CHAPTER 1

INTRODUCTION
1.1 INTRODUCTION

Pharmaceutical Analysis is the branch of science which deals with identification of substances and determination of amount present in particular sample. Pharmaceutical analysis covers the bulk materials, dosage forms and more recently, biological samples in support of bio-pharmaceutical and pharmaco-kinetic studies. Analysis can be divided into areas called qualitative and quantitative analysis. Pharmaceutical products synthesized and identified using instrumental techniques.\(^{(1)}\)

The Laboratory controls shall include the establishment of sound and appropriate specification standards and test procedure to assure that the final drug product conforms to the required standards of identity, strength, quality and purity. Modern physical methods of analysis are extremely sensitive, providing precise and accurate information about the standards of chemicals or drugs up to nanogram levels like HPLC\(^{(2)}\). These methods are used extensively in the quality assurance of raw materials, in process quality assessment, stability of the drugs on storage and monitoring drugs concentrations in various body fluids or tissues.

1.2 ANALYTICAL CHEMISTRY

The types of analysis can be distinguished in two ways:

**Qualitative analysis:** To refer identity of product, i.e., it yields useful clues from which the molecular or atomic species, the structural features or the functional groups in the sample can be identified.

**Quantitative analysis:** To refer the purity of the product, i.e., the results are in the form of numerical data corresponding to the concentration of analytes.

**Types of analytical methods**

The various methods of analysis\(^{(3)}\) can be grouped into two categories. They are:

1. Chemical methods.
2. Instrumental methods.
Chemical methods:
In these methods, volume and mass are used as means of detection.
1. Titrimetrical methods like acid-base, oxidation-reduction, non-aqueous, complexometric, precipitation titrations.
2. Gravimetric and thermo gravimetric methods.
3. Volumetric methods.

Instrumental methods:
Based on principles different instrumental methods are available. These methods are based on the measurement of specific and non-specific physical properties of a substance. Table-1.1 represents the instrumental methods and principles.

Table-1.1: Table showing list of instrumental methods.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Principle</th>
<th>Instrument Method</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Fluorescence spectrometry.</td>
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<td></td>
<td></td>
<td>Differential scanning calorimetry.</td>
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<td></td>
<td></td>
<td>Thermogravimetry.</td>
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</table>
1.3 CHROMATOGRAPHY

Chromatography (from Greek *chroma*, color and *graphein* to write) is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on different partitions between the mobile and stationary phases. Suitable differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation. Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

The history of chromatography begins during the mid-19th century. Chromatography, literally "color writing", was used and named in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll. New types of chromatography developed during the 1930s and 1940s made the technique useful for many types of separation process. Some related techniques were developed during the 19th century (and even before), but the first true chromatography is usually attributed to Russian botanist M.Tswett, who used columns of calcium carbonate for separating plant pigments during the first decade of the 20th century during his research of chlorophyll.

**Chromatography terms**

- The analyte is the substance to be separated during chromatography.
- Analytical chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a sample.
- A bonded phase is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.
A chromatogram is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.

Plotted on the X-axis is the retention time and plotted on the Y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

A chromatograph is equipment that enables a sophisticated separation e.g. gas chromatographic or liquid chromatographic separation.

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.

The eluent is the mobile phase leaving the column.

An eluotropic series is a list of solvents ranked according to their eluting power.

An immobilized phase is a stationary phase which is immobilized on the support particles, or on the inner wall of the column tubing.

The mobile phase is the phase which moves in a definite direction. It may be a liquid (LC), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated (or) analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromatography and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.

Preparative chromatography is used to purify sufficient quantities of a substance for further use, rather than analysis.
The retention time is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.

The sample is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.

The solute refers to the sample components in partition chromatography.

The solvent refers to any substance capable of solubilizing other substance, and especially the liquid mobile phase in LC.

The stationary phase is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography.

