Chapter -1

Introduction
1.1.1 Abstract

Analytical chemistry is a scientific discipline that develops and applies methods, instruments and strategies to obtain information on the composition and nature of matter. It deals with quantitative analysis of composition of substances and complex materials in various matrices by measuring physical and chemical or physicochemical properties of a distinctive constituent of the component or components of interest. So in this thesis the new analytical method is developed and validated as per the pharmaceutical guidelines for Lopinavir & Ritonavir Soft gelatin Capsules and Montair Tablets both are completely different dosages forms.

Analytical chemistry has played the major role in the changes faced by the pharmaceutical industry today. The analytical department has becomes significant partner in the drug development process. Pharmaceutical analysis is branch of pharmaceutical sciences which deals with analysis of drugs and excipient used in manufacture of drug formulation assuring the quality of drugs and their formulation i.e. Pharmaceuticals. Analytical chemist or quality control chemists have the responsibility to develop sensitive, reliable and accurate methods for the test analysis.

Development efficient analytical method and its validation are critical elements in the development of pharmaceuticals. An analytical method is selected on the basis of criteria of Method Validation and the amount of available sample, the amount of analyte in the sample, time, cost, and the availability of equipment. These criteria are not mutually independent, and it often necessary to find an acceptable balance among them. In developing a procedure or protocol, consideration is given to compensating for interferences, calibrating equipment and standardizing the method, obtaining an appropriate sample, and validating the analysis. Poorly designed procedures and protocols produce result that is insufficient to meet the needs of the analysis. Hence analytical method development needs systematic plan of work with all specifications and characterization of specific drug substances and drug products.

Analytical approach
Fig.1.1: Flow diagram for analytical approach

There are various ways by which concentration of analyte determined.

According to need of analysis analytical chemistry can be classified as following types:
Qualitative analysis in which determine identity of the constituent species in a sample, which may be of organic or inorganic.

Quantitative analysis use to determine how much of a constituent species is present in a sample.

Characterization analysis which deals with evaluation of a sample’s chemical or physical properties.

Fundamental analysis is an analysis whose purpose is to improve an analytical method’s capabilities.

Analytical methods are broadly classified into instrumental method and classical or wet-chemical methods according to the property that is observed in the final measurement process.

Chemical methods of analysis involve measurements of the mass of a substances or the volume of reacting Sample e.g. Gravimetric Method, Volumetric Method.

Instrumental methods which depend upon the determination of different electrical property and also dependant on absorption of radiation or measurement of intensity of emitted radiations. for these all instrument required.

e.g. Spectrophotometer, HPLC.

Techniques of instrumental analysis
Several instrumental methods are used in pharmaceutical analysis, of which important methods are –

- Separation Techniques: These are nothing but chromatographic methods i.e. TLC, GC or HPLC which may applicable for an analysis depends on several parameter like volatility or solubility of the sample, separation efficiency, concentration of analyte, detection limit, cost of analysis etc. These techniques depend upon rate of migration of analyte through stationary phase by movement of liquid or gaseous mobile phase.

- Spectrometric Techniques: UV, IR, NANOMETERR, MASS etc. spectrometric techniques are usually used in analysis of drug of interest alone in excipients
matrix, additives, degradants, impurities etc. It also includes Flame, Atomic, X-Ray, Emission Spectrometry, etc.

- **Electro-analytical techniques**: Electro-analytical method of analysis deals with electrical signal to the sample and/or monitor the electrical property of the sample e.g. Potentiometry, Conductometry, electrogravimetry, Polarography, Amperometry etc.

- **Thermo-analytical techniques**: These analytical technique deals with interaction of heat with material. e.g. Thermo gravimetric technique, Differential scanning Colorimetry and Differential thermal analysis etc.

- **Modern hyphenated techniques**: These techniques deal with advancement in drug analysis by combination of one instrumental technique with other in order to refine the analysis of drug, impurity, degradation product etc.

  E.g. Liquid Chromatography with Mass spectrometry Gas Chromatography with MS, GC with infrared spectroscopy.

