CHAPTER I

INTRODUCTION

Drugs play a vital role in the progress of human civilization. The word “drug” has been derived from the French word “drogue”, which means a “dry herb”. It is defined as any substance that is used to control or cure disease(s). Mankind owes much to pharmaceutical chemistry that deals with the chemistry of drugs, medicinal and pharmaceutical formulations. Advances in the field of chemistry and other branches of chemometrics have made it possible to design new drugs that give maximum therapeutic effects and minimum side effects. In recent years, more and more synthetic organic and inorganic compounds are being used as drugs for the treatment of diseases.

Classification of drugs:

Based on therapeutic actions, the drugs may be classified into two categories as shown below:

1. Chemotherapeutic agents:

   The term “chemotherapy” was introduced in 1891 by German chemist, Paul Ehrlich. Chemotherapeutic agents are designed to kill the invading organisms without causing harmful effects on the tissues of the recipient. These may be further sub-divided into different classes viz., antibacterials, antitubercular, antifungal, organometallic compounds, antineoplastics, anthelmintic, antimalarial, antiprotozoals, antiseptics, antileprotics, birth control and antiviral drugs.
2. Pharmacodynamic or Functional agents:

These are the drugs which exhibit certain effects on animal organs, but are not specific remedies for particular diseases. Some pharmacodynamic agents include central nervous system modifiers (depressants and stimulants), adrenergic stimulants and blocking agents, cholinergic and anticholinergic agents, antihistamines, cardiovascular agents, diuretics, local anesthetics, haematological agents etc.

Types of pharmaceutical formulations:

The drugs are used in various dosage forms viz., tablets, capsules, dry syrups, liquid orals, creams/ointments, parenterals (injections in dry or liquid form), lotions, dusting powders etc. In tablets, one or more among the diluents viz., starch, lactose, cellulose derivatives, sucrose, acacia, gelatin, stearic acid, talc, magnesium stearate, sodium benzoate, mannitol, sorbitol, calcium phosphate etc., besides colors and flavors will be added. In capsules, one or more among the diluents, gelatin, plasticizers, certified dyes, preservatives, starch, lactose, talc etc., may be added.

Official status of drugs:

In all countries, pharmacopoeal standards are promulgated by Government agencies and are called ‘pharmacopoeia’ (e.g. Indian, IP; United Kingdom, BP; United States, USP; European, EP; Japan, JP; Martindale Extra Pharmacopoeia, Merck Index etc). The Official monographs for drugs and formulations are both descriptive and informative in addition to prescribing standards for active product and conditions for storage of drugs.
Assay:

The word assay refers to the determination of active component(s) in the unit quantity of medicinal preparation. It can be divided into three classes as shown below:

1. Chemical assay: The determination of potency of the active component present in drug preparations by chemical methods is known as chemical assay.

2. Bio assay: It is defined as a method of determination of the potency of a physical, chemical or biological agent by means of a biological indicator.

3. Immunological assay: It is based on the principle that a hormone antigen reacts with its specific antibody.

Generally, chemical assay is more reliable and precise compared to biological and immunoassays.

Analytical chemistry plays an important role in the quality assurance and control of bulk drugs and their dosage forms. Excellent progress in the field of analytical instrumentation coupled with advances in the field of information technology has led to automation of many instrumental methods of analysis. The pharmaceutical industries have employed several analytical techniques to penetrate chemical determinations not only of the active ingredient but also the quantification of related compounds or impurities in incoming chemicals, drug materials and formulations. Pharmaceutical industry is highly regulated industry. It will be under increased scrutiny from the
Government regulatory authorities and public interest groups to curtail cost and consistently deliver to market the safe and efficient products. Thus, the quality of drugs has become the focus of both industry and regulatory authorities.

Physical, chemical, physico-chemical and biological methods are being employed for the assay of drug samples in bulk and formulations. Among these, physical and physico-chemical methods are commonly employed. Physical methods of analysis are based on the physical properties of a substance such as color, solubility, degree of turbidity or transparency, moisture content, freezing and boiling point (for liquids), melting point (for solids), density or specific gravity while chemical methods of analysis include gravimetric and volumetric procedures (which are based on complex formation, precipitation, acid-base and redox reactions). Physico-chemical methods depend on the physical phenomena that occur as a result of chemical reactions.

