CHAPTER I

GENERAL INTRODUCTION
Chapter-1 General Introduction

GENERAL INTRODUCTION

Biological Importance of Organic Compounds:

Quest for the knowledge of importance of organic compounds has revolutionised their synthesis, isolation, and applications. No field of science is so closely related with our daily activities as organic chemistry. The vast number of organic compounds isolated from nature and synthesised in the laboratories by man, have not only provided the basic amenities of life but also made his life very comfortable. The use of organic compounds is perhaps as old as the human civilization. For understanding the life processes, the knowledge of organic compounds in the form of proteins or fats or carbohydrates are not only the main bulk of our food system but also constituents of our body. Life without soap, paper, petrol, detergents etc. which represent simple organic compounds is inconceivable. In short, we can say that life and civilization would not have been what it is today without the presence of organic compounds. The organic compounds, which are biologically active and possess physiological and therapeutic values are called "Drugs".

Advances of modern medicines has demonstrated in the successful eradication and control of many deadly diseases. In the past few decades, pharmaceutical chemistry is widely exploring new avenues in order to find and
develop organic compounds those are now available in pharmaceutical formulations for the treatment of various diseases and often for the maintenance of better human health. In recent decades, more and more synthetic & semi-synthetic organic and inorganic compounds are used as drugs for treatment of various ailments and they are principally classified into two basic types on the basis of their therapeutic action as mentioned below:-

(i) Chemotherapeutic agents: In 1891, the word ‘chemotherapy’ was introduced by the famous German chemist, Sir Paul Ehrlich. It is a specific treatment using chemical agents to arrest the progress or eradicate diseases caused by micro-organisms like bacteria, helminths, protozoa, viruses etc., without causing irreversible injury to healthy tissues in the body. The drugs so used are called the chemotherapeutic agents which include organometallic compounds, antibiotics, antiprotozoals, antifungals, antibacterials, antineoplastics, and antiviral drugs.

(ii) Functional or pharmacodynamic agents: These are the drugs that act on various functions of the body, but are not specific remedies for particular diseases. Some pharmacodynamic agents include central nervous system modifiers (depressants and stimulants), cholinergic and anticholinergic agents, adrenergic stimulants and blocking agents, antihistamines, local anaesthetics, cardiovascular agents, haematological agents etc.
The organic compounds isolated from vinca rosea plants- Vinblastine and Vincristine are indole alkaloids, which are antineoplastic agents and these compounds are receiving wide attention for their therapeutic application especially in leukemia.

Some of the compounds belonging to this group are also dopamine and levodopa, which are neuro transmitters and used in the treatment of a debilitating disease known as "Parkinsonism". Methyl dopa is an antihypertensive compound whereas adrenaline is a cardiac stimulant and a vasodilator.

In view of the above facts, the investigator of the present work has made a sincere attempt to assay some of the therapeutically important drugs with new spectrophotometric methods for their quantitation.
SECTION 1.1

Role of Analytical chemistry and its techniques:

Analytical chemistry has had a big role to play in separating, isolating and quantifying various chemical compounds. Virtually, every item of commerce has been subjected to analytical testing at one or more stages in its manufacture. It serves as a tool for scholarly efforts of several investigators [1]. Many instrumental techniques have been developed apart from classical methods, such as titrimetry and gravimetry, for the determination of not only the active ingredient, but also the quantification of related compounds or impurities associated with it. The latest analytical techniques have made the determinations rapid and accurate. The analytical methods have the advantage of not only in consumption of small quantities of analytes, reagents and less time, but they also produce accurate results. The analytical techniques may be (i) physico-chemical methods or (ii) electro analytical methods or (iii) chromatographic methods. The physico-chemical methods like spectroscopy [2] include colorimetry and spectrophotometry covering U.V. and visible region [3, 4] or fluorimetry [5], nephelometry or turbidimetry and NMR & mass spectrometry. The electro analytical methods cover potentiometry, amperometry, voltammetry [6], electrophoresis [7] and polarography.
The chromatographic techniques involve HPLC [8], LC [9], HPTLC [10], CEC [11], and GC [12]. The combination of GC with mass spectroscopy [13] is one of the most powerful tools employed in the quantification and identification of the analytes in pure as well as in associated forms.

