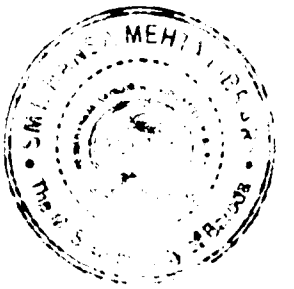
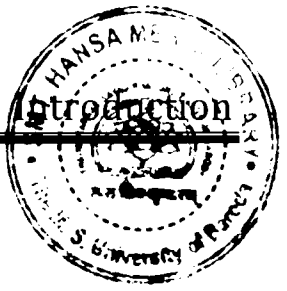


Chapter 1

Introduction

Chapter 1 Introduction



1. Introduction

1.1 Importance of Analytical Methods:

Quality is important in every product or service, but it is vital in medicine as it involves life. Unlike other consumer goods, there can be and there is no second quality.

Therefore analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, is a stable and will continue to meet purported quality throughout its shelflife[1].

These methods should be selective and sensitive to monitor the known and unknown impurities, have to be written in a format such that they can be produced over a period of time and from laboratory to laboratory, i.e. these methods should be validated.

1.2 Introduction to Analytical Methods:

Analytical methods are required to characterize drug substance and drug product composition during all phases of pharmaceutical development[2] .Early phase methods must support changes in synthetic routes and dosage form and elucidate the structures and levels of impurities. In later phases, goals change to the development of rapid and robust methods for release and stability evaluation.

Analysis includes a wide range of simple and instrumental analytical methods, but the most widely most used analytical methods for quality assurance are spectroscopy and chromatography based. Most quantitative analysis require, measuring specified components in the presence of sample matrix and /or related substances, therefore isolation or separation of the components are required preceding quantitative analysis. In such cases chromatographic techniques are used for quantitative analysis. In cases where matrix interference is not observed quantitative measurements are made using spectroscopic or titration methods directly[3].

For the present studies analytical methods based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Reserved Phase and Normal Phase High Performance Thin Layer Chromatography HPTLC and Infra Red Spectrophotometry have been developed

1.2.1 Chromatography Techniques:

In chromatographic methods, separation is based on variation in the distribution of different compounds between two dissimilar phases -a stationary phase and a mobile phase. Further differentiation can be made between chromatographic procedures in which the individual

components are monitored on line (HPLC) and procedures in which the components are measured in situ on the chromatographic stationary phase(TLC)[4].

A. High Performance Liquid Chromatography (HPLC):

High-performance liquid chromatography (HPLC) is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to understand better the role of individual molecules. In liquid chromatography, a mixture of molecules dissolved in a solution (mobile phase) is separated into its constituent parts by passing through a column of tightly packed solid particles (stationary phase). The separation occurs because each component in the mixture interacts differently with the stationary phase. Molecules that interact strongly with the stationary phase will move slowly through the column, while the molecules that interact less strongly will move rapidly through the column. This differential rate of migration facilitates the separation of the molecules.

HPLC utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector to signal the characteristic retention time for the analyte.Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase[5]. A block diagram of HPLC System is as given below.