Techniques by chromatographic bed shape:
- Column chromatography
- Planar chromatography
- Paper chromatography
- Thin layer chromatography

Displacement chromatography

Techniques by physical state of mobile phase:
- Gas chromatography
- Liquid chromatography

Affinity chromatography
- Supercritical fluid chromatography

Techniques by separation mechanism:
- Ion exchange chromatography
- Size exclusion chromatography
Special techniques:
❖ Reversed-phase chromatography
❖ Two-dimensional chromatography
❖ Simulated moving-bed chromatography
❖ Pyrolysis gas chromatography
❖ Fast protein liquid chromatography
❖ Counter current chromatography
❖ Chiral chromatography.

1.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High pressure liquid chromatography (HPLC) sometimes called high performance liquid chromatography is a separation that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases.

The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The techniques of HPLC are so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

HPLC is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the
interactions between the stationary phase, the molecules being analyzed and the solvent(s) used.\textsuperscript{(7)}

It offers the following advantages:

\begin{itemize}
  \item Speed (many analysis can be accomplished in 20 minutes or less)
  \item Greater sensitivity (various detectors can be employed)
  \item Reusable columns (expensive columns but can be used for many analysis)
  \item Ideal for the substances of low volatility.
  \item Easy sample recovery, handling and maintenance.
  \item Instrumentation leads itself to automation and quantitation.
  \item Precise and reproducible.
  \item Calculations are done by integrator itself.
  \item Suitable for preparative liquid chromatography on a much larger scale.
\end{itemize}

1.5 Types of HPLC\textsuperscript{(9)}

\begin{itemize}
  \item Based on the mode of separation
    \begin{itemize}
      \item Normal phase chromatography
      \item Reverse phase chromatography
      \item Partition chromatography
      \item Displacement chromatography
    \end{itemize}
  \item Based on principle of separation
    \begin{itemize}
      \item Adsorption chromatography
      \item Ion exchange chromatography
      \item Size exclusion chromatography
      \item Affinity chromatography
      \item Chiral phase chromatography
      \item Ion pair chromatography
    \end{itemize}
  \item Based on elution technique
    \begin{itemize}
      \item Isocratic separation
      \item Gradient separation
    \end{itemize}
  \item Based on the scale of operation
    \begin{itemize}
      \item Analytical HPLC
    \end{itemize}
\end{itemize}
Preparative HPLC

Based on the mode of separation:
- Normal phase chromatography
- Reverse phase chromatography
- Partition chromatography
- Displacement chromatography

Normal phase chromatography:
It was one of the first kind of HPLC in which stationary phase is polar in nature and the mobile phase is non-polar and non-aqueous in nature. If the affinity between the stationary phase and the analyte increases the retention time (RT) of the analyte also increases and vice versa. The interaction strength depends not only on the functional groups in the analyte molecule but also on steric factors. The effect of sterics on interaction strength allows this method to resolve (separate) structural isomers.

Reversed phase chromatography:
In reversed phase technique, a non-polar stationary phase is used and the mobile phase is polar in nature. Hence polar components get eluted first and non-polar compounds are retained for a longer time. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster, columns used in the mode of chromatogram are ODS (Octadecyl silane) or C18, C8, C4, etc.

Partition chromatography:
Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid-liquid applications. Partition chromatography uses a retained solvent, on the surface or within the grains or fibers of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional cumblic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent separate analytes based on the polar
differences is known as Hydrophilic Interaction Chromatography (HILIC). Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences. However, HILIC has the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

**Displacement chromatography:**

The basic principle of displacement chromatography is a molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites and thus displace all molecules with lesser affinities. http://en.wikipedia.org/wiki/High_performance_liquid_chromatography-cite_note-0 In elution mode, substances typically emerge from a column in narrow gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds and thereby be resolved there must be substantial differences in some interaction between the bio molecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”.