An important feature of modern pharmaceutical chemistry is the introduction of more refined and sensitive methods of physico-chemical analyses such as optical (atomic absorption spectrophotometry, emission and fluorescence methods, polarimetry, photometry including photocolorimetry and spectrophotometry covering UV, visible and IR regions or turbidimetry), electro-chemical (coulometry, conductometry, polarography, voltammetry, potentiometry and amperometry) and chromatography (thin layer, HPLC, LC, HPTLC, GC and CEC) for the assay of individual components of a mixture. Other analytical techniques
including kinetic-spectrophotometric\textsuperscript{31}, electrophoresis\textsuperscript{32}, thermal\textsuperscript{33} and flow injection\textsuperscript{34} methods are also being used for the assay of drugs. In addition, NMR technique\textsuperscript{35} is also used for the assay of individual components of a mixture. The combination of mass spectroscopy with gas/liquid chromatography\textsuperscript{36,37} is one of the most powerful tools employed in identification and quantification of analyte in bulk or dosage forms.

The existing methods of analysis of drugs often need improvements to suit laboratory requirements and facilities available in a particular laboratory set up. Modern methods of analysis (HPLC, GLC, NMR and Mass) involve sophisticated equipments which are costly and pose problems of maintenance. Hence, they may not be in the reach of most of the laboratories and small scale industries, which produce bulk drugs and pharmaceutical formulations. Among various techniques, spectrophotometry still enjoys significant role in the assay of several class of drugs at micro or nano gram levels. It is simple, economically viable and easy to carryout. The importance of a spectrophotometric method lies in the chemical reaction(s) upon which the procedures are based, rather than upon the sophistication of the instrument. Many reactions which yield colored species for a particular drug are found to be selective or can be rendered selective through the introduction of masking agents, control of pH, use of solvent extraction techniques, by prior removal of interfering substances and so on. Hence, spectrophotometry is generally preferred in small scale industries and most of the laboratories for routine quality assurance.
**Theory of spectrophotometric method:**

Spectrophotometric methods are remarkable for their versatility, sensitivity, selectivity, accuracy and precision. These methods are based on chemical reactions, characteristic of various functional groups. The variation of color with change in concentration of an active component forms the basis of spectrophotometric method. A close relationship exists between the color of a substance and its electronic structure. Every functional group in a molecule is characterized by the absorption of light in a particular region of spectrum and this is being used for identification and assay of a substance in bulk and dosage forms. The color of a molecule with one or more chromophoric groups may be intensified by substituents called auxochromic groups.

The purpose of this research work is to provide practicing analysts with procedures that are carefully tested for selected drugs.

**Beer-Lambert law:**

This is the fundamental law governing the absorption of all types of electromagnetic radiation and is variously known as the Lambert-Beer, Bouguer-Beer or Beer’s law. The basic law of spectrophotometry is represented by the equation:

\[ \log \frac{P}{P_0} = 
\]

where \( P \) = radiant power of the beam transmitted by the sample,

\( P_0 \) = radiant power of the beam striking the sample,
c = concentration of the absorbing constituent of the sample expressed in moles/litre
b = thickness of the absorbing sample in cm
\( \varepsilon \) = a constant, extinction coefficient, whose value depends on the identity of the absorbing species, the wavelength of the light, the nature of the solvent, the temperature etc., is called the molar absorptivity. Units of \( \varepsilon \) are l/mol/cm.

It is evident from the above equation that the absorbance of a solution is directly proportional to the concentration of absorbing species when the length of light path is fixed and directly proportional to light path when the concentration is fixed.

**Calibration curve:**

Construction of a calibration curve is one of the important steps in chemical analysis. For this purpose, suitable quantities of an analyte are taken and treated in exactly the same way as the sample solution for development of the color. A plot of values of absorbance *versus* concentration yields a straight line if Beer's law is obeyed. This line passes through the origin, since any absorption due to the solvent is cancelled out in the usual method of making the measurement.

**Sensitivity of a spectrophotometric method:**

Sensitivity refers to the slope of a calibration curve, but is frequently used to mean the least determinable concentration or amount of the species to
be determined. According to Beer’s law, $A = e l c$, where ‘$e$’ is the molar absorptivity at the wavelength of maximum absorbance ($\lambda_{\text{max}}$) of the colored species and ‘$l$’ is the length of the solution. The ‘$e$’ is a valuable index in knowing the relative sensitivity of different spectrophotometric methods. For sensitive spectrophotometric methods, ‘$e$’ would be greater than $1 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and the values of ‘$e$’ below $1 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ correspond to less sensitive methods.

In order to make a comparison, which is independent of atomic weights, the sensitivity is often expressed in terms of sensitivity index as given by Sandell\textsuperscript{38}, which represents the number of micro/nanogram of the analyte/determinant per ml of a solution having the absorbance of 0.001 for a path length of 1 cm. Sandell’s sensitivity (SS) is expressed in $\mu$g cm$^{-2}$ or ng cm$^{-2}$.

