CHAPTER – 1

GENERAL INTRODUCTION ON THE METHOD DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROPHOTOMETRIC AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUES IN PHARMACEUTICAL ANALYSIS
Formulations containing various drugs and combinations of drugs for potentiating or complementing one another in therapy are available in market. Pharmaceutical equivalents containing identical amounts of the same active ingredient(s) in the same dosage form and targeted to give in the same route of administration are called as generics drugs. For a generic drug to be approved it must be shown to be pharmaceutically equivalent and bioequivalent to the Reference Listed Drug (RLD). They must also meet all relevant standards of strength, quality, purity, and identity. In some cases, no analytical method is reported and the reported procedures need.

In view of the foregoing discussion the assaying and stability testing in pharmaceutical analysis[1-6] occupies an important role to meet the requirement of statutory certification of drugs and their formulations by the industry. The analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. The quality of the analytical data depends on the quality of the methods employed in generation of the data.

The best way to characterize the quality of a bulk drug is to determine its purity. There are two possible approaches to reach this goal. The determination of the active ingredient content with a highly accurate and precise specific method or the determination of its impurities. In the early years of drug analysis, when chromatographic techniques were not yet available the characterization of the purity of drugs was based on the determination of the active ingredient content by non-specific titrimetric and photometric methods supported by the determination of physical constants and some limit tests for known impurities based mainly on color reactions. The deficiencies of this approach are well known. As a consequence of the enormous development of the analytical technology in the last two
decades entirely new possibilities have been created for the determination of the purity of drug materials.

Among the several instrumental techniques [HPLC/UPLC GC, CE (Capillary electrophoresis), Fluorimetry, NMR, mass spectroscopy, spectrophotometry covering IR, NIR, Raman, UV and visible regions] available for the assay of drugs, usually visible spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of chemical reactions involved in color development and not on the sophistication of the equipment. Spectrophotometric analytical procedures are not generally stability indicating. Most widely used methods are based on HPLC / UPLC, GC. Capillary electrophoresis and Super critical fluid chromatography are slowly gaining ground in recent years

Keeping in view of the above discussion, in the present study the author made some attempts in the development and validation of such analytical methods for the assay of some selected drugs. In the present thesis the author has proposed few economical, sensitive visible spectrophotometric methods and stability indicating RP-HPLC methods for assay the some selected drugs by exploiting their characteristics, physical and chemical properties
PART-A

UV-VIS ABSORPTION SPECTROPHOTOMETRY

A) INTRODUCTION:

The principle of UV-Vis spectrophotometry [7-14] is based on the ability of molecule to absorb ultraviolet and visible light. The absorption of light corresponds to the excitation of outer electrons in the molecule. When a molecule absorbs energy and the outer electrons in the molecule excited from the Highest Occupied Molecular Orbital (HOMO) to Lowest Unoccupied Molecule Orbital (LUMO). The occupied molecular orbitals with lowest energy are known the σ orbitals, at slightly higher energy are called π orbitals and at still higher energy are known non-bonding orbitals (unshared pair electrons). The π* and σ* are called the highest energy state.

The absorption can be measured at a single wavelength or on spectral extended range. Ultraviolet and visible spectroscopy are enough energetic to excite outer electrons to high energy level and it is very useful for quantity measurement. The Beer – Lambert Law is used to determine the concentration of analyte by measuring the absorbance at various wavelengths. Beer – Lambert Law is the relationship between absorbance and concentration. It can be written as.

\[ A = \varepsilon cl \]

Where ‘A’ is the absorbance, ‘\varepsilon’ is the molar absorbtivity and expressed in units L mol\(^{-1}\) cm\(^{-1}\), ‘c’ is the concentration of the sample (compound) and expressed as mol L\(^{-1}\) and ‘l’ is length of cell and expressed in units cm.