**Based on principle of separation:**

**Adsorption chromatography:**

When a mixture of compounds (adsorbate) dissolved in the mobile phase (eluent) moves through a column of stationary phase (adsorbent) they travel according to their relative affinities. The compound which has more affinity towards stationary phase travels slower, if less affinity towards stationary phase travels faster.
Ion exchange chromatography:

It is the process by which a mixture of similar charged ions can be separated using ion exchange resin. There is a reversible exchange of ions between the ions present in the column. And those present in the ion exchange resin. For cations, cation exchange resin and for anions, an anion exchange resin is used.

1. Cation exchange eg: Sulfonated polystyrenes
2. Anion exchange resin eg: Carboxylic methacrylate

Size exclusion chromatography:

It is the process by which mixture of compounds with molecular sizes are separated by using gels. The gel used acts as molecular sieve. It can be separated by steric and diffusion effects of pores in the gels. The compound can separate according to the molecular sizes and the stationary phase is a porous matrix.

Eg: separation of proteins and polysaccharides.

Chiral phase chromatography:

In this type of chromatography, separation of optical isomers can be done by using chiral stationary phases i.e., levo and dextro form can be separated by using chiral stationary phases.

Eg: chemically bonded silica gel.

Ion pair chromatography:

In this chromatography, a reversed phase column is converted temporarily into ion exchange column by using ion pairing agents like pentane or hexane.

Based on the scale of operation:

Analytical HPLC: It is used for analysis of samples. But recovery of samples is not done since the samples used are at very low level.

Eg: microgram quantities.

Preparative HPLC: It is used for separation and collection of compound mixture, where the individual and pure compounds can be collected using fraction collector. And the collector samples are reused.

Eg: separation of few grams of mixtures.
The main components of HPLC are:

- Solvent reservoir
- Pump
- Injection port
- Column
- Detector
- Data acquisition system

**Solvent reservoir**

Solvent reservoirs are used to store mobile-phase. Scott Duran bottles are commonly used as solvent reservoirs. The solvent reservoir must be made of inert material such as glass and must be smooth so as to avoid growth of microorganisms on its walls. It can be transparent or can be amber colored. A graduated bottle gives a rough estimate of mobile-phase volume in the bottle. Solvent reservoirs are placed above HPLC system (at higher level) in a tray. They should never be kept directly above the system as any spillage of solvent on the system may damage electronic parts of HPLC. Schematic diagram represented in figure-1.1 and waters make HPLC instrument represented in figure-1.2.
Fig-1.1: Systematic diagram of HPLC.
**HPLC pump:**

The HPLC pump is very important component of the system. The pump delivers the constant flow of the mobile phase or phases so that the separation of the components of the mixture occurs in a reasonable time. There are two types of pumping systems Isocratic and Gradient. The gradient type pumps also classified into two types, those are:

- High pressure mixing. Eg: Mechanical and piston pumps.
- Low pressure mixing. Eg: Pneumatic and quaternary pumps.

**Injection port**\(^{(10)}\):

The sample introduction device such as injector is to introduce the sample in a flow of mobile phase at high pressure. The valve injection through fixed or variable loop is a common way of introducing the sample. The Rheodyne valve is the mostly used devise. HPLC injector loop represented in figure-1.3.
HPLC column:

The HPLC column holds the stationary phase for separating the components of the sample. The columns are usually made up of SS-316 grade steel. Apart from columns, the material of construction of tubing and fittings, plumbing and connections are also very critical. Apart from resistive to corrosion, connections and plumbing should have very low dead volume. Table-2 represents the column selection details. Column hardware represented in figure-1.4. And columns selection represented in table-1.2
Table-1.2: List of column selection chart.