**Theory of solvent extraction:**

Solvent extraction, often termed as liquid-liquid extraction, is considered to be the most versatile method amongst the various methods of separations. It is based on the distribution of a solute in a certain ratio between two immiscible solvents, aqueous and organic phase. The technique has proved to be an indispensable in inorganic-analytical and pharmaceutical chemistry\textsuperscript{39-41} for quantitative separation and determination.

Ion-association complex extraction has been applied for the assay of several bioactive compounds possessing basic moieties (secondary or tertiary...
aliphatic amino groups) using a suitable acidic dye as a reagent and a chlorinated solvent as an extractant. The physical forces such as dipole and induced dipole interactions, London dispersion forces, hydrogen bonding and dative bonding interactions lead to the formation of colored molecular complexes, which exist in solutions in equilibrium with their components. The ion-association complex or adduct is a special form of molecular complex resulting from two components and is extractable into organic solvents from aqueous phase at an appropriate pH. Out of the two, one component is a chromogen (dye or drug complex) possessing either cationic or anionic charge and so insoluble in an organic solvent whereas the other is colorless, possessing opposite charge (anionic or cationic) to that of chromogen.

**Limit of detection:**

The limit of detection\(^42\), expressed as the concentration, \(C_L\), or the quantity, \(q_L\), is derived from the smallest measure, \(X_L\), that can be detected with reasonable certainty for a given analytical procedure. The value of \(X_L\) is given by the equation:

\[
X_L (k = 3) = x_{bl} + k s_{bl}
\]

Where, \(x_{bl}\) is the mean of blank measures and \(s_{bl}\) is the standard deviation of the blank measures.

The minimum concentration detectable, \(C_L\), is given by the equation

\[
C_L = (X_L - x_{bl})/S
\]

where “S” is the slope of the analytical calibration curve.
The method of least squares:

Least-squares regression analysis\textsuperscript{43,44} is used to describe the relationship between the response say absorbance (y) and concentration (x). It may be represented by the general function:

\[ Y = f(x, a, b_1, \cdots, b_m) \]

where \(a, b_1, \cdots, b_m\) are the parameters of the function.

The convention that the x values relate to the controlled or independent variable (e.g. the concentration of a standard) and the Y values to the dependent variable (the absorbance measurements) is adopted generally. This means that the x values have no error on the condition that errors made in preparing the standards are significantly smaller than the measuring error. The values of the unknown parameters \(a, b_1, \cdots, b_m\) must be determined in such a way that the model fits the experimental data points \((x_i, y_i)\) to the best possible.

The true relationship between \(x\) (say concentration) and \(y\) (say absorbance) is considered to be given by a straight line. The relation between each observation pair \((x_i, y_i)\) can be represented as:

\[ y_i = \alpha + \beta x_i + e_i \]

The signal \(y_i\) is composed of a deterministic component predicted by linear model and a random component \(e_i\). One must now find the estimates \(a\) and \(b\) of the two values \(\alpha\) and \(\beta\). This is done by calculating the values of \(a\) and \(b\) for which \(e_i^2\) is minimal. The component \(e_i\) represents the differences
between the observed $y_i$ values and the predicted $y_i$ values by the model. The $e_i$ values are called the residuals, and $a$ and $b$ are the intercept and slope respectively.

$$a = \frac{n \sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n \sum_{i=1}^{n} x_i^2 - \left( \sum_{i=1}^{n} x_i \right)^2}$$

$$b = \frac{\sum_{i=1}^{n} y_i \sum_{i=1}^{n} x_i^2 - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} x_i y_i}{n \sum_{i=1}^{n} x_i^2 - \left( \sum_{i=1}^{n} x_i \right)^2}$$

**Correlation coefficient, $r$:**

The correlation coefficient $r(x, y)$ is useful to arrive at the relationship between two variables. It is calculated using the equation:

$$r = \frac{n \sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\sqrt{\left[ n \sum_{i=1}^{n} x_i^2 - \left( \sum_{i=1}^{n} x_i \right)^2 \right] \left[ n \sum_{i=1}^{n} y_i^2 - \left( \sum_{i=1}^{n} y_i \right)^2 \right]}}$$

where, $x_i$ and $y_i$ are the individual values of the variables, $x$ and $y$, and $n$ refers to the number of observations. The value of $r = 1$ reveals the exact correlation between the true variables while $r = 0$ indicates the complete independence of the variables. The value of $r > 0.99$ indicates excellent linearity between two variables.
**Precision and accuracy:**