Pharmaceutical analysis is a very important branch of analytical chemistry which effectively safeguards the last line of quality assurance in the purchase and sampling of bulk drugs, control of manufacturing processes, detection of trace impurities and analysis of the contents of formulated drugs as well as their subsequent stability studies monitoring. It also plays a pivotal role in quality assurance and control of pharmaceutical drug moieties and their various dosage forms. The advent of sophisticated instruments has marked a new epoch in analytical chemistry and offered technical assistance in the routine work of the analyst in a modern pharmaceutical laboratory.

However, even though the costly sophisticated instruments like HPLC, HPTLC, GLC, AAS etc. are available by virtue of their scope, speed, and reliability, they require use of highly sophisticated and costly equipments and many times involve tedious multiple extraction procedures, which are time consuming. Therefore, Spectrophotometry is still preferred by many
laboratories for its simple, economical and easy handling techniques since the methods developed are usually rapid, precise, accurate and cost effective. The spectrophotometer is one of the most easily accessible instruments and the spectrophotometric methods equally compete with the latest techniques in determination of the analyte in micro quantities, thereby increasing its popularity among analysts. Hence, many small-scale industries and most of the laboratories as well as many compendial methods utilize the spectrophotometric techniques for the determination of several drugs as well as organic compounds.
SECTION 1.2

Theory of spectrophotometric determinations

Spectrophotometry is an important technique used in qualitative and quantitative analysis. The spectrophotometric methods are known for their versatility, sensitivity, and accuracy. They are also used in the determination of composition, stability, besides studying complexes. The basis of spectrophotometric methods is the simple relationship between the absorption of radiation by solution and the concentration of the coloured species in the solution. The absorption of light in the UV-visible region causes the electronic excitations (i.e., $\pi-\pi^*$ or n-$\pi^*$) due to the presence of unsaturated groups called "Chromophores", which are the specific portions of the molecules, that can absorb radiant energy in UV or visible region. The intensity of the colour increases with conjugation. Some of the chromophoric groups responsible for the colours are,

\[
\begin{align*}
\text{\textcolor{red}{C=C}}, \quad \text{\textcolor{green}{C=O}}, \quad \text{\textcolor{blue}{-COOH}}, \quad \text{\textcolor{purple}{-N=O}}. \\
\text{\textcolor{red}{-N=N}}, \quad \text{\textcolor{green}{=NH}}, \quad \text{\textcolor{blue}{-N\Rightarrow O}}. \\
\text{\textcolor{red}{O}}, \quad \text{\textcolor{green}{\text{\vphantom{0}O}}}, \quad \text{\textcolor{blue}{\text{\vphantom{0}O}}}, \quad \text{\textcolor{purple}{\text{\vphantom{0}O}}}.
\end{align*}
\]
Each functional group has characteristic region for absorption of light. In addition to chromophoric groups, there are certain functional groups, which by themselves fail to absorb in the visible region and produce colour, can affect the behavior of chromophores when they are conjugated with them. These groups are called 'Auxochromes', which may deepen the colour and shift the absorption maximum towards longer wavelength (bathochromic shift) or may cause opposite effect and shift the absorption maximum to shorter wavelength (hypsochromic shift). These are usually salt forming groups like -OH, -OR, -NH₂, -NHR, and -NR₂, -X (halogens).

The spectrophotometric method of analysis is based on the fundamental law, "Beer & Lambert's law" which governs the absorptions of all types of electromagnetic radiation. The mathematical expression of the law is represented as [14],

\[
\log \frac{I_0}{I_t} = A = \varepsilon b c
\]

Where, \( I_0 \) = intensity of incident radiation
\( I_t \) = intensity of transmitted radiation.
\( A \) = absorbance.
\( \varepsilon \) = molar absorptivity
\( b \) = breadth of the cell or thickness of absorbing samples expressed in cm.
c = concentration of the absorbing species expressed in moles per litre.