<table>
<thead>
<tr>
<th>LC mode (packing)</th>
<th>Water soluble</th>
<th>Organic solvent soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP bonded (C₈, phenyl, C₄)</td>
<td>aqueous gel filtration (SEC)</td>
<td>Ion exchange</td>
</tr>
</tbody>
</table>

Stationary phases:

There are many types of stationary phases employed in liquid chromatography including:

1. Silica, alumina or porous graphite in normal-phase chromatography where the separation is based on differences in adsorption and/or mass distribution.
2. Resins or polymers with acid or basic groups in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase.
3. Porous silica or polymers in size-exclusion chromatography, where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion.
4. A variety of chemically modified supports prepared from polymers, silica or porous graphite, used in reversed-phase liquid chromatography, where the separation is based principally on partition of the molecules between the mobile phase and the stationary phase.
5. Special chemically modified stationary phases, for example cellulose or amylase derivatives, proteins or peptides, cyclodextrins etc., for the separation of enantiomers (chiral-chromatography). Column packing types were represented in figure-1.5 and stationary phase represented in figure-1.6.
Structures of various stationary phases

- \( C_{18} \) - \((CH_2)_nCH_3\)
- \( C_8 \) - \((CH_2)_nCH_3\)
- \( C_4 \) - \((CH_2)_nCH_3\)
- TMS - \(CH_3\)
- CN - \((CH_2)_nCN\)
- \(NH_2\) - \((CH_2)_nNH_2\)

Fig-1.5: HPLC column packing types.

- Solute interacting with second layer of solvent (B) SORPTION
- Solute interacting with first layer of solvent (B) by displacing solvent from second layer DISPLACEMENT
- Solute interacting directly with first layer of solvent (B) SORPTION
- Solute interacting with silica surface by displacing solvent (B) from first layer DISPLACEMENT
- Solute interacting with silica surface by displacing solvent (A) from first layer DISPLACEMENT
- Solute interacting directly with layer of solvent (A) SORPTION

Fig-1.6: Column stationary phase.
HPLC Detectors\(^{(12)}\)

Detectors detect various compounds as they elute out from column. The detector gives response in terms of a millivolt (mv) signal that is then processed by the computer (integrator) to obtain a chromatogram. Basically detector consists of a flow-cell through which the mobile phase and resolved sample moves optic shine through the detector cell and variation in optical properties are detected.

The photo diode array detector (PDA) is the most used detector in LC today. The PDA gives a three dimensional view of chromatogram (Intensity Vs Time) and spectra (Intensity Vs Wavelength) simultaneously. It can be called as spectro-chromatogram. The detailed analysis of the data reveals more information on the complexity of co elution and helps in identifying the merged peaks and gives information on peak purity.

Various types of HPLC detectors:

There are several types of detectors available in the market. Those are

- UV-Visible detector,
- Photo-diode array detector (PDA),
- Fluorescence detector,
- Conductometric and calorometric detector,
- Mass detector,
- Evaporative light scattering detector (ELSD)

Among these detectors photo diode array detector (PDA) is the most widely used detector. The PDA gives a three dimensional view of chromatogram (Intensity Vs Time) and spectra (Intensity Vs Wavelength) simultaneously. It can be called as spectro-chromatogram. The detailed analysis of the data reveals more information on the complexity of co elution and helps in identifying the merged peaks and gives information on peak purity.

Ideal characteristics of a detector:

- Equally sensitive to all eluted peaks,
- Gives the response (area) proportional to the amount injected, respective of the size of sample.
Cheap, reliable and easy to use.
Will not be affect by change in temperature or mobile phase composition.
Able to monitor small amounts of compound.

i. Bulk property detector:
These provide a differential measurement of bulk property possessed by both the solute and mobile phase Eg: Refractive index. It is not suitable for gradient elution and is less sensitive.

ii. Solute property detector:
This measures physical or chemical property that is specific to the solute only. Eg: UV detector, conductivity detector. These can be used for gradient elution. The detection of the separated compounds in the elute from the column is based up on the bulk properties of the elute are the solute property of the individual components generally, a detector is selected so that it will respond to a particular property of the substances being separated, and ideally it should be sensitive to atleast $10^{-8}$g/mL and give a linear response over a wide concentration range.

Detector selection depends on the nature of molecule structure, refractive index, fluorescence. Generally used detectors are UV, RID, ELSD, fluorescence and chemical detector.