So nalytical science deals with use of various techniques for determination of analyte in sample matrices. Hyphenated technique put the added advantage in analytical sciences in order to determination of analyte specifically.

### 1.1.2 INTRODUCTION TO CHROMATOGRAPHY:
The great innovation was discovered by Michael Tswett in 1906. He was working on separation of green pigment from green leaves of the plant. He used chalk columns to determine this colour pigment and described the term ‘chromatography’ used by Tswett to describe the colour which comes down from the column. Nowadays, chromatography in industries is driven by chemistry as well as all types of instrumentation. Chemistry is vastly used for developing instrumental methods via their chemical appearance, physical properties. At the time of chromatographic development, molecular structure, pH, pKA consideration in method determination. Chemistry of column is also important in chromatography for method establishment.

**Principle:**

Chromatography is a separation method which employs two phases, one stationary and other mobile. As a mixture of analytes being carried through the system by the mobile phase passes over and through the stationary phase, individual components of the mixture equilibrate or distribute between the two phases,

\[ X_m = X_s \]

The corresponding thermodynamic distribution coefficient \( K \) is defined as the concentration of component (X) in the stationary phase divided by its concentration in the mobile phase.

\[ K = \frac{X_s}{X_m} \]

The constant \( k \) of equilibrium is very useful in chromatography. It proves that the system operates at equilibrium stage. Any substance or main drugs passed through the stationary phase column through the mobile phase, the absorption of active molecules shows aperticular retention time. Each drug has its individual ability to control thermodynamically distribution and the elution time. Thos through the proper separation show effectiveness of both thermodynamics and kinetics. Thus the fundamental principle of the chromatography deals with unequal distribution and division of components in sample thorough mobile phase by using a perfect stationary phase. It depends on their respective physicochemical properties.
1.1.3 Modes of separation:

In chromatography there are different Methods available for separation of components are given as follows,

- Type-1 – Adsorption-chromatography
- Type-2-Partition-chromatography
- Type-3-Ion exchange-chromatography
- Type-4-Permeation-chromatography

Discription of liquid high pressure or performance chromatography

separation of different compound is nothing but a chromatography, to separate out component from mixture by using the combination of solvents with different types of liquid buffers. In liquid chromatography mobile phase is pump through column containing stationary phase bearing a large pressure. As a result of significant development during the past two decades, which have brought significant improvements to instrumentation and column packing, HPLC has useful in quantitative as well as qualitative types of analysis.

This ability of separation is plays important role in medicinal and biological chemistry. The technique offers less time for analysis with perfect accuracy and useful in any determination of analysis. Modern HPLC techniques became available in 1969, but from 1990s HPLC become most popular instrument for drug analysis which is presently used in pharmaceutical research and development;

- chromatography is useful in natural product and for synthetic molecules.
- Metabolites characterization also carried out.
- used to determine assay, related impurities in to sample.
- it is also in pharmacodynamic and different pharmacokinetic study.

Recent improvements in HPLC deal with:

changing of partical size, packing material, different types of silica with lower u size.

- separation technique with high speed..
HPLC assets with computer to operate automatically
i.e. hyphenated detection systems.

1.1.4 INSTRUMENTATION:

Fig. 1.3: Block diagram of High-pressure (Performance) Liquid Chromatography

HPLC-Columns:

Different types of column are available in market. The column mostly made up of stainless steel. Which are withstand against high pressure. The column available different length, different in particle size. Different types of column packing also used in it. Mostly C18 and C8 HPLC Columns are used

C18 and C8 HPLC Columns

- In reverse phase chromatography these column generally use.
- Perfect peak shape compared to competitive column.
- High selectivity to this column
- C18 column are most retentive than C8
### Table 1: Characteristics of C\textsubscript{18} and C\textsubscript{8} Column

