is much reduced for halogenated compounds.
- The response varies with the temperature of the detector and for high temperature like 240 ^{0}c, voltages of 1000v or less are usually necessary.

APPLICATION

- ✓ Qualitative analysis- by comparing the retention time or volume of sample to the standard/ by collecting the individual components as they emerge from the chromatograph and identify these compound by other methods like UV,IR,NMR.

Pharmaceutical Application

- Quality control and analysis of drug products like antibiotics(pencilline) antivirals (amantdine),general anesthetics(chloroform, ether), sedative /hypnotics(barbiturates) etc.
- Assay of drugs-Purity of a compound can be determined for drugs like,
  -atropine sulphate
  -clove oil
  -stearic acid
- In determining the level of metabolites in the body fluids like serum, plasma,urine etc.
- Analysis of foods like carbohydrates, proteins ,lipids ,vitamines,

METHOD OF VALIDATIION

Validation is the documented act of proving that any procedure ,process ,equipment ,material ,activity or system actually leads to the expected result.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength,quality, purity and potency of the drug substance or drug product.

The main objective of analytical validation is to ensure that a selected analytical procedure will give reproducible and reliable results that are adequate for he intended purpose.

Validation of a method must be performed for the following reasons

- ✓ Assuring high quality of the result.
- ✓ Reaching acceptance of the products by international agencies.
- ✓ Achieving the range of " official/reference method " approved by regulatory agencies.
- ✓ Improve the financial bottom line of the laboratory.
- ✓ Compulsory condition for registration of any pharmaceutical product or pesticide formulation.

Parameters recommended by FDA, USP and ICH,

- Specificity
- Linearity &Range
- Precision
  - Method of precision
  - Intermediate precision
- Accuracy
- Solution stability
- Limit of detection

- Limit of qualification
- Robustness
- System suitability

SPECIFICITY

International council for Harmonization (ICH) defines specificity as "the ability to assess the analyte unequivocally in the presence of components which may be expected to be present.

Specificity is validated by comparing the retention time of individual solvent peak of standard solution to those in sample solution.

The blank solution should not interfere with the analysis in the GC method.

Determination

- Identification testes
- Assay and impurity tests

LINEARITY

ICH defines linearity of an analytical procedure as its ability (with a given range) to obtain test results that are directly proportional to the curve concentration of analyate in the sample.

Method : dilution of stock solution of Drug.

Minimum 5 concentrations are used

Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components.

Linearity test solutions were prepared at six concentrations level from LOQ to 40%,60%,80%,100% and 120% of working level through the dilution of standard mixture of stock solution .As a result , the linearity study was carried out. Linear regression equation was conducted on the peak area of each solvent versus its calculated concentration.

RANGE

The interval between the upper and lower concentrations of analyte in the sample that have been demonstrate to have a suitable level of precision, accuracy, and linearity.Specific range dependent upon intended application of the procedure.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. precision is consider at three levels , repetabiliy, intermediate ,and reproductively precision.

Repeatability-It expresses the precision under the same operating conditions over a short interval of time.

Intermediate precision-It expresses variations within laboratories,such as different days,different analysts,different equipment.

Reproducibility-Reproducibility expresses the precision between laboratories.

- ✓ **System precision** –The system precision is checked by using a standard chemical substance to ensure that the analytical system is working properly. In method precision a homogeneous sample of the single batch should be analyzed six times.
- ✓ **Method precision**- Method precision should be performed by determining % assay on six homogeneous sample at working concentration level on one single batch and determining the % RSD of assay value.

ACCURACY

ICH defines the accuracy of analytical procedure as the closeness of agreement between the actual conventional value or accepted reference value and the value found.

The FDA guidance states that accuracy must be determined using quality control (QC)sample with a known amount of the target analyate .The purity of the analyate standard must be accurately known..Three types of reference standareds are usually used, by decreasing order of preference:certified reference standards, commercially supplied reference standards from a reputable source and in laboritary synthesized standared by a reliable noncommercial establishment. The source, expiration date lot number ,certificate of analysis and identification ,purity test should be analysized by five replicates.

ROBUSTNESS

ICH defines the robustness of an analytical procedure as a measure of it's capacity to remain unaffected by small but deliberate variations in method parameters.

It studies aim to examine the influence of the potential source of variations in the responses of the method.

It is recommended to include the robustness during the appropriate step of the method validation, instead of at the end of the validation, and document all the critical results. A validation of a scarcely robust method would provide inadequate results throughtout

the overall validation process and will result in loss of efficiency during routine quality control testing,with the subsequent loss of time and fund.

It refers to the range of parameters and stress to test a particular analytical method.

The importance of demonstrating robustness in a particular analytical method is to meet regulatory and manufacturing standards.

LIMIT OF DETECTION

The limit of detection (LOD) is the statistical value that establishes the minimal concentration that provide a signal that can be reliable differentiated from the background noise, with a specified significance level (**α**=5%).Therefore, signals over that produced by the LOD are assigned to the analyate , where as inferior values are attributed to the background. Finally at LOD the presence of analyte can be assessed, but not qualified with reliable accuracy and precision.

The qualification of the analyate at <LOD would provide uncertainties larger to the value itself .In this case the result must be referred to as "concentration<LOD"instead of "concentration=0". The LOD is logically under the calibration range . A LOD under the minimal value expected in a real sample is usually required.

The LOD must be calculated in simple matrix , as the base line noise depends on the physical properties and chemical composition of the matrix.

LIMIT OF QUANTIFICATION

LOQ is the lowest concentration that can be quantitatively determined with accuracy and precision under the fixed acceptance criteria. The qualification of the analyate in LOD and LOQ range is possible, but with a too high associated uncertinity. Thus the reported confidence interval would be uninformative , Thus the result must be simply reported as "concentration between LOD &LOQ" The LOQ would be reasonably close to the LLOQ. The LOQ must be under the minimal concentration expected in the real sample.

STABILITY OF SOLUTION

All the test concentrations of impurities and test solution shall be prepared and injected at periodic interval and area shall be evaluated.

The solutions are considered stable till the area is changed from initial area by 5%

Established duration for which solution is stable shall be mentioned in STP.

CONCLUSION:

Gas chromatography is one of the most important tools in chemistry because of its simplicity, sensitivity, and effectiveness in separating components of mixtures. It is widely used for quantitative and qualitative analysis of mixtures and for the purification of compounds. Gas chromatography can be effectively coupled with uv/visible detectors for monitoring dye labels, and infra- red spectroscopy to more effectively analyze mixtures.

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