The molar absorptivity (ε) was designated earlier as the molar extinction coefficient and has the unit $1 \text{ mol}^{-1} \text{ cm}^{-1}$. The value of ε depends upon the nature of absorbing species, the wavelength of light, nature of solvent temperature etc. The relationship between absorbance (A) and the transmittance (T) is given by,

$$A = \log \frac{1}{T} = \log \frac{I_0}{I_t} \quad \text{[Where, } T = \frac{I_t}{I_0}]$$

This equation shows that the absorbance of a solution is directly proportional to the concentration of the absorbing species, when the length of light path is fixed and directly proportional to light path, when concentration is fixed.

It is evident, that a straight-line graph passing through the origin is produced, when absorbance is plotted against concentration at a fixed path length if Beer's law is obeyed.

**Calibration curve:**

Spectrophotometric method of determination involves construction of calibration curve. To check whether the method adheres to Beer's law or not,
suitable quantities of constituents under test are taken, colours are developed and absorbance values are measured which are plotted against concentration. A straight-line graph passing through origin confirms the adherence to the Beer's law.

**Deviations from Beer's law:**

There are number of instances where the coloured species do not obey Beer's law. The deviations are ascribed to physical and chemical changes. They are,

(i) Non-monochromatic nature of radiation,

(ii) Shift in $\lambda_{\text{max}}$ due to dissociation, association, or complex formation of absorption species,

(iii) Reflection of radiation by the solution,

(iv) Fluorescent nature of the solution and

(v) Change of temperature at the time of measurement.

**Photometric accuracy:**

The conditions for photometric accuracy can be deduced by equating the Beer's law equation to zero. If Beer's law is obeyed, then the relative error in the concentration $\Delta C/C$ for the unit photometric error $\Delta P$ is given by [15]

$$\frac{\Delta C/C}{\Delta P} = \frac{1}{2.3 \ A \ (10^{-A})}$$
Ringbom plot:

In the event Beer's law is obeyed or not, Ringbom showed that the ratio between relative error in concentration ($\Delta C/C$) and photometric error ($\Delta P$) can be visualised by plotting absorbance or percentage of absorbance against logarithm of concentration. Such a plot is known as Ringbom Plot [16]. The plot has a sigmoid shape with a virtual linear segment at intermediate absorbance or concentration values. This segment represents the optimum range of concentrations.

When the system obeys Beer's law,

$$\frac{\Delta C/C}{\Delta P} = \frac{2.30}{\Delta P/\Delta \log c}$$

Where $\frac{\Delta P}{\Delta \log c}$ gives the slope of Ringbom's plot.

Sensitivity:

It is necessary to distinguish between the sensitivity of an analytical method from the sensitivity of photometric reaction. Sensitivity depends solely upon selection of the reagent and the reaction conditions. The sensitivity of the photometric method is expressed in terms of Sandell's sensitivity [17], which represents the number of microgram of the determinant per ml of a solution,
having the absorbance of 0.001, for a path length of 1.0 cm. The sensitivity [18] is expressed in (µg.cm\(^{-2}\)).

The objective numerical expression of the sensitivity of spectrophotometric methods [19, 20] is the molar absorptivity (ε) at the wavelength of maximum absorption (λ\(_{max}\)) of the coloured species.

\[
\varepsilon = \frac{A}{b \cdot c}
\]

The molar absorptivity is a valuable index in evaluating the relative sensitivity of various available colorimetric methods. Beer's law limits and molar absorptivity values are expressed as µg ml\(^{-1}\) and 1 mol\(^{-1}\) cm\(^{-1}\) respectively. For sensitive spectrophotometric method, ε is greater than 1 X 10\(^4\) 1 mol\(^{-1}\) cm\(^{-1}\) and values of σ below 1 X 10\(^3\) correspond to less sensitive method [21]. The sensitivities of spectrophotometric methods for the compounds to be determined can be compared in terms of ε.