<table>
<thead>
<tr>
<th>Properties</th>
<th>C\textsubscript{18}</th>
<th>C\textsubscript{8}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonded Phase</td>
<td>Octadecylsilane materials</td>
<td>Octylsilane, materials.</td>
</tr>
<tr>
<td>Silica</td>
<td>Spherical shape highly pure</td>
<td>Spherical shape highly pure</td>
</tr>
<tr>
<td>Particle Size</td>
<td>5-µm</td>
<td>5-µm</td>
</tr>
<tr>
<td>Pore Size</td>
<td>180 (Å)</td>
<td>180 (Å)</td>
</tr>
<tr>
<td>Surface Area</td>
<td>200 m\textsuperscript{2}/g</td>
<td>200 m\textsuperscript{2}/g</td>
</tr>
<tr>
<td>% C</td>
<td>~12.0 %</td>
<td>~7.50 %</td>
</tr>
<tr>
<td>Coverage</td>
<td>~3.0 µmoles/m\textsuperscript{2}</td>
<td>~3.40 µmoles/m\textsuperscript{2}</td>
</tr>
<tr>
<td>Structure</td>
<td><img src="image1" alt="Structure Diagram" /></td>
<td><img src="image2" alt="Structure Diagram" /></td>
</tr>
</tbody>
</table>

**HPLC pumps:**

The retention time of any peak is also dependant on the pump. Pump is useful to delivered mobile phase through the system. Through the pump Mobile phase passed through the columnn with specified flow which separate out component present in mixture or compound at respective absorbance. nowa days different types of pumps are use.

**HPLC injectors:**

Injector is most important part of the HPLC system it long narrow thin tube which take sample and introduced it in to column via continuous flow of Mobile phase. It take out diffren injection volume from 5 ul to 100 ul. But some times dosages forms are very low in these cases higher injection volume injected through columnn by using different types of loops. Because higher injection volume are found broadening
of peaks. In this loop injection, valve injection, column injection are useful types to introduce sample.

**HPLC detectors:** The different types of detectors used in HPLC detector system. It simplifies chromatography in different components on the detector response of compound. It separates out multi components from any mixture on the basis of absorption. Detectors are classified in on its bulk and solute property.

The different types of detectors used in HPLC given as follows:

- **Ultraviolet-Visible Absorbance Detectors:**
  This is most important and widely used detector. In new Analytical method development
  Scanning is most important and use photo diode detector also called PDA.

- **Refractive Index Detectors**
  This is a detectors useful in isomeric component separation.

- **Fluorescence Detectors**
  These detectors are useful for nearly molecule which show molecular fluorescence. These detectors are very sensitive and high detection limits.

- **Electrochemical Detectors**
  These detectors are useful in electron transfer process in very sensitive detection of solute.

- **Conductance Detectors**
  These detectors are useful in electron transfer takes place in reaction.

- **Other Detectors**
  - Post-column Reaction Detector (derivatisation and separation reaction product use to detect)
  - Hyphenated Techniques (LC-MS, GC-MS, and FT-IR).
1.1.5 FUNDAMENTALS OF HPLC

A. SYSTEM SUITABILITY:

Definition
Any analysis carried out on instruments needs to determine its system suitability. The instrument use at time of analysis are highly calibrated in chromatography. The different parameter would be determine as per different pharmacopical bodies. Without suitability of system results has no value. So different tests are carried out to prove that system is suitable for analysis. Before starting of analysis system suitability parameter would be check.

B. DIFFERENT SYSTEM SUITABILITY REQUIREMENT IN GOOD CHROMATOGRAPHY:

As in any method development, the different system suitability criteria plays very important role in chromatography. Most of method depends on these criteria, like theoretical plates, factor for tailings, Re-solution (separation between two peaks), peak to valley ratio, signal to noise ratio and many other parameters. These all parameters are shows column performance.