UV-Visible detector:
UV detectors function on the capacity of many compounds to absorb light in the wavelength range 180 to 400nm with deuterium and tungsten lamp. Figure-1.7 shows the UV photo diode array detector (PDA). The sensor cell usually consists of a cylindrical cavity about 1.0mm internal diameter (ID) and a few mm long, having a capacity that ranges from about two micro-liters to eight micro-liters. Light from a UV light sources passes through the sensor onto a photoelectric cell, the output from which is electronically modified and presented on a potentiometer recorder, a computer screen or printer. Alternatively a broad band light source can be used light after passing through the cell can be optically dispersed by prism or grating and allowed to fall onto a diode array. Schematic diagram represented in figure-1.7.
Refractive index detector (RID):

The refractive index detector is one of the least sensitive LC detectors. It is very sensitive to changes in ambient temperature, pressure changes and flow-rate changes; and cannot be used for gradient elution. Despite these disadvantages, this detector is extremely useful for detecting those compounds that are nonionic, do not adsorb in the UV and do not possess the fluorescence. Below Figure represents the refractive index detector (RID). Schematic diagram represented in figure-1.8.
**Fluorescence detector:**

Fluorescence detectors are used in modern HPLC detectors. It is possible to detect even a presence of a single analyte molecule in the flow cell. Typically, fluorescence sensitivity is 10-1000 times higher than that of the UV detector for strong UV absorbing materials. Fluorescence detectors are very specific and selective among the others optical detectors.

To improve the specificity of an LC analysis, a fluorescent derivative of the substance of interest may be prepared (employing an appropriate fluorescent reagent). The substance may then be selectively detected from other solutes which, (if they do not fluoresce) need not be resolved from each other by the chromatographic column. Schematic diagram represented in figure-1.9.

![Fig-1.9: Schematic diagram of fluorescence detector.](image)

**Mass-spectrometric detector:**

Mass detector actually weighs the mass of solute eluted from the chromatographic column continuously over a predetermined period of time (usually the complete period of chromatographic development). It can only function with packed columns, as adequate sample must be placed on the column for the mass measuring device to respond. Schematic diagram represented in figure-1.10.
Evaporative light scattering detector (ELSD):

ELS detector employs a unique method of detection. The detection process involves three steps: nebulization, evaporation and detection.

**Nebulization:**

Detector transforms the liquid phase leaving the column into an aerosol cloud of fine droplets. The size and uniformity of the droplets are extremely important in achieving sensitivity and reproducibility.

**Evaporation:**

In the evaporation tube the solvent is volatilized to produce particles or droplets of pure analyte. The temperature of the drift tube is set at the temperature to evaporate the solvent. Drift tube provides evaporation of solvents at low temperatures to minimize the evaporation of the compound of interest.

**Detection:**

The particles emerging from the evaporation tube enter the optical cell, where the sample particles pass through a beam of light. Figure-1.11 represents the schematic
diagram of evaporative light scattering detector (ELSD). Schematic diagram represented in figure-1.11.

**Fig-1.11:** Schematic diagram of ELS detector.
**Thermal conductivity detector (TCD):**

Certain ionic solutes can be detected in aqueous mobile phases using a conductivity detector and devices with low-volume cells suitable for use with high performance columns are commercially available. In principle, this device should be applicable to non-aqueous systems; however, applications of conductivity cells with high-performance columns and non-aqueous media have not been adequately studied. Response of conductivity detectors is temperature dependent; consequently, temperature must be controlled carefully. Certain conductivity detector designs are also susceptible to changes in mobile-phase flow rate. The response of this detector is predictable from conductivity data and these devices exhibit a linear response to solute concentration when properly designed. Schematic diagram represented in figure-1.12.

![Schematic diagram of thermal conductivity detector.](image)

**Fig-1.12:** Schematic diagram of thermal conductivity detector.

**Data collection devices:**

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The data storage capacity of these devices is usually limited. Modern data stations are computer based and have a large storage capacity to collect process and store data for possible subsequent reprocessing. Analytical reports can often be customized to the needs of the analyst.