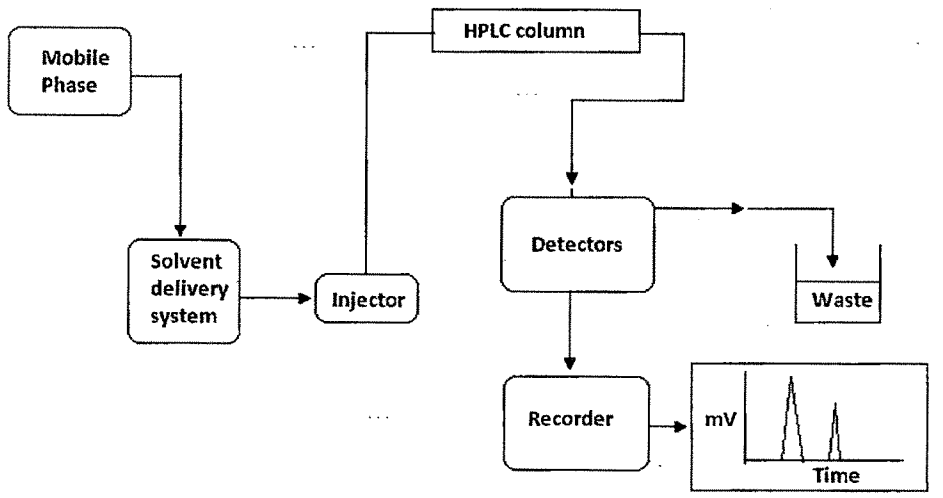


Fig: 1.1 Block diagram of HPLC System

In HPLC, a pump provides the higher pressure required to move the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes.

This allows for a better separation on columns of shorter length when compared to ordinary column chromatography.

The sample to be analyzed is introduced, in small volumes, into the stream of mobile phase. The solution moved through the column is slowed by specific chemical or physical interactions with the stationary phase present within the column. The velocity of the solution depends on the nature of the sample and on the compositions of the stationary (column) phase. The time at which a specific sample elutes (comes out of the end of the column) is called the retention time; the retention time under specified conditions is considered an identifying characteristic of a given sample.

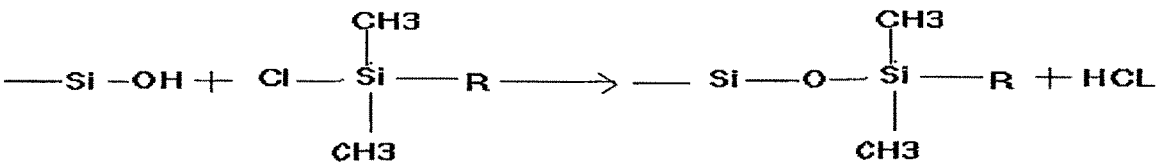
There are two types of stationary phases mainly reverse phase and normal phase. The nature of the stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography.

a. Normal phase HPLC:

Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds

b. Reversed phase HPLC:

About 75% of current HPLC analysis is performed using the reverse phase[6]. In reversed phase chromatography the Stationary phase is mainly silica chemically bonded through a siloxane (Si-o-Si-C) linkage to a low polar function group. These phases are prepared by treating the surface silanol groups of silica with an organochorosilane reagent[7].The polarity of the column can be changed by varying the alkyl chain length in R.



Where R= C_6H1_3 (Hexyl), C_8H_{17} (Octyl) or $C_{18}H_{17}$ (Octadecyl).

For our studies mainly we have used C-18 columns

Thus Reversed phase HPLC has a non-polar stationary phase and an aqueous, moderately polar mobile phase. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.

Retention time can be increased by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent.

HPLC Detectors[8]

Based on the method or principle used in detection the detectors available are RI detectors, UV detectors, Fluorescent detectors, Electrochemical detectors and Photo diode-array detectors (PDA).For the present study UV and PDA detectors are mainly used.

Refractive index detector is known as Universal detector, but it is not a very sensitive detector

UV Detectors

In these systems detection depends on absorption of UV ray energy by the sample.The equipment comprises of accessories in order as UV source, grating (for light defraction),sample passing through a tubing exposed to rays, photo cell, charge conductor etc.

When the UV rays emitted by lamp pass through gratings, rays split into different wavelengths. One specific wavelength rays are passed through sample. Some amount of light is absorbed by sample and the unabsorbed rays which fall on photo cell.

These rays on collision on photo cell produce electrons whose current is recorded. This is indicative of nature and quantity of sample. This UV wavelength range of absorption is specific for sample . These are the HPLC detectors used in general, unless there is requirement for analysis of special compounds. They are capable to detect very wide range of compounds. The sensitivity ranges till microgram quantity of estimation.