B) INSTRUMENTATION OF UV-VISIBLE SPECTROPHOTOMETER:

The general arrangement of an UV-Visible spectrophotometer is presented in Fig.1.01 and its usual components are given below.
1. **Radiation Source:** Two radiation sources are generally used in UV-Visible spectrometers which together cover the range from 200-800nm. For measurements below 320 nm a deuterium or a hydrogen lamp at low pressure is used for emitting a continuous spectrum. For measurements above 320 nm compact tungsten halogen sources in quartz envelope are often used. This type of source is used in the wavelength range of 350–2500 nm. Tungsten/halogen lamps are very efficient, and their output extends well into the ultraviolet region.

2. **Filters/Monochromators:** If a tungsten halogen lamp is used to emit radiations below 400 nm, special filters are often included in the optical path, to reduce the stray radiation. Wavelength selectors are needed to guarantee monochromatic radiation, since a narrow bandwidth is required in order to enhance the sensitivity of the absorbance measurements.

3. **Sample container (Cuvette):** The sample containers [cuvettes] usually used are made of a material which is transparent to the radiation concerned – silica or quartz for the UV-visible region and glass or plastic to the visible region.

4. **Detectors:** A variety of detectors are available for UV-Visible measurements. High performance UV-Visible spectrophotometers utilize photomultiplier tube technology from the ultraviolet into the visible region.

**C) THE CRITERIA FOR A SATISFACTORY COLORIMETRIC ANALYSIS:**
1. **Specificity of the color reaction:** Very few reactions are specific for a particular substance, but many give colors for a small group of related substances only, that is they are selective. By altering and controlling of pH, close approximation to specificity is obtained.

2. **Proportionality between color and concentration:** For visual colorimeters it is important that the color intensity should increase linearly with the concentration of the substance to be determined, since a calibration curve may be constructed relating the instrumental reading of the color with the concentration of the solution. It is desirable that the system follows Beer’s law even when photo electric colorimeters are used.

3. **Optimization of analytical variables:** The color procedure should be sufficiently stable to permit an accurate reading to be taken. This applies also to those reactions in which colors tend to reach a maximum after a time; the period of maximum color must be long enough for precise measurements to be made. In this connection the influence of other substances and of experimental conditions (temperature, pH, stability in air etc) must be known.

4. **Reproducibility:** The colorimetric procedure must give reproducible results under specific experimental conditions. The reaction need not necessarily represent stoichiometrically quantitative chemical change.

5. **Clarity of the solution:** The solution must be free from precipitate if comparison is made with a clear standard. Turbidity scatters as well as absorbs the light. High sensitivity: It is desirable, particularly when minute amount of substances are to be determined, that the color reaction be highly sensitive and that the reaction product absorbs strongly in the visible rather than the UV region. The interfering effect of other substances in the UV region is usually more pronounced.

6. **Choice of solvent:** The solvent to be used in colorimetric or spectrophotometric determination must be a good solvent for the substance under determination. It should not
interact with the solute and must show significant absorption at the wavelength to be employed in the determination.

7. **Calibration:** Calibration is one of the most important steps in spectrophotometric analysis. Good precision and accuracy can only be obtained when a good calibration procedure is used.

7.1. **Correlation and regression:** A calibration curve is constructed by measuring the instrumental signal for each standard and plotting this response against concentration.

a) **Correlation coefficient:** In order to establish whether there is a linear relationship between two variables \( x \), and \( y \), the Pearson’s correlation coefficient is used.

\[
 r = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{\sqrt{\left( n \sum x_i^2 - (\sum x_i)^2 \right) \left( n \sum y_i^2 - (\sum y_i)^2 \right)}}
\]

b) **Linear Regression:** Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient \((r)\), then the best straight line through the data points has to be estimated. This can often be done by evaluating the best straight line by linear regression (the method of least squares)

\[
 Y = a x + b.
\]

Where \( Y \), the dependent variable, is plotted as a result of changing \( x \), the independent variable. To obtain the regression line ‘y on x’, the slope of the line and the intercept on the \( y \)-axis \((b)\) are given by the following equations

\[
 a = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{n \sum x_i^2 - (\sum x_i)^2} \quad b = \bar{y} - a \bar{x}
\]

Where \( x \) is the mean of all the values of \( x \) and \( y \) is the mean of all values of \( y \).