C. PEAK TIME (TIME FOR RETATION) AND RETENTION VOLUME:

Retention measurements in elution chromatography may be given as the Elution time \( t_r \) directly defined From the Elution time, the retention volume \( V_r \) may be calculated.

\[
V_r = t_r n
\]

Where,

\( t_r \) - Elution time

\( n \) - Flow rate of the mobile phase.
D. MASS DISTRIBUTION RATIO:

The mass distribution ratio \( (Dm) \) (also defined as factor of capacity \( k' \)) is defined as:

\[
Dm = \frac{\text{Amount of solute in stationary phase}}{\text{Amount of solute in mobile phase}} = k \frac{V_s}{V_m}
\]

Where,

\( k \) - Equilibrium Distribution co-efficient,
\( V_s \) - Stationary phase volume,
\( V_m \) - mobile phase volume.

The mass distribution ratio determined from the chromatogram from following expression

\[
Dm = \frac{t_r - t_d}{t_d}
\]

Where,

\( t_r \) - Elution time
\( t_d \) - dead time

DISTRIBUTION COEFFICIENT:

The characteristics of elution of particular column in component, in size-exclusion chromatography, may be given by the distribution co-efficient \( (K_d) \) which is calculated from the expression:

\[
K_d = \frac{t_r - t_d}{t_r - t_d}
\]

Where,

\( t_r \) - Elution time.
\( t_d \) -dead time or distance (or exclusion volume) of the baseline from injection point to the unretained baseline to the maximum of the peak. \( t_r \) - Elution time or distance (or total permeation volume) along the baseline from the injection point and perpendicular to maximum peak height. Which has full assessed to pore size of column material?
E. CHROMATOGRAPHIC DATA:

The peaks define by peak area (A), peak height (h), peak width for half- height (w_{0.5}), peak height (h) and width of peak between inflection (w_{infl}) point. Gaussian peaks there is the relationship: w_{0.5} = 1.18 w_{infl}

1) Tailing factor :-

The tailing factor (As) (or factor of Asymmetry) for any peak is calculated by following formula:

\[
A_s = \frac{w_{0.05}}{2d}
\]

Fig. 1.4 symmetry Factor

Where,

\( w_{0.05} \) - one-twentieth peak Width from the peak height,

\( d \) - Perpendicular drop distance from the maximum peak height and leading edge in peak at height as seen in figure. 1.4
2) Column performance: (Theoretical Plate)

This is important parameter to know the performance of column known theoretical plates higher the theoretical plates then Sharpe peak shape observed .this terminology expressed as $N$, $t_r$, and $w_{0.5}$ have expressed in time, distance, volume.

\[ N = 5.54 \left( \frac{t_r}{w_{0.5}} \right)^2 \]

Fig. 1.5 Theoretical Plate

Where,

- $t_r$ - Elution time
- $w_{0.5}$ - width of the peak at half-height.

The apparent number of theoretical plates varies with the component as well as with the column and the time for retention.
F. SEPARATION DATA:

1) Re-solution or Re-solution factor:

The Re-solution or Re-solution factor ($R_s$) similar height of component may be calculated from the expression:

$$R_s = \frac{1.18(t_{r,b} - t_{r,a})}{w_{0.5,a} + w_{0.5,b}}$$

where $t_{r,b} > t_{r,a}$

Where, $t_{r,a} & t_{r,b}$ - Elution time of both peak.

High re-solution prove the separation between two component. The expression given above may not be applicable if the peaks are greatly dissimilar in height. Planar chromatography (quantitative), distances of migration $z_a$ and $z_b$ are used in stead of peak time (RT) and the Re-solution using the following expression:

$$R_s = \frac{1.18z_f(R_{f,b} - R_{f,a})}{w_{0.5,a} + w_{0.5,b}}$$

Where, $R_{f,a} & R_{f,b}$ - Distances ratios from application point to centers of spots and distance of solvent travelled from application point.
2) **Peak-valley ratio** :-

The peak-valley ratio use in system suitability parameter for related substances test. separation of impurity not achieved from the Main peak.

\[
\frac{p}{v} = \frac{h_p}{h_v}
\]

**Where**, 

- \(h_p\) - height of the peak from base line of impurity peak.
- \(h_v\) - height of lowest point of curve due to main peak and impurity peak in chromatograph as shown in figure.