Integration of peak areas and the setting of threshold levels are not normally problematic in an assay since the peak of the substance to be analysed should be free of interference. However, in a test for impurities, the selection of the peak area
integrator parameters becomes very important, particularly when baseline separations are not always attainable. If baseline separations cannot be obtained, valley-to-valley integration should be employed.

HPLC allows limits to be set for individual impurities and for the sum of impurities, but there is a level at which peaks should not be integrated. This "disregard level" is set in relation to the area of the peak in the chromatogram of the prescribed reference solution and is usually equivalent to 0.05% of the substance being examined. Typical HPLC system software represented in figure-1.13.

**Fig-1.13**: HPLC data collector
1.7 HPLC DATA INTERPRETATION (13)

System suitability:

The system suitability test represents an integral part of the method and is used to ensure the adequate performance of the chosen chromatographic system. Efficiency, capacity factor resolution factor, and symmetry factor are the parameters that are normally used in assessing the column performance. Factors that can affect chromatographic behavior include mobile phase composition, temperature, ionic strength, apparent pH, flow rate and column length and stationary phase characteristics such as porosity, particle size and type, and specific surface area.

Efficiency (N) (14):

The efficiency of a chromatographic column is defined in terms of the number of theoretical plates (N) and can be calculated using the following formula:

\[
N = 5.54 \frac{t_R^2}{W_h^2}
\]

Where, \( t_R \) = retention time or the baseline distance between the point of injection and the perpendicular dropped from the maximum of the peak of interest.

\( W_h \) = the width of the peak of interest determined at half peak height, measured in the same units as \( t_R \).

\( N \) = the number of theoretical plates per meters.

The column plate number increases with several factors:

1. Well-packed columns (column “quality”)
2. Longer columns
3. Lower flow rates (but not too low)
4. Smaller column-packing particles
5. Lower mobile-phase viscosity and higher temperature
Capacity factor (mass distribution ratio, $D_m$):

This factor determines the retention of a solute and can be calculated from the chromatogram using the following formula:

$$D_m = \frac{(t_R - t_M)}{t_M}$$

Where, $t_R$ = retention time of the solute

$t_M$ = retention time of an unretained component

A low $D_m$ value indicates that the peak elutes close to the solvent front, which may compromise selectivity. A minimum $D_m$ value of 1 is recommended for the peak of interest.

The retention time of the test substance can be varied, if necessary, by changing the relative proportion or composition of solvents in the mobile phase. Generally, an increase in the proportion of a more polar solvent will lead to a shorter retention time on a normal-phase column and a longer retention time on a reversed-phase column.

Resolution factor ($R_s$):

It is measure of the extent of separation of two compounds and the baseline separation is achieved.

The resolution between two peaks of similar height in a chromatogram can be calculated using the following formula:

$$R_s = \frac{1.8(t_{R2} - t_{R1})}{(W_{b1} + W_{b2})}$$

Where, $t_{R1}$ and $t_{R2}$ = retention times or baseline distances between the point of injection and the perpendicular dropped from the maximum of each of the two peaks.

$W_{b1}$ and $W_{b2}$ = the respective peak widths determined at half peak height, measured in the same units as $t_{R1}$ and $t_{R2}$.

The value of $R_s$ for a baseline separation between peaks of similar height should be at least two. Resolution graphs represented in figure-1.14.
Relative retention:
The relative retention \((r)\) is calculated as an estimate using the following formula:

\[
r = \frac{t_{R2} - t_M}{t_{R1} - t_M}
\]

Where, 
- \(t_{R2}\) = retention time of the peak of interest
- \(t_{R1}\) = retention time of the reference peak
- \(t_M\) = retention time of an unretained component

Retention time (\(R_t\)):
Retention time is the difference in time between the points of injection and eluted from a column. Retention time is measured in minutes or seconds. Retention time is also proportional to the distance moved on a chart paper, which can be measured in cm or mm.