PDA detectors: These are detectors which follow principle similar to UV detectors but the only advantages are higher sensitivity and measure the entire absorption range i.e. it gives scan of entire spectrum.

HPLC method development[9]

The wide variety of equipment, columns, eluent and operational parameters involved makes high performance liquid chromatography (HPLC) method development seem complex. The process is influenced by the nature of the analytes and generally follows the following steps:

- Step 1 – Initial studies
- Step 2 - Selection of initial conditions
- Step 3 - Selectivity optimization

- step 4 - System optimization

Step-1 Initial studies

When developing an HPLC method, the first step is always to consult the literature to know the physico chemical properties of analyte..T he properties like solubility,polarity,molecular weight and partition coefficient are key parameters in selecting the right column and mobile phase for a successful chromatographic separation.

Sample preparation. The sample matrix determines whether the sample requires dissolution, filtration, extraction, preconcentration or clean up. Chemical derivatization may be required to assist detection sensitivity or selectivity.

Types of chromatography. Reverse phase is the choice for the majority of samples, but if acidic or basic analytes are present then reverse phase ion suppression (for weak acids or bases) or reverse phase ion pairing (for strong acids or bases) should be used. The stationary phase should be C_{18} bonded. For low/medium polarity analytes, normal phase HPLC is a potential candidate, particularly if the separation of isomers is required.

Gradient HPLC.

This is only a requirement for complex samples with a large number of components (.20–30) because the maximum number of peaks that can be resolved with a given resolution is much higher than in isocratic HPLC.

Gradient HPLC will also give greater sensitivity, particularly for analytes with longer retention times, because of the more constant peak width (for a given peak area, peak height is inversely proportional to peak width).

Detectors UV detectors ,single channli,multi channel or Photodiode array are the most common detectors used in HPLC .

Fluorescence or electrochemical detectors are used for trace analysis. For preparative HPLC, refractive index is preferred because it can handle high concentrations without overloading the detector.

UV wavelength.he analytical sensitivity is maximum at λ_{max} ,but the wavelengths showing high absorbance can be used as analytical wavelengths. The UV wavelengths below 200 nm are avoided because mobile phase interferences and detector noise increase in this region. Higher wavelengths give greater selectivity.

Step 2 - Selection of chromatographic conditions.

This step determines the optimum conditions to adequately retain all analytes; that is, ensures no analyte has a capacity factor of less than 0.5 (poor retention could result in peak overlapping) and no analyte has a capacity factor greater than 10–15 (excessive retention leads to long analysis time and broad peaks with poor detectability). Selection of the following is then required.

Mobile phase solvent strength. The solvent strength is a measure of its ability to sweep analytes from the column. It is generally controlled by the concentration of the solvent with the highest strength; for example, in reverse phase HPLC with aqueous mobile phases, the strong solvent would be the organic modifier; in normal phase HPLC, it would be the most polar one. The aim is to find the correct concentration of the strong solvent.

Gradient HPLC. With samples containing a large number of analytes (20–30) or with a wide range of analyte retentivities, gradient elution becomes necessary to avoid excessive retention.

Step 3 - Selectivity optimization.

The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To select these, the nature of the analytes must be considered.

Once the analyte types are identified, the relevant optimization parameters may be selected The optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization.

Step 4 - system parameter optimization.

This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

Step 5 - method validation. Proper validation of analytical methods is important for pharmaceutical analysis when ensurance of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria.

B. High Performance Thin Layer chromatography (HPTLC) [10]:

Thin Layer chromatography utilizes a flat (Planar) stationary phase for separation therefore is also called as Planar Chromatography. In Thin Layer Chromatography (TLC) this stationary phase is supported by a glass plate or foil (plastic or aluminium).Again unlike column separations, the TLC plate constitutes an open system, which passes through the individual steps of the TLC analysis in an off-line mode.

The most advanced form of instrumental TLC is called **High Performance Thin Layer chromatography (HPTLC)**. HPTLC includes sophisticated instruments, controlled by integrated software.