In the determination of ε, the absorbance measured should be within the range, over which, the colour system conforms to Beer's law and within that range of values the error in the measurement is minimal. Sensitivity also
depends upon the monochromaticity of radiation with monochromatic light of
very narrow bandwidth corresponding to wavelength \( \lambda_{\text{max}} \); the maximum
value of molar absorptivity (at \( \lambda_{\text{max}} \)) is obtained.

**Composition or Stoichiometry of complexes:**

A spectrophotometric technique is useful in knowing the empirical
formulae of the complexes formed by organic compounds and as well as
reagents in solution. This can be established by different procedures such as
Job's method of continuous variation [22] modified by Vosburgh and Cooper
[23], Mole ratio method developed by Yoe and Jones [24]. Slope ratio methods
of Harvey and Manning [25], Bent and French [26], and Asmus' method [27].
For the present work, Job's method of continuous variation and Molar ratio
methods, is employed.

**Job's method of continuous variation:**

This is a widely used method for elucidating the composition of the
complexes. The method is based on the variation of absorbance of both the
organic compound (OC) and the reagent [R] (equimolar concentrations) as a
function of volume of organic compound and the reagent (keeping the total
volume constant). A plot of absorbance against the mole traction of the organic compound in the mixture,

\[ f = \frac{[\text{OC}]}{[\text{OC}] [\text{R}]} \]

shows a maximum at the stoichiometric composition of the complexes. When a 1:1 complex (OC-R) is formed, the curve shows maximum at \( f = 0.5 \), if it is 1:2 complex (OC-R₂) the maximum occurs at \( f = 0.33 \) and if the formula is OC₂-R, the maximum is expected at \( f = 0.67 \) and so on. Vosburgh et al. [23] suggested a modification for Job's method, as Job's method is suitable only when a single complex species is present in the concentration range of study.

The method of continuous variations applies, provided, (i) there is only one complex formation under the experimental conditions, (ii) there is no volume change on mixing the solutions of organic compound and reagent, (iii) Beer's law is obeyed throughout, and (iv) the mole ratio (OC:R) is not greater than three. Job's method is simple and hence very popular for the study of complexes and has been extensively adopted for the investigations of complexes in solution.
Mole ratio method:

In this method, the absorbance values are measured for a series of solutions containing varying amounts of one constituent with a constant amount of the other. A plot is drawn between its absorbance and the ratio of moles of reagent (varied) to moles of organic compound (fixed). This gives a straight line from the origin to the point, where equivalent amounts of constituents are present. The following portion of the curve then becomes horizontal. When the complex formed is stable, it shows no appreciable dissociation and the plot gives a sharp break. A curved plot is obtained if the complex formed is weak. Harvey and Manning [25] later showed that a weak system could also give a sharp break on changing the ionic strength of the solution.

Precision and Accuracy:

The duty of an analyst is to obtain a valid result as near to the true value as possible by the correct application of the analytical procedure employed. For carrying out this, the knowledge of precision and accuracy to determine the error of an individual determination is important, and they are the most important criteria for judging the analytical procedures and the results.
Precision:

Precision refers to the reproducibility of a measurement within a set, i.e., is to scattering or dispersion of a set about its central value [28]. The term "set" refers to a number (n) of the independent replicate measurements of same property. One of the most common statistical terms employed is the standard deviation of a population of observations. It is also called as root mean square deviation as it is the square root of mean of the sums of squares of the differences between the values and the mean of those values and is mathematically expressed as,

\[ S = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n-1}} \]

Standard deviation has the same limit as the property being measured. The square of the standard deviation is called Variance (\( S^2 \)). A further measure of precision, known as Relative standard deviation (RSD), is given by \( S/\bar{X} \). This measure is often expressed as a percentage known as co-efficient of variation (C.V.),

\[ C.V. = \frac{S \times 100}{X} \]
The precisions of the spectrophotometric methods depend on the concentrations of the determinant and on the techniques of measurement. The precision of the objective spectrophotometric method is higher and varies from 0.5-2.0%, under suitable measuring conditions. By the differential method, a precision of 0.2-5.0 % is obtainable which enables macro amounts of components to be determined. The precision attainable is a function of absorbance measured & when very low concentrations are measured, the error is very large.