3) **Relative retention time: (RRT)**

The relative retention \(r_{ab}\) is calculated following formula by using:

\[
r_{ab} = \frac{t_2 - t_1}{t_0 - t_1}
\]
\[ r_{a/b} = \frac{t_{r,b}}{t_{r,a}} \]

*Where,*

\( t_{r,b} \) - Elution time of the main peak,
\( t_{r,a} \) – Reference peak elution time.

In planar chromatography, the retention factors \( R_{f,b} \) and \( R_{f,a} \) are used instead of \( t_{r,b} \) and \( t_{r,a} \).

G. **RECISSION OF QUANTIFICATION:**

1) **Signal-to-noise ratio**

The signal-to-noise ratio is calculated by formula as its equation:

\[ S / N = \frac{2H}{h} \]

*Fig.1.7 Signal-to-noise ratio*

\( H \) - The peak height from baseline,
2) Repeatability

The repeatability calculates in RSD injection series. The RSD calculated from the equation is as below:

\[
RSD_\% = 100 \sqrt{\frac{\sum (y_i - \bar{y})^2}{n-1}}
\]

The maximal permitted \( RSD_{\text{max}} \) is calculated injection series which inject during analysis, which is also calculated for repeatability injection.

\[
RSD_{\text{max}} = \frac{KB\sqrt{n}}{t_{90\%, n-1}}
\]

Where,

- \( k \) - Constant, formula as \( K = \frac{0.6}{\sqrt{3}} \times \frac{100}{\chi^2_{0.95}} \) in which \( \chi^2_{0.95} \), show RSD after six injection for \( B = 1 \),
- \( B \) - Highest limit found from each of
- \( n \) - Number of replicate injections of the reference Sample (3 \( \leq n \leq 6 \)),
- \( T_{90\%, n-1} \) - Student’s \( t \) at the 90 per cent probability level (double sided) with \( n-1 \) degrees of freedom
1.1.6 QUANTIFICATION IN HPLC:

- External standard method: In this method the concentration of standard Sample or Reference Sample compare with the sample concentration. It is type of quantitative method and called as external standard quantification method.

- Internal standard method: In this method comparison is carried out between the area observed in standard sample and area observed in sample sample and also comparison between it peak height. A same or equal amount of substance to be determine was introduced in standard sample and sample.

- Peak Are Method (Area normalization): The impurity also determine by area normalization method. This is calculated by using peak area divided by total area. Mostly impurity determination carried out by this method during chromatographic analysis.

- Calibration Method: The measured relationship between its signal and it weight i.e. mass of respective substance evaluated and the function of calibration also calculate. The all results are evaluated from the evaluated signal of analyte due to the inverse function. For determination of quantitatively assay through the internal standard method or the calibration procedure may be used generally and the normalization procedure is not normally applied. In impurity determination either single standard injection method for sample or the normalization procedure is generally applied.

  Hence by using both method the normalization procedure and the external standard method, when a dilution of the test sample is used for comparison, the responses of the related substances are similar to the substance itself (response factor of 0.8 to 1.2), otherwise correction factors, the reciprocal of the response factors, are included in the text.
1.1.7 METHOD DEVELOPMENT BY HPLC:

Analytical method development is very useful in pharma industries. This process of technique is used to determine accurate results by using different instrument. HPLC method development depends upon different character such as solubility of molecule, pH of molecule, and other properties. The validated method used in quality department for analysis of drug substances, assay, dissolution, related substances, impurity determination, content uniformity and many other tests carried out during method development. The developed method should be validated. For stability indicating method forced degradation study has been carried out. Dissolution method development also a critical parameter but it useful at the time of formulation of new drugs.

Effective HPLC method development mainly deals with 3 critical components:

1. sample preparation,
2. HPLC analysis and
3. Standardization (Calculations).
The method development has validated after new method created and it is essential to prove. It mainly concerns with samples (drug constituents) physicochemical properties (pH, pKa, solubility) in order to select best mobile phase for elution. Selection of best stationary phase is also much important. Consideration on interaction between stationary phase and mobile phase has great importance in order to select absolute mode of separation. Likewise selection of best detector, proper column length, internal diameter, pH-buffer (or Mobile phase) etc. assures the development of effective and sensitive method development for pharmaceuticals which must be easy to validate.