It uses HPTLC plates featuring small particles with narrow size distribution. HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and are used for pharmaceutical densitometric analysis. Normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform methanol has been used for pharmaceutical analysis.Lipophilic C-18, C-8, C-2; phenyl chemically-modified silica gel plates developed with a more polar aqueous mobile phase, such as methanol-water or dioxane are used for reverse phase.

C. Infra Red Spectroscopy[11]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used for qualitative analysis by organic and inorganic chemist. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

Quantitative[12,] IR spectroscopy was generally considered to be able to provide only qualitative and semi quantitative analyses of common samples, especially when the data were acquired using the conventional dispersive instruments. However, the developments of reliable FTIR instrumentation and strong computerized data-processing capabilities have greatly improved the

performance of quantitative IR work. Thus, modern infrared spectroscopy has gained acceptance as a reliable tool for quantitative analysis.

The basis for quantitative analysis of absorption spectrometry is the Bouguer–Beer–Lambert law, commonly called Beer's law. For a single compound in a homogeneous medium, the absorbance at any frequency is expressed as

$A = abc$

Where, A is the measured sample absorbance at the given frequency, a is the molecular absorptivity at the frequency, b is the path length of source beam in the sample, and c is the concentration of the sample.

In the present work,it has been tried to quantitate albendazole and ivermectin in the combined dosage form(Tablet) by IR spectroscopy,because solubility of albendazole is the major problem for its analysis in the solution form. However taking advantage of DRS or ATS ,IR technology the IR spectra of samples can be directly taken with less sample preparation and without using mineral acids for solublizing albendazole and also avoiding degradation of ivermectin if any in acidic medium.

1.3 Validation of analytical methods

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories[13].

"Validation is the process of collecting documented evidence that the method performs according to its intended purpose" .This is based on analytical experiments performed according to the validation protocols that comply with the international guidelines i.e. ICH guidelines on method validation. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a unique project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration.

Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the

International Conference on Harmonization (ICH) guidelines (Q2A and Q2B)[14,15] The US Food and Drug Administration (FDA)[16,17]and US Pharmacopoeia (USP) both refer to ICH guidelines, i.e. these methods should be validated.

All analytical procedures require some form of validation, regardless of whether the method is used for stability, in –process analysis ,release, or acceptance testing.

The extent of guidelines for validation requirements provided by different organizations varies widely, but the objective of validation is always to achieve valid analytical test results. This is important to ensure the quality and safety of products that are measured using the analytical method.

Method validation has received considerable attention in literature from various industrial committees and regulatory agencies. There is a wide variety of information and guidance available, as mentioned below;

Literature from industrial committees and regulatory agencies

Committees and regulatory agencies	**Guidelines available**
ICH	a) $Q2R_1$Guidelines are guidelines for new method development and its validation. b) Q_1R_1 Guidelines are for development and validation of stability indicating analytical methods includes methodology
The United States Food and Drug Administration	Two industry guidelines: a)for the validation of analytical methods2 b)forthe validation of bioanalytical methods 3.
IUPAC	"Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis".
EURACHEM	detailed guide for method validation primarily developed for ISO/IEC accredited laboratories but because of its completeness it is also a good source for (bio)pharmaceutical laboratories
AOAC	technical document for the verification of analytical methods for the ISO 17025 accreditation.

Huber	Has published a technical document for the verification of analytical methods for the ISO 17025 accreditation.
Viswanathan and co-authors	An overview for validationof bioanalytical methods.

1.3.1 Validation Parameters[18]

As per ICH guidelines following types of analytical procedures need to be validated:

- Identification test
- Quantitation tests for impurities content
- Limit test for the control of impurities
- Quantitative tests of the active ingredient or other main components of the drug

These various parameters for validation of any analytical method are:

1. Accuracy

The accuracy of an analytical procedure ***expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.***

Accuracy can also be described as ***the extent to which test results generated by the method and the true value agree.***

The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (for example, three concentrations with three replicates each). Accuracy should be reported as percent recovery by the assay of known added amount of standard in the sample or as the difference between the mean and the accepted true value, together with the confidence intervals.