7.2. **Sensitivity of the method:** Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing sensitivity[15,16].
The absorbance (A) is proportional to the concentration (c) of absorbing species if absorptivity (ε) and thickness of the medium (t) are constant. When concentration is in moles per litre, the absorptivity is called molar absorptivity (ε). Molar extension coefficient is expressed as l. mole\(^{-1}\) cm\(^{-1}\). Sandell’s sensitivity[17] is the number of μg of the drug to be determined, converted to the coloured product, which in a column solution of cross section 1.0Cm\(^2\) shows an absorbance of 0.001 which is expressed as μg.Cm\(^{-2}\).

**7.3. Accuracy:** The accuracy [18,20] of an analytical result is considered to be the closeness with which mean estimates the populations mean \(\mu\).

\[
\bar{x} = \frac{\sum x_i}{n}
\]

Where \(x_i\) is the value of the \(i^{th}\) observations and \(n\) is the number of observations.

The population mean \(\mu\), represents the most frequently occurring value of the variable and therefore the midpoint of the symmetrically disposed observations, is regarded as the true value of the quantity being measured. The absolute error (E) of a determination is the difference between the observed or measured value and the true value of the quantity being measured.

\[
E = \bar{x} - \mu
\]

Smaller the error, higher is the accuracy of the measurements.

**7.4. Precision:** It is a synonym for reproducibility[14,15]. It is associated with the population standard deviation, \(s\).
\[ s = \sqrt{\frac{(x_1 - x)^2 + (x_2 - x)^2 + \ldots + (x_n - x)^2}{n-1}} \]

It is the square root of the mean of the sum of the squares of the differences between the values and the mean. The quantity \((n-1)\) is called the degree of freedom, for it represents the number of independently assignable quantities necessary to fix the system once the other parameters are fixed. A small value of \(s\) is associated with high precision and a narrow distribution curve. The square root of standard deviation is called the variance. A further measure of precision, known as the relative standard deviation (RSD) is given by:

\[ RSD = \frac{s}{\bar{x}} \]

7.5. **Comparison of results**: The comparison of the values\([21-24]\) obtained from a set of results with either the true value or other sets of data makes it possible to determine whether the analytical procedure has been accurate and/or precise or if is superior to the other methods.

These are two common methods for comparing results:

a) Student’s t-test.

b) Variance ratio test (F-test).

**a) Student’s t-test**: The test is used for small samples. Its purpose is to compare the mean from a sample with some standard value and to express some level of confidence in the significance of the comparison.

\[ t = \frac{(\bar{x} - \mu)\sqrt{n}}{s} \]

Where, \(\mu\) is the true value. It is related to a set of t- tables in which the probability (p) of the t-value falling within certain limits is expressed, either as a percentage or as a function of unity, relative to the number of degree of freedom.
b) F-test: This is used to compare the precisions of two sets of data, for example, the results of two different analytical methods:

\[
F = \frac{S_1^2}{S_2^2}
\]

The value obtained for F is then checked for its significance against values in the F-table calculated from an F-distribution corresponding to the number of degree of freedom for the two sets of data.

**PART-B**

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

High Performance Liquid Chromatography [HPLC] [24-31] is one mode of chromatographic techniques widely used to separate a wide variety of chemical mixtures and pharmaceutical compounds in mixtures. HPLC utilises a stationary phase that can be a liquid or a solid phase and a liquid mobile phase to separate the components of a mixture.

There are three basic types of molecular forces: ionic forces, polar forces and dispersive forces on which each specific technique capitalizes one of these specific forces. Polar forces are the dominant type of molecular interactions employed in Normal Phase-HPLC. Dispersive forces are employed in Reversed Phase-HPLC.