Steps involved in the method development:

1. Define method objectives and understand the chemistry
2. Initial HPLC conditions
3. Sample preparation procedure
4. Standardization
5. Final method optimization
6. Method validation
INTRODUCTION OF SPECTROPHOTOMETRY

Spectroscopy concerns different methods of analysis (Optical Methods): Optical absorption radiant energy to determine different wavelength or Optical emission radiant energy to determine at specified wavelength of component or sample.

Absorption methods are given as follow.

1. Visible spectrophotometry
2. UV (Ultraviolet Spectrophotometry)
3. IR (Infra-red Spectrophotometry.)

Atomic absorption spectroscopy involves the nebulization of sample in flame as it aspirate the molecules goes towards excited state and for that they absorbs some energy the absorbed energy would determine for quantification.

A Turbidimetric and Nephelometric method determines scattering of energy. Emission method give heat to sample so that atom go to exited state and emits energy.

Methods are as follow:

a) Emission spectroscopy,

b) Flame photometry

c) Fluorimetry

The analysis of sample containing single component can be carried out using one of the following modes-

a) Using standard absorptivity values:
The absorptivity value \( A (1\%, \, 1\text{cm}) \) of a standard at selected wavelength (usually \( \lambda_{\text{max}} \)) in particular solvent is established and concentration of sample is determined by comparison of absorbance of sample with standard value.
b) **Using standard calibration graph:**

In this method absorbance is determined at different concentrations against reference samples. By this method calibration graph plots absorbance of concentration of samples and absorbance of reference samples. Generally, standard calibration is used in various aspects. The concentration of the main drug in any component is respected to absorbance of that sample.

c) **Single- double point standardization:**

In this method, absorbance of a component to the reference substance is calculated. The sample having some concentration is resulted from the relationship of absorbance and concentration.

\[
C_{\text{test}} = \frac{A_{\text{test}} \times C_{\text{std}}}{A_{\text{std}}}
\]

*Where,*

- \(C_{\text{test}}\) and \(C_{\text{std}}\) – desired concentration of sample with reference standard, respectively.
- \(A_{\text{test}}\) and \(A_{\text{std}}\) – Absorbance’s of the sample and standard samples, respectively.

The determination of the substance(s) in the multicomponent formulation can be done by one of the following methods –

- Vierodt’s method or simultaneous equation method
- Absorption ratio method or isobestic point method
- Absorption factor method or absorption correction method
- Derivative spectroscopy
- Difference spectroscopy
- Geometric correction method
- Orthogonal polynomial method
- Two-wavelength method
Area under the curve method
Multicomponent mode of analysis.
1.1.8 METHOD VALIDATION:

Validation contains different types of parameter, which proved that the develop method issue to analysis. for respective method doing different test included in ICH guideline. Any new develop method of analysis required to validate before its application. it would carried out to reproducibility of results on any system and at any condition. The HPLC Method Will be validated ac per ICH and pharmacopeial Guide line

- Method precision and system precision
- Selectivity i.e. specificity
- LOD and LOQ
- Ruggedness (robustness)
- Linearity-Range
- Study of filter paper
- Study Degradation

The target of validation is to estimate method such way that method use every where with any instrument and with given condition having its Lower and upper criteria

VALIDATION TEST:

a) Recovery ie.accuracy :
The Recovery is carried out by adding of drugs with placebo at Lower level to higher level of the test concentration for assay and with limit concentration for related substances. Recovery also calculated by spiking of sample through a stock. This parameter is useful to prove the recovery of sample from 50.0-% to 150.0-%

b) Study of method precision:
In method precision multiple sample is prepare by same method the criteria is to achieve % RSD below than2.0 %. Precision is a parameter that measures the degree of repeatability of method from the sample population under the normal operating circumstance. Relative standard Deviation (RSD) in percent is generally used to represent the precision of the method for sample between
two sets of experiments (different days /different analyst/different instrument or combination of any of them).