Thus, accuracy of the method was studied by recovery experiments using standard addition method at three different levels (80%, 100% and 120. Known amounts of Standard solutions containing analyte were added to prequantified sample solutions to get 80,100 and 120 %. These samples were analyzed by injecting the sample solution and % recovery was calculated.

In the present study %recovery was calculated by the following formula.

$$\frac{\text{AREA OF SPIKED SAMPLE-AREA OF UNSPIKED SAMPLE}}{\text{AREA OF STANDARD}} \times \frac{\text{CONC.OF STANDARD}}{\text{CONC. OF ADDED DRUG}} \times 100$$

Acceptance limit for % recovery is 98-100%

2. Precision

The precision of an analytical procedure expresses ***the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions***. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility

Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results (i.e. between 6 - 1 0). Therefore for present work sample were assayed six times and the %RSD for obtained assay values was obtained. Precision is then expressed as the relative standard deviation.

$$\%RSD=\frac{STD\ DEV}{MEAN} \times 100$$

Assay values were calculated with the following formula

$$\%\ Assay = \frac{Area\ of\ Sample}{Area\ of\ Standard} \times \frac{Concentration\ of\ Standard}{Concentration\ of\ Sample} \times \frac{Purity\ of\ Standard}{100} \times 100$$

Repeatability

Repeatability ***expresses the precision under the same operating conditions over a short interval of time***. Repeatability is also termed intra-assay precision.

Intermediate precision

Intermediate precision ***expresses within-laboratories variations: different days, different analysts, different equipment, etc.***

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

3. Specificity

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc."USP<1225> refers to the same definition but also comments that other reputable authorities such as IUPAC and AOAC use the term "selectivity" for the same meaning.

This reserves the use of "specific" for those procedures that produce a response for a single analyte only. ISO/IEC most likely has the same understanding because it requires a method to be "selective" rather than specific. Our goal is to distinguish and quantify the response of the target compounds from the responses of all other compounds Selectivity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix.

Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and/or impurities. Determination of this can be carried out by assessing the peak identity and purity.

4. Detection Limit

The detection limit of an individual analytical procedure ***is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.***

Limit of detection (LOD) was calculated by using the formula

LOD = 3.3 x σ/S Where, σ = Standard deviation of response

S = Slope of regression equation.

5. Quantitation Limit

The quantitation limit of an individual analytical procedure ***is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.*** The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

LOQ = 10 x σ/S Where, σ = Standard deviation of response, S = Slope of regression equation.

6. Linearity

The linearity of an analytical procedure ***is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.*** This is the method's ability (within a given range) to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range.

Linearity is determined by calculating the regression line using a mathematical treatment of the results (i.e. least mean squares) vs. analyte concentration.. Calibration curve was constructed by plotting peak area vs concentrations of analyte.

7. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The range is normally expressed in the same units as the test results. For assay tests, ICH requires the minimum specified range to be 80 to 120 percent of the test concentration.

8. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Thus Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (i.e. from laboratory to laboratory, from analyst to analyst.)

Table:1.1 Important validation parameters suggested by regulatory agencies

Parameter	ICH	USP	ISO 17025
Specificity	X	X	-
Selectivity	-	-	X
Precision		X	-
Repeatability	X	-	X
Intermediate precision	X	-	-
Reproducibility	X	X (RUGGEDNESS)	X
Accuracy	X	X	X
Linearity	X	X	X
Range	X	X	-
Limit of detection	X	X	X
Limit of quantitation	X	X	X
Robustness	X	X	X
Ruggedness	X	X	-

1.3.2 System Suitability Tests (SST)[19,20]

System suitability testing is an integral part of HPLC procedures..The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Once a method or system has been validated the task becomes one of routinely checking the suitability of the system to perform within the validated limits.