The term precision\(^45\) describes the reproducibility of a result. It can be defined as the agreement between the numerical values of two or more measurements that have been made under identical conditions. One of the most common statistical terms employed is the standard deviation of a population of observations. The standard deviation (S) is the square root of the sum of squares of deviations of individual results (\(x_i\)) from the mean (\(x\)) divided by one less than the number of results in a set. It is calculated using the following equation:

\[
S = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x)^2}{n - 1}}
\]

The square of standard deviation is called the variance (\(S^2\)). Relative standard deviation is the standard deviation expressed as a fraction of the mean i.e. S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation to make it a more reliable expression of precision.

\[
\text{% Relative standard deviation} = \frac{S \times 100}{x}
\]

Accuracy describes how close a measured value is to 'true value' and it is expressed in terms of error. Generally, it refers to the difference between the mean, x, of a set of results and the true or correct value for the quantity measured. According to IUPAC\(^46\), accuracy relates to the difference between a result (or mean) and the true value.
For analytical methods, there are two possible ways of determining the accuracy viz. absolute method and comparative method.

**Absolute method:**

Accuracy of a method is checked by taking varying amounts of the analyte and proceeding according to the chosen procedure. The difference between the mean of an adequate number of results and the amount of analyte actually present, is usually expressed as parts per hundred (%) i.e. % error.

Generally, the analyte in question has to be determined in presence of foreign substances and therefore it is necessary to examine the effects of these in the assay method. This requires testing the influence of a large number of probable compounds in selected samples, each in varying amounts.

**Comparative method:**

In order to compare the results of a proposed method with those of an official/reported method, it is necessary to determine the content of the bioactive compound by two or more (proposed and official/reported methods) supposedly “accurate” methods. These methods which are of essentially different character, can be accepted provided there is no appreciable determinate error.

**Recovery experiments (Standard addition method):**

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. A known amount of the drug to be determined was added to each one
of the previously analyzed samples and the total amount of the drug was once again determined by the proposed methods. The amount of the added drug was determined by the difference. Satisfactory recovery values will enhance the accuracy of the proposed procedures.

**Student t-test:**

This is used to compare the means of two related (paired) samples analyzed by reference and proposed methods. It gives answer to the correctness of the null hypothesis with a certain confidence at 90% or 95% or 99%. If the number of pairs (n) are smaller than 30, the condition of normality of x or at least the normality of the difference (d_i) is required. If this is the case the quantity

$$ t = \frac{d}{S_d / \sqrt{n}} $$

The calculated “t” values are compared with the tabulated value for a given number of replicates at the desired confidence level. If the calculated t values are smaller than the tabulated t values, one can conclude that the two methods are not significantly different at a given confidence level.

**F-test:**

The significance of the difference in variances of reference and proposed methods can be tested by F-test. Suppose that one carries out ‘n_1’ replicate measurements using the proposed method and ‘n_2’ replicate measurements using the reference method. If null hypothesis is true, then the estimates $S_T^2$ (variance of proposed method) and $S_R^2$ (variance of reference method)
method) do not differ very much and their ratio should not differ much from unity. In fact, one uses the ratio of the variance: \( F = \frac{S_r^2}{S_k^2} \)

It is conventional to calculate the F-ratio by dividing the large variance by the smallest in order to obtain a value equal or larger than unity. If the calculated F-values are smaller than the tabulated F-values, it could be concluded that the procedures are not significantly different in precision at a given confidence level.

**Confidence limits:**

Calculation of the standard deviation for a set of data provides an indication of the precision inherent in a particular procedure or analysis. It is difficult to know the nearness between experimentally determined mean value \( (x) \) and to the true mean value \( (\mu) \) in the absence of a large number of observations/measurements. Statistical theory however allows us to estimate the range within which the true value might fall, within a given probability defined by the experimental mean and the standard deviations. This range is called the confidence interval and the limits of this range are called the confidence limit\(^\text{47}\)

\[
\text{Confidence limit} = x \pm \left[ \frac{t s}{\sqrt{N}} \right]
\]

where, \( t \) is a statistical factor that depends on the number of degrees of freedom and the confidence level desired, \( s/\sqrt{N} \) is the standard deviation of the mean, \( N \) is the number of replicates and \( x \) is the mean value.
Stoichiometry of the complexes:

The empirical formula of a complex formed between analyte and reagent(s) in solution state can be determined from spectrophotometric results. The most extensively used spectrophotometric methods for the determination of composition of the complexes are Job’s method of continuous variation, mole ratio method, slope ratio method, equilibrium shift method and logarithmic method. Job’s method of continuous variations was employed in the present study by the investigator.