b) Specificity:
Selectivity is parameter in which there is study to prove the method has selective by injecting all impurities present in drug with different types of placebo materials used at time of formulation. Selectivity shall be performed by injecting blank Sample, placebo Sample and all the known impurities at limit concentration as a part of selectivity (specificity).

c) Limit of Detection(LOD)and limit of quantification:
The Lowest concentration is determine in LOD but it does not needs to quantified while LOQ is concentration determine such way that it would be quantified. This parameter carried out in impurity determination test. That can be determining with acceptable range. This is calculated by regration or dependent on S/N ratio found during analysis through PDA detector. The stated experimentation used in determination of impurity.

d) Linearity and Range :
Linearity is mathematical expression through graph plotted for average against concentration. The linearity proves that sample would prepare in any given concentration is useful to analysis. The linearity study is generally selected on the type of Experiment. Linearity would be performed from 50% to 150% of the test concentration.
1. In Assay test linearity graph shall be plotted peak area response against standard concentration
2. In impurity determination Linearity graph of principle peak and known impurity shall be plotted for peak area response against standard or impurity concentration.
3. In Sample linearity graph of principle peak shall be plotted for peak area response against analyte concentration.

f). Robustness:
The robustness is carried out to applying the plus, minus criteria to the method. In robustness different types of parameter are used such as temperature of column oven, flow rate of instrument, different types or lots of
column, at different RPM. The robustness also carried out with different composition of mobile phase, solvents which are stated in method.

g). Sample Stability:
It is essential when validating an analytical method to confirm that the analyte has adequate stability in both the standard and Sample during analytical measurement stages of the testing. Prepare the standard and sample given as in method of analysis. Analyze the standard Sample and sample at the different time intervals and calculate the %RSD.

h). Forced Degradation:
The method used in analysis during self life have to perform forced degradation studies by applying appropriate accelerated stress conditions to the sample. The proposed stress condition is:

- Heat-solid degradation study
- Heat-Solution degradation study
- Hydrolysis by using acids and base.
- Peroxide degradation study

If the drug product is in dose proportionate then forced degradation study will be performed on higher strength only, but if the drug product is not in dose proportionate then force degradation study will be performed based on qualitative composition of the drug product.

i). Filter paper study:
It will be performed using suitable filters like PVDF, Nylon, GHP. Overall % RSD shall be NMT 2 with of the method precision.

J). System Suitability Testing:
During regular analysis it not necessary to prepare standard and impurity standard on regular basis. as this materials are very costly. Hence the solution stability study carried out by keeping sample and standard at normal temperature or freezed condition when it is used. Some times mobile phase stability needs to evaluate as buffers of method getdurbided or not. Suitability parameters testing is important part of many new develop analytical method.
This proved that all test carry out under suitability parameters to be established in a particular Method depend on the type of Method validated.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery /trueess</td>
<td>Accuracy found between 80 to 120 percent specified levels.</td>
</tr>
<tr>
<td>Precision</td>
<td>For this %RSD Not more than 2.00</td>
</tr>
<tr>
<td>Repeatability</td>
<td>For this %RSD Not more than 2.00</td>
</tr>
<tr>
<td>Intermediate-Precision(IP)</td>
<td>For this %RSD Not more than 2.00</td>
</tr>
<tr>
<td>Specificity/Selectivity</td>
<td>No peak at time of interested peak.</td>
</tr>
<tr>
<td>Forced Degradation</td>
<td>Purity of peak must been pass</td>
</tr>
<tr>
<td>Filter paper study</td>
<td>% RSD for sample not more than 2.00</td>
</tr>
<tr>
<td>Linearity</td>
<td>Correlation coefficient r Not exceeded 0.999</td>
</tr>
<tr>
<td>Range</td>
<td>Lower 80- higher 120%</td>
</tr>
<tr>
<td>Sample stability</td>
<td>Hourly basis or day basis</td>
</tr>
</tbody>
</table>