System suitability test parameters are:

- Plate number or number of theoretical plates (n)
- Capacity factor (capacity ratio) k
- The selectivity or Separation Factor (relative retention) α
- Peak Resolution R
- Peak asymmetry factor (As).

These are measured on a peak or peaks of known retention time and peak width.

1. Plate number or number of theoretical plates (n)

This is a measure of the sharpness of the peaks and therefore the efficiency of the column. This can be calculated in various ways, for example the USP uses the peak width at the base and the BP at half the height.

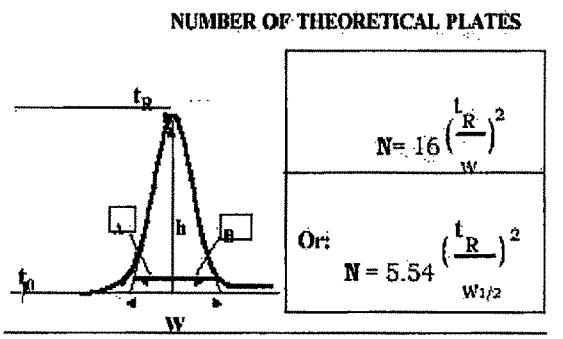


Fig: 1.2 Plate number or number of theoretical plates

WhereWhere, tR is the retention time and W is the peak width.

$W_{1/2}$= peak width at peak height

Wb = peak width at base

t = retention time of peak

Therefore the higher the plate number the more efficient the column. The plate number depends on column length - i.e. the longer the column the larger the plate number.

2. Capacity factor (capacity ratio) k

Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor, k", is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k" ranges from 2-10. Capacity factor can be determined by using the formula,

RETENTION FACTOR or CAPACITY RATIO

$$k' = \frac{t_R - t_0}{t_0} \qquad k' = \phi \frac{C_s}{C_m}$$

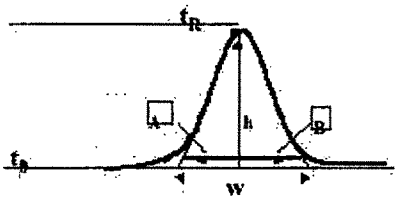


Fig: 1.3 Capacity factor (capacity ratio) k

Where, tR = retention volume at the apex of the peak (solute) and t0 = void volume of the system.

3. The selectivity or Separation Factor (relative retention) α

The selectivity (or separation factor), **α**, is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components.

This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, and eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.

The ideal value of **α** is 2. It can be calculated by using formula,

$\alpha = V2 - V1 / V1 - V0 = k1''/ k2''$

Where, V0 = the void volume of the column,

V1 and V2 =the retention volumes of the second and the first peak respectively

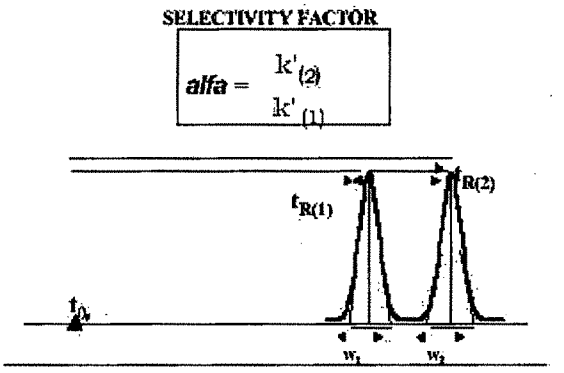


Fig: 1.4 The selectivity (or separation factor), **α**

4. Peak Resolution R

Resolution (Rs): Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture.

The resolution, Rs, of two neighboring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of Rs is 1.5. It is calculated by using the formula

This is not only a measure of the separation between two peaks, but also the efficiency of the column. It is expressed as the ratio of the distance between the two peak maxima. (At) to the mean value of the peak width at base (Wb).