**NORMAL PHASE - HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (NP-HPLC):** NP-HPLC explores the differences in the strength of the polar interactions of the analytes in the mixture with the stationary phase. The stronger the analyte-stationary phase interaction, the longer the analyte retention. Analyte molecules compete with the mobile phase molecules for the adsorption sites on the surface of the stationary phase. The stronger the mobile phase interactions with the stationary phase, the lower the difference between the stationary phase interactions and the analyte interactions, and thus the lower the analyte
retention. Mobile phases in NP-HPLC are based on nonpolar solvents (such as Hexane, Heptane, etc.) with the small addition of polar modifier (i.e., Methanol, Ethanol). Packing materials traditionally used in NP-HPLC are usually porous oxides such as Silica (SiO$_2$) or Alumina (Al$_2$O$_3$). Surface of these stationary phases is covered with dense population of OH groups, which makes these surfaces highly polar. Chemically modified stationary phases can also be used in NP-HPLC.

**REVERSED PHASE - HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC):** RP-HPLC employs mainly dispersive forces (hydrophobic or vander wall’s interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of Chromatography. Almost 90 % of all the analysis of low-molecular-weight samples are carried out using RP-HPLC. Adsorbents employed in this mode of Chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous Silica.

**A) INSTRUMENTATION OF HPLC:**

HPLC is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result, the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography. The basic HPLC instrumentation [11] is shown in the Fig.1.02.
i) SOLVENT DELIVERY SYSTEM: This is the most important component of HPLC because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems, (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc. The pumping systems used in HPLC can be categorized in three different ways. The first classification is according to the eluent flow rate that the pump is capable of delivering. The second classification is according to the construction materials, and the final classification is according to the mechanism by which the pump delivers the eluent. Each of these classifications are given below.

a) PUMP CLASSIFICATION ACCORDING TO FLOW RATE: Standard bore systems are the most commonly used pumping systems for analytical HPLC because they provide reliable operation at flow rates ranging from 100µl / min to 10µl/ min.
b) PUMP CLASSIFICATION ACCORDING TO MATERIALS OF CONSTRUCTION:
These pumps are classified as metallic or non-metallic, depending on the material used for the eluent flow path. The most commonly used material for HPLC metallic pumping systems is 316 stainless steel, because of its mechanical strength, corrosion resistance, good thermal stability and malleability.

Pumps are also constructed from non-metallic materials using PEEK (Poly Ethyl Ethyl Ketone), Teflon (Poly Tetra Fluoro Ethylene) and Ceramics.

c) PUMP CLASSIFICATION ACCORDING TO MECHANISM OF ELUENT DISPLACEMENT: The third classification of pumps is according to the mechanism by which the liquid is forced through the chromatograph. The pumps are classified into two types. They are syringe pumps and reciprocating-piston pump.

SOLVENT DEGASSING SYSTEM: HPLC systems are also provided an online degassing system which continuously removes the dissolved gases from the mobile phase. In HPLC system it is achieved by heating, stirring, vacuum degassing with an aspirator, filtration through 0.45 μm filters, vacuum degassing with an air-soluble membrane, Helium purging, ultra sonification

ii) SAMPLE INTRODUCTION SYSTEM: Two means for analyte introduction sytems are used in the HPLC systems. (i) flowing stream and (2) a stop flow injection. These two techniques are used with a syringe or an injection valve. The most useful and widely used sampling device for modern LC is the micro sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow even at elevated temperatures.
iii) COLUMN: The heart of the any HPLC system is the column which decides the efficiency of separation. Silica (SiO₂. X H₂O) is the most widely used packing materials inside the column. It consist of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connected pores. Thus, a wide range of commercial products are available in the market with surface areas ranging from 100 to 800 m²/g and particle sizes from 3 to 50 µm.

Silica is altered by reaction with organo Chloro Silanes or organo Alkoxy Silanes giving Si-O-Si-R linkages with the surface. This attachment of hydrocarbon chain to Silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluents. The most popular material is Octa Decyl Silica (ODS) which contains C₁₈ chains, but material with C₂, C₆, C₈ and C₂₂ chains are also available.