Retention volume (\(V_r\)):
Retention volume is the volume of mobile required to elute 50% of the component from the column. It is the product of retention time and flow rate.

\[
Retention volume (V_r) = Retention time (R_t) \times flow rate
\]

Column efficiency (\(N\)):
It is called as the number of theoretical plates. It measures the band spreading of a peak. When band spread in smaller, the number of theoretical plates is higher. It indicates a good column and system performance.

\[
N = 16 \left( \frac{R_t}{W} \right)^2
\]
Where \( N = \) plates per meter

\( RT = \) retention time of the components.

\( W = \) width of the base of the component peak using tangent method.

**HETP (High equivalent theoretical plates):**

A theoretical plate can be of any height, which decides the efficiency of separation. If HETP is less the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

\[
\text{HETP} = \frac{L}{N}
\]

\( L = \) column length in meters

\( N = \) plates per meter

**Symmetry factor (\( A_s \)):**

The symmetry factor for a peak can be calculated using the following formula:

\[
A_s = \frac{W_x}{2d}
\]

Where, \( W_x = \) width at 5% of peak height measured from the baseline.

\( d = \) baseline distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height, measured in the same units as \( W_x \).

Values of \( A_s \) which are greater than 2 may lead to incorrect integration, resulting in erroneous quantitation. The main factors that influence peak symmetry depend upon retention, solvent effects, incompatibility of the solute with the mobile phase or development of an excessive void at the inlet of the column. In reversed-phase chromatography, adsorption phenomena due to the presence of residual silanol groups in the stationary phase may lead to tailing (poor peak symmetry).

**Tailing Factor (\( T \)):**

The tailing factor (\( T \)), a measure of peak symmetry is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced. In some cases, values less than 1 may be observed. As peak asymmetry increases integration and hence precision becomes less reliable.
Where, \( W_{0.05} \) = width of peak at 5% height

\( f \) = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline. Limit: \( \leq 2 \).

### 1.8 HPLC METHOD DEVELOPMENT

The need to save method development time and improve accuracy is forcing today's analytical chemists to look for better, faster ways to develop stability indicating methods. Starting with HPLC columns that offer excellent reproducibility, column lifetime and sensitivity this step-by-step protocol can save the method development chemist time and money required to establish new method. This approach is consistent with developing process.

**Procedure (protocol) for method development:**

**Literature survey**

Conducted literature survey and collected information available from the following references

- Chemical abstracts
- Analytical abstracts
- Journals
- National library of medicines etc,

And collected the following literature from survey

**Solubility profile:** Solubility of drug in different solvents at different pH conditions which is useful while selecting the diluents for standard solution and extraction solvents for test solution.

**Analytical profile:** Physico-chemical and spectroscopic properties, impurity and degradation profile of drug substance. Spectral profile is useful in the selection of detector wavelength for analysis, where as degradation profile helps to develop the method for separation of all possible impurities and degradants from API.

**Stability profile:** Stability of the drug with storage conditions. This helps to adopt suitable and adequate precautions while handling drug substances and its solutions.
The various parameters that include to be optimized during method development are:

- Selection of mode of separation
- Selection and optimization of mobile phase
- Selection of detector wavelength
- Selection of column
- Selection of solvent delivery systems
- Selection of flow rate
- Selection of column temperature
- Selection of diluent and extraction procedure
- Selection of test concentration and injection volume
- Establishment of stability of the solutions
- Establishment of system suitability

**Selection of mode of separation**

In reversed phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A second factor is the nature of the matrix.

**Selection and optimization of mobile phase**

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all individual impurities and degradants from analyte peak. The selection of mobile phase is done always in combination with selection of column (stationary phase). The following are the parameters, which shall be taken into consideration during the selection and optimization of mobile phase.

- Buffer, if any and its strength
- pH of buffer or pH of mobile phase
- Mobile phase composition
Buffer if any and its strength

Buffer and its strength play an important role in deciding the peak symmetries and separation. Various types of buffers can be employed for achieving the required separation. The following are some of the most commonly used ones.