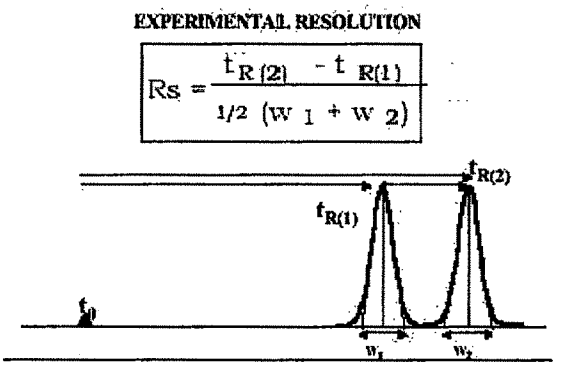


Fig: 1.5 Peak Resolution R

Where, Rt1 and Rt2 are the retention times of components 1 and 2 and W1 and W2 are peak width of components 1 and 2

5. Peak asymmetry factor (Tf): Peak asymmetry factor, Tf, can be used as a criterion of column performance. The peak half width, b, of a peak at 10% of the peak height, divided by the corresponding front half width, gives the asymmetry factor.

Asymmetric factor or Tailing factor

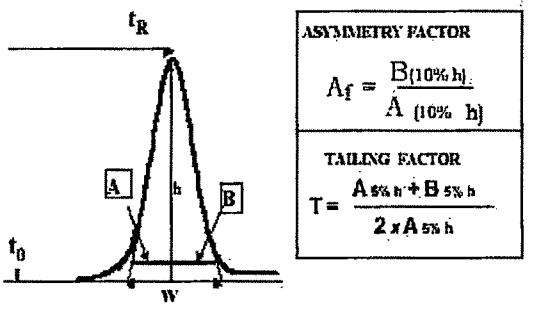


Fig: 1.6 Peak Asymmetry facor(Tf)

Table 1.2 SYSTEM SUITABILITY TEST PARAMETERS AND RECOMMENDATIONS

Parameters	**Recommendation**
Theoretical Plates (N)	should be > 2000
Capacity Factor ($k^{\prime}$)	The peak should be well-resolved from other peaks and the void Volume ,generally $k^{\prime}$>2.0
Relative retention	Not essential as long as the resolution is stated.
Resolution (Rs)	R_S of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard) etc.
Tailing Factor (T)	T of </= 2

1.3.3 Stability indicating assay method

Patients taking a pharmaceutical product for a particular malady, expect the product to be safe and efficacious. Pharmaceutical regulatory agencies worldwide demand that the product retains its identity, quality, purity, and potency for the time the product is commercially available. Consequently the agencies expect to see stability data supporting the proposed expiration date of the product in the marketing submission. Therefore it is necessary to conduct stability studies to predict, evaluate, and ensure drug product safety[21].

Stability studies of drug substances via acid hydrolysis, base hydrolysis, oxidation and thermal and photolytic stress testing are a part of development strategy under the ICH requirements[22].

These studies provide information on a drug's inherent stability and help to validate analytical methods to be used for evaluation stability. Stability assays are currently being developed by using the stress testing approach of the ICH guidelines, Q1 A[R2].The approach has been further extended to stress test of drug combinations. These tests allow accurate and precise quantification of drugs, their degradation products, and their interaction products.

Stability-indicating methods according to 1987 guideline were defined as the '***Quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured.***'

The revised drug stability guideline Q1AR from the International Conference on Harmonization (ICH) requires that stability samples be tested using validated stability-indicating assay methods. The guideline suggests that stress testing be performed to establish the intrinsic stability of the drug substance and to validate the stability-indicating nature of the analytical method. It also recommends that stress testing include the evaluation of the effect of temperature, humidity (when appropriate), oxidation, and photolysis on the drug substance plus its susceptibility to hydrolysis across a wide range of pH values when in solution or suspension. Although applicable to new drugs, the ICH guidelines have recently been sought to be extended to generic drugs.

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