During manufacture various organic compounds which contain groups such as Phenyl, Nitro, Amino and Hydroxyl are reacted with a small mono functional silane (eg: Trimethyl Chlorosilane) of the column to reduce further number of silanol groups remaining on the surface (End -Capping).

In HPLC, generally two types of columns are used, normal phase column and reversed phase column. In normal phase chromatography, analysis is carried out on the passage of a relatively non polar mobile phase over a polar stationary phase, while in reversed phase the analysis is carried out using a polar mobile phase such as Methanol, Acetonitrile, Water, Buffer etc.

iv) DETECTORS: PDA (photodiode Array) detector is most common and exclusively useful in all types of HPLC systems which is based on the chromophores present in the
compounds to be separated and moreover if the compounds are not having chromophores, other detectors like RI, ELSD/CCAD are used.

v) PERFORMANCE CALCULATIONS:
To access overall system performance of an HPLC instrument the following calculating values are used. These include

Relative retention
Theoretical plates
Capacity factor
Resolution
Peak asymmetry
Plates per meter

This information furnishes the parameters that are used to calculate these system performance values for the separation of two Chromatographic components. (Note: Where the terms w and t both appear in the same equation they must be expressed in the same units).

1. Relative retention (selectivity): \( \alpha = \frac{(t_2 - t_a)}{(t_1 - t_a)} \)

Where, \( \alpha \) = Relative retention; \( t_1 \) = Retention time of the first peak measured from point of injection; \( t_2 \) = Retention time of the second peak measured from point of injection; \( t_a \) = Retention time of an inert peak not retained by the column, measured from point of injection.

2. Theoretical plates: \( n = 16 \left( \frac{t_R}{w} \right)^2 \)

Where, \( n \) = Theoretical plates; \( t_R \) = Retention time of the component; \( W \) = Width of the base of the component peak using tangent method.

3. Capacity factor: \( K^1 = \left( \frac{t_2}{t_a} \right) - 1 \)
Where, $K^1 = \text{Capacity factor}; t_a = \text{Retention time of an inert peak not retained by the column, measured from point of injection.}$

4. **Resolution:** $R = 2 \left( t_2 - t_1 \right) / \left( w_2 + w_1 \right)$

Where, $R = \text{Resolution between a peak of interest (peak 2) and the peak preceding it (peak 1)}; W_2 = \text{Width of the base of component peak 2}; W_1 = \text{Width of the base of component peak 1}$

5. **Peak asymmetry:** $T = W_{0.05} / 2f$

Where, $T = \text{Peak asymmetry, or tailing factor}; W_{0.05} = \text{Distance from the leading edge to the tailing edge of the peak, measured at a point 5% of the peak height from the baseline}; f = \text{Distance from the peak maximum to the leading edge of the peak.}$

6. **Plate per meter:** $N = n / L$

Where, $N = \text{Plates for meter}; L = \text{Column length in meters.}$

7. **Height equivalent to theoretical plate (HETP):** $L / n$

Where, $N = \text{Plates for meter}; L = \text{Column length in meters.}$

**B) ANALYTICAL METHOD VALIDATION[31-37]:**

Method validation can be defined as (ICH) “Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics”.

HPLC method validation studies include system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification, stability of samples.
1. SYSTEM SUITABILITY: Prior to the analysis of samples by HPLC, the operator must establish that the HPLC system and the procedure developed should be capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and Precision. The requirements for system suitability are usually developed after method development.

2. LINEARITY: The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data so obtained is then processed using a linear least-squares regression and the resulting plot slope, intercept and correlation coefficient provide the desired information on linearity.

3. PRECISION: Precision can be defined as “The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample”. A more comprehensive definition proposed by the International Conference on Harmonization (ICH) divides precision into three types:

Repeatability

Intermediate precision and

Reproducibility

Repeatability is the precision of a method under the same operating conditions over a short period of time.