Accuracy:

Accuracy of a determination may be defined as the concordance between the result and the true or most probable value [1]. According to IUPAC [29], the 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, i.e., (i) Absolute method (ii) Comparative method.

(i) Absolute Method:

In this method, a varying amount of constituents under investigation are taken and proceeded according to specified instructions. The difference between mean of adequate number of results and the amount of constituents
actually present is usually expressed as parts per hundred (%) i.e., % error. The constituents in question will be usually determined in presence of other substances, and is therefore necessary to know the effect of them upon the determination. These require testing the influence of a large number of probable compounds in the chosen samples, in each in varying amounts.

In few instances, the accuracy of the method is controlled by separations (usually by solvent extraction or chromatographic techniques) in chosen samples.

(ii) Comparative Method:

In the analysis of pharmaceutical formulations or laboratory prepared solid samples of desired composition, the content of the constituent sought is determined by two or more (proposed, official or reference) supposedly "accurate" methods. These methods of essentially different character are being accepted usually as indicated in the absence of appreciable determinate error. The general procedure for the assay of pharmaceutical formulations, either in proposed or reference methods, comprises of various operations, which include sampling, preparation of solutions, separation of interfering ingredients, if any and also a method for quantitative assay.
Recovery Experiments (Standard addition method):

A known amount of the constituent being determined is added to the sample, which is analyzed for the total amount of constituents present. The difference between the analytical results for samples with and without the added constituent gives the recovery of the amount of added constituent. If recovery is satisfactory, the confidence in the accuracy of the procedure is enhanced.

Validation of the analytical methods [30]:

Validation of the analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the methods meet the requirements for the intended analytical applications. Typical analytical performance characteristics that should be considered in the validation of the methods are (i) accuracy (ii) precision (iii) specificity (iv) detection limit (LOD) (v) quantification limit (vi) linearity and (vii) range.

Detection limit:

It is a characteristic of limit tests. It is the lowest amount of the analyte in the sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, the limit tests merely substantiate whether the amount of analyte is above or below a certain level. It is usually expressed
as the concentration of the analyte in the sample i.e., \( \mu g \text{ ml}^{-1} \). Mathematically, it is obtained from the equation:

\[
LOD = \frac{3 \times \sigma}{m}
\]

Where \( \sigma \) is the standard deviation of the blank measured with reference to distilled water for a particular number of measurements and \( "m" \) the slope of the calibration curve.

**Quantification Limit (LOQ):**

It is a characteristic of the quantitative assays for low concentrations in the sample matrices, such as impurities in bulk substances and degradation products. In this, the lowest amount of the analyte with acceptable precision and accuracy under the stated experimental conditions can be determined. The Quantification limit is expressed as concentration of analyte in the sample i.e., \( \mu g \text{ ml}^{-1} \). Mathematically, it is expressed as:

\[
LOQ = \frac{10 \times \sigma}{m}
\]

Where \( \sigma \) is the standard deviation of the blank measured with reference to distilled water for a particular number of measurements and \( "m" \) the slope of the calibration curve.
Linearity and Range:

Data from regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation co-efficient, slope of the regression line and residual sum of squares should be considered to account for linearity and range.

Ruggedness:

Ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different assay temperatures, different lots of reagents, different days etc. The degree of reproducibility of test results is determined as a function of assay variables. This reproducibility is then compared with the precision of assay under normal conditions to obtain the measure of ruggedness of the analytical method.