**Job’s method of continuous variations:**

Job’s method of continuous variations\(^{48-50}\) is based on the variation of absorbance of both the bioactive compound and the reagent of equimolar concentrations keeping the total volume of the compound and the reagent constant. A plot of absorbance versus the mole fraction of the compound in the mixture, \(f = [C]/[C] + [R]\) shows a maximum value at the composition of the complex. The curve shows maximum value at \(f = 0.5, 0.33\) and \(0.67\) for \(1:1, 1:2\) and \(2:1\) complexes, respectively.

**Chemistry of the colored species:**

The chemistry of the colored species formed in each method is ascertained either through probability with the existing experimental evidences or through analogy with the reported methods.
The ever increase use of various classes of drugs in pharmaceutical formulations makes their determination a matter of foremost importance. The following drugs have been selected in the present study:

- **Sildenafil Citrate (Viagra)** [Ajanta Pharma. Ltd., India].
- **Trazodone hydrochloride** [Protec, Mumbai, India].
- **Methyldopa** [Indian Drugs and Pharmaceuticals Ltd].
- **Levodopa** [Sun Pharmaceuticals Ltd., India].
- **Adrenaline bitartarate** [Merck].
- **Dopamine hydrochloride** [Merck].
- **Nortriptyline hydrochloride** [Wallace Pharmaceuticals. Ltd., India].
- **Indomethacin** [Micrro Laboratories, India].
- **Buzepide metiodide** [Janssen Biotech., Belgium].
- **Cinnarizine** [Wallace, Pharmaceuticals, India].
REAGENTS USED IN THE PRESENT INVESTIGATION

The selection of a reagent for the determination of a particular drug is made after a literature survey for methods that have been used or that show reasonable promise for the assay of drug under consideration.

Different oxidizing agents, coupling agents, drug samples as analytical reagents, dyes and chemicals used in the present work are listed below:

<table>
<thead>
<tr>
<th>Materials</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocresol purple</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Bromocresol green</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
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<tr>
<td>Thymol blue</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
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<tr>
<td>Bromocresol blue</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>3-methylbenzothiazolin-2-one hydrazone</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Ferric chloride (AR)</td>
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<tr>
<td>1,10-phananthroline</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>2,2'-bipyridyl</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>Qualigens Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Hydrochloric acid (AR)</td>
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<td>Sulphuric acid (AR)</td>
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<td>o-phosphoric acid</td>
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<td>Sodium nitrite</td>
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<tr>
<td>Potassium chloride</td>
<td>Merck, India</td>
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<tr>
<td>Potassium hydrogen phthalate</td>
<td>s.d. fine chemicals Ltd., Mumbai, India</td>
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<tr>
<td>Sodium acetate</td>
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<tr>
<td>Sodium hydroxide</td>
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<td>Chloroform (AR)</td>
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<td>Acetone (AR)</td>
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<tr>
<td>Methanol (AR)</td>
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SCOPE OF THE PRESENT WORK

New drugs are being pushed into market in large numbers so as to cure/control diseases. It has become difficult to keep abreast of these drugs. Hence, a strict control on the quality evaluation of the drugs and their therapeutic actions assume significance. For this purpose, pharmaceutical industries or drug regulatory authorities require novel and easy methods of analysis for routine quality control. These methods should be less time consuming, accurate, economical, precise, selective and sensitive. The survey of existing analytical methods reveals that not much attention was paid for the development of spectrophotometric methods for the assay of selected drugs. Hence, the investigator has made some attempts in this direction and succeeded in developing the following new spectrophotometric methods for the assay of selected drugs:

❖ Bromo cresol purple as an analytical reagent for spectrophotometric determination of sildenafil citrate (Viagra) in pharmaceutical formulations
❖ Sensitive spectrophotometric methods for the assay of an antidepressant in bulk powder and dosage forms
❖ Diazocoupling reactions of catecholamines for their spectrophotometric determination in bulk and pharmaceutical preparations
❖ Extractive spectrophotometric determination of tricyclic antidepressant drug in bulk and dosage forms
❖ New spectrophotometric methods for the assay of a non-steroidal anti-inflammatory drug in bulk and dosages
❖ Novel spectrophotometric methods for the assay of buzepide metiodide in formulations
❖ Spectrophotometric assay of an anti-allergic agent in pure and pharmaceutical preparations using thymol blue

The results of the proposed methods were compared with those of official/reported methods. In addition, the results were subjected to rigorous statistical data treatment.
REFERENCES