Intermediate precision is the agreement of complete measurements (including standards) when the same method is applied many times within the same laboratory.

Reproducibility examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments.
4. ACCURACY: The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose “true value” is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies. There are three ways to determine accuracy:

Comparison to a reference standard

Recovery of the analyte spiked into blank matrix or

Standard addition of the analyte.

5. SPECIFICITY / SELECTIVITY: The terms selectivity and specificity are often used interchangeably. According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selective refers to a method which provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate. The analyte should have no interference from other extraneous components and be well resolved from them. To achieve this either a representative Chromatogram or a profile should be generated that should show the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte.

6. RUGGEDNESS: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in
more than one laboratory. For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

7. ROBUSTNESS: The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters”. The variable method parameters adapted to carryout robustness in HPLC technique may involves the studies on the flow rate, column temperature, sample temperature, pH and mobile phase composition.

8. LIMIT OF DETECTION: Limit of Detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Several approaches are made for determining the LOD are possible, depending on whether the procedure is a non-instrumental or instrumental.

Based on Visual Evaluation
Based on Signal-to-Noise
Based on the Standard Deviation of the Response and the Slope

The LOD may be expressed as: \( \text{LOD} = 3.3 \frac{\sigma}{S} \)

Where, \( \sigma \) = Standard deviation of Intercepts of calibration curves; \( S \) = Mean of slopes of the calibration curves; The slope \([S]\) may be estimated from the calibration curve of the analyte.

9. LIMIT OF QUANTIFICATION: Limit of Quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and
accuracy under the stated experimental conditions. Several approaches for determining the LOQ are possible depending on whether the procedure is a non-instrumental or instrumental. Based on Visual Evaluation

Based on Signal-to-Noise Approach

Based on the Standard Deviation of the Response and the Slope

The LOQ may be expressed as: $\text{LOQ} = \frac{10 \sigma}{S}$

Where, $\sigma = \text{Standard deviation of Intercepts of calibration curves}; S = \text{Mean of slopes of the calibration curves};$ The slope $[S]$ may be estimated from the calibration curve of the analyte.

10. STABILITY: To generate reproducible and reliable results, the samples, standards, and reagents used for the HPLC method must be stable for a reasonable time (e.g., one day, one week, and one month, depending on need). Therefore, for short separation a few hours of standard and sample solution stability can be required. When more than one sample is analyzed (multiple lots of one sample or samples from different storage conditions from a single lot), automated, overnight runs often are performed for better lab efficiency.

Keeping in view the above discussion, the author had reviewed the present state of development of such analytical methods for some of the widely used drugs and found only a very few analytical methods that are not economical and stability indicating. The present thesis aimed to develop and validate procedures of some selected drugs using UV–Visible spectrophotometric and HPLC techniques as analytical tools that meet most of the present demands of pharmaceutical industry in terms of selectivity, simplicity, reliability and cost of analysis of the selected drugs in pure and pharmaceutical dosage forms ensuring the safety and efficacy of the dosage forms which is directly related to public health”.

19
AIM & OBJECTIVE

Keeping in view the above discussion, the author had reviewed the present state of development of such analytical methods for some of the widely used drugs and found only a very few analytical methods that are not economical and stability indicating.

In the present research the author has aimed in the development and validation of some economical visible spectrophotometric methods and few stability indicating RP-HPLC methods for assay some selected drugs in either single or combined dosage forms by exploiting their reactive functional groups with various chromogenic reagents(visible spectrophotometry) and by exploiting their characteristics, physical and chemical properties (HPLC) as the there is wide scope for the development of new analytical methods for their assay. The new visible spectrophotometric and RP-HPLC methods developed by the author meets most of the demands of pharmaceutical industries in assuring quality in terms of selectivity, simplicity, reliability and cost of analysis of many drugs in pure and pharmaceutical dosage forms.
REFERENCES


34. International Conference on Harmonization, *Validation of Analytical Procedures*, Methodology. Federal Register, 1-8, **1996**.