Robustness:

Robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in parameters and provide an indication of its reliability during normal usage.
Statistical analysis of the results [31]:

The comparison of the values obtained from a set of results with true value or other set of data makes it possible to determine whether the analytical procedure has been accurate and/or precise or if it is superior to another method. There are two common methods for comparing the results (i) student's - t-test (ii) variance ratio test (F-test).

(i) Student's t-test: This is a test [32] used for samples in micro amounts. 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. It is also used to test the difference between the mean of the two sets of data. The value of 't' it obtained from the equation:

$$ t = \frac{(\bar{X} - \mu)\sqrt{n}}{S} $$

where \( \mu \) is the true value.

Whenever a new analytical method is being developed, it is a usual practice to compare the values of mean and precision of new (test) method with
those of established (reference) method. For comparing the two methods, with
two sample means $X_1$ and $X_2$, 't' is given by

$$ t = \frac{X_1 - X_2}{Sp \sqrt{1/n_1 + 1/n_2}} $$

Where $Sp$ = the pooled standard deviation and is calculated from two sample
standard deviations $S_1$ and $S_2$ as

$$ Sp = \sqrt{\frac{(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2}{n_1 + n_2 - 2}} $$

For the method to be accurate, there should not be any significant difference
between theoretical (Tabulated) and calculated t-values.

(ii) **Variance ratio test (F-test):** This is used to compare the precisions of
the two sets of data [33]. The results are of two different analytical methods or
results from two different laboratories. It is given by

$$ F = S_A^2 / S_B^2, \quad \text{where} \quad S_A \text{ and } S_B \text{ represent standard}
\text{deviations of two sets of results.} $$

23
Paired-t-test:

Another method of validating, a new procedure is to compare the results using samples of various compositions, with the values obtained from the accepted method. The t-value is calculated by the equation.

\[
t = \frac{\bar{d}\sqrt{n}}{S_d}
\]

Where, \( \bar{d} = \frac{\sum d}{n} \)

\( d \) = difference between each pair of results

\( n \) = number of determinations

\( \bar{d} \) = mean of the difference

\( S_d \) = Standard deviation

\[
S_d = \sqrt{\frac{\sum (d - \bar{d})^2}{n - 1}}
\]

Chemistry of the coloured species formed:

The chemistry of the coloured species formed in each of the method was ascertained either through probability with the existing experimental evidence or through analogy with the literature methods.
SECTION 1.3

A) Drugs used for the present study:

This section deals with the drugs and reagents used for the present investigations. The brief account of the drugs chosen for the present investigation is given below:

1. Sulfonamide derivatives:

   a) Sulfadiazine (SFD) [Molecular weight = 250.28]

   {4-amino-N-2-pyrimidinyl benzene sulfonamide}

   SFD, chemically named as 4-amino-N-2-pyrimidinyl benzene sulfonamide is synthesised by condensing 2-aminopyrimidine with acetylsulfonyl chloride followed by the hydrolysis of acetyl group with NaOH. A comprehensive description of the drug is given by Stober et al. SFD is a good antibacterial drug and the structure is given below:

   ![SFD structure](image_url)
b) Sulfacetamide (SFA) [Molecular weight = 214.25]

\{N-[(4-aminophenyl) sulphonyl]-acetamide\}

SFA, is a sulfonamide, antibacterial and chemically known as N-[(4-amino phenyl) sulphonyl]-acetamide. It is synthesised by the reaction of N³-acetyl sulfanilamide and acyl halide in the presence of dry pyridine. Houisby studied the antimicrobial activity of the drug. Sulfacetamide is used for the eye infections such as conjunctivitis, blepharitis, stye, trachoma etc.
d) Sulfamethoxazole (SFMx) [Molecular weight = 253.28]

{4-amino-N-(4-methyl-2-thiazole) benzene}

SFMx is a good antibacterial and antipneumocytis. Also, in combination with pyrimethamine, it is used as a potential anti-malarial drug. Chemically, it is designated as 4-amino-N(4-methyl-2-thiazole) benzene sulfonamide. It is synthesised by using ethyl 5-methylisoxazole-3-carbamate. Rudy and Senkowski elaborated comprehensive description of the drug. The structure of SFMx is given below:

![SFMx](image)


e) Sulfaguanidine (SFG) [Molecular weight = 214.25]

{4-amino-N-(amino-iminomethyl) benzene sulfonamide}

Sufaguanidine is chemically known as 4-amino-N-(amino-iminomethyl) benzene sulfonamide. SFG is synthesised by condensing acetylsulfonyl chloride with guanidine nitrite in the presence of excess of NaOH in water-acetone. Several other synthesis for the
sulfaguanidine are given in sulfonamide monograph. It is used as a potential antibacterial drug and has the following structure:-

\[
\text{SFG}
\]

f) Sulfamethazine (SFMt) \([\text{Molecular weight } = 278.33]\)

\{4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene sulfonamide\}

Chemically, SFMt is designated as 4-amino-N-(4, 6-dimethyl-2-pyrimidinyl) benzene sulfonamide. It is synthesised by refluxing hydroxyl methyl ketones with guanidine carbonate in alcohol or by the reaction of acetyl sulfonyl chloride with requisite aminopyrimidine in dry pyridine. SFMt is used as a good antibacterial drug and has the following structure:-

\[
\text{SFMt}
\]

g) Sulfamerazine (SFMr) \([\text{Molecular weight } = 264.31]\)

\{4-amino-N-2-thiozolyl benzene sulphonamide\}
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**SFMr** is chemically designated as 4-amino-N-2thiazolyl benzene sulphonamide. SFMr is synthesised by the reaction of sulfonyl chloride and 2 or 4-aminopyrimidine. SFMr is closely related to SFMt in its antimicrobial properties and its therapeutic uses. The structure is as shown below:

![SFMr Structure](image)

2. **Pindolol**: [Molecular weight = 248.32]

1-(1H-indol-4-yloxy)-3-(1-methylethylamino) propan-2-ol

![Pindolol Structure](image)
3. Chloramphenicol: [Molecular weight = 323.13]

[R-(R*,R*)]-2,2-Dichloro-N-[hydroxy-1-(hydroxyl-methyl)-2-(4-nitrophenyl)ethyl] acetamide.

4. Vincreistine VCS [Molecular weight = 824.97]

and Vinblastine VBS [Molecular weight = 810.99]
B) Reagents used for the present investigation

The brief account of the reagents chosen for the present investigation is given below:

1. Imino Dibenzyl (IDB) [Molecular weight = 195.3]
   \{10, 11-dihydro-5H-dibenzazepine\}
   ![IDB](image)

2. Sodium molybdate (MoNa$_2$O$_4$)
   [Molecular weight = 205.92]

3. 3-Amino phenol [Molecular weight = 109]
   ![3-Amino phenol](image)

4. Pyrocatechol [Molecular weight = 108]
   ![Pyrocatechol](image)
5. Sodium nitroprusside (Na₂[Fe(CN)₆NO].2H₂O)  
[Molecular weight = 261.92]

6. 8-Hydroxyquinoline [Molecular weight = 145.0]

7. P-phenylenediamine dihydrochloride  
[Molecular weight = 180.0]

C) Scope of the present work:

Demand for the new and rapid methods of analysis of drugs whose numbers are increasing in multiple folds, is forcing the analyst to develop sensitive reagents for their assay. The methods developed should be accurate, economical, rapid, precise, selective and sensitive. Although a number of analytical techniques are available, the technique adopted should be economical, should have an easy access to laboratories and should also satisfy
the above enlisted conditions. Spectrophotometric method is one such analytical technique used for the assay of drugs. The investigator has made sincere attempts and has successfully developed few new chromogenic reagents for the assay of following classes of drugs such as:-

a) Antineoplastic,

b) Antibacterial,

c) Antihypertensive drugs,

d) Antibiotic

The results obtained by the proposed methods were comparable with pharmacopoeial and reported methods.
REFERENCES


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