- Phosphate buffers: KH₂PO₄, K₂HPO₄, Na₂HPO₄, H₃PO₄
- Acetate buffers: Ammonium acetate, Sodium acetate
- Amine buffers: Triethyl amine/Diethyl amine
- Buffers with various ion pair reagents like tetra butyl ammonium hydrogen sulphate
- Butane sulphonic acid, Hexane sulphonic acid, Heptane sulphonic acid etc.

It is important to use the buffers with suitable strength to cope up for the injection load on the column otherwise peak tailing may arise due to changes in ionic form during chromatography. The retention times also depends on the molar strength of the buffer since molar strength is inversely proportional to retention time.

Ideally, the strength of the buffer shall be adopted in-between 0.05M to 0.2M. The selection of the buffer and its strength is done always in combination with selection of organic phase composition in mobile phase. The strength of the buffer can be altered if necessary to achieve the required separation. But it has to be ensured that the higher strength of the buffer shall not result in precipitation/turbidity either in mobile phase or in standard and test solution while allowed standing in bench top or in refrigerator. Experiments shall be conducted using different buffers having different strength to obtain the required separation.

The buffer having a particular strength, which gives separation of all individual impurities from API peak, shall be selected. Then strength of the buffer can be varied by about 10 to 20% from the selected buffer strength and the effect of variation shall be studied. After reviewing the results of variation, the buffer and its strength shall be selected, this is rugged for at least 2% variation in strength.
**pH of the buffer or pH of the mobile phase:**

pH plays an important role in achieving the chromatographic separation as it control the elution properties by controlling the ionization characteristics. Depending on the pKa, drug molecule changes retention. E.g.: Acids show an increase in retention as pH decreases, while bases show decrease in retention time.

Experiments shall be conducted using buffers having different pH to obtain the required separation. It is important the pH of the mobile phase in the range of 2.0 to 8.0 as most of the columns doesn’t withstand to a pH outside this range. This is due to fact that the siloxane linkages are cleaved below pH 2.0, while at pH values above 8.0, silica may dissolve.

If a pH outside this range is found necessary, packing materials, which can withstand these ranges shall be chosen. pH of the buffer, which gives separation of all individual impurities from each other and from API, shall be selected. Then pH is varied by ±0.2 from the selected pH and effect of variation shall be studied. After reviewing the results, a pH is selected which is rugged at least for ±0.2 of the selected pH.

**Mobile phase composition:**

In reverse phase chromatography, the separation is mainly controlled by the hydrophobic interactions between drug molecule and the alkyl chains on the columns packing material. Most chromatographic separations can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions. Most widely used solvents in reverse phase chromatography are methanol and acetonitrile. Tetra hydro furan is also used but to a lesser extent.

Experiments shall be conducted with mobile phase having buffers with different pH and organic phases to check for the best separation between the impurities. A drug solution having all possible known impurities can be used for checking the extent of separations with different mobile phase ratios. Alternatively solution of stressed drug substance can be used to check for the separation of impurities. A mobile phase composition which gives separation of all possible
impurities and degradants from API peak and which is rugged at least for ± 0.5 in both aqueous and organic phase shall be selected.

In reversed phase, methods are developed using buffers at the specified mobile phase pH. Optimum buffering capacity occurs at a pH equal to the pKa of the buffers. Also it shows little effect on retention time. Potassium salts are more soluble than sodium salts. Diammonium hydrogen orthophosphate is used as phosphate buffer pH 2.3 and acetonitrile.

**Selection of detector wavelength**

Selection of detector wavelength is a critical step in finalization of the analytical method. To determine the exact wavelength standard API is injected into chromatographic system with photo diode array detector and the wave length, which gives higher response for the compound will be selected. An ideal wavelength is one that gives good response for the drugs to be detected. In order to ascertain the optimum wavelength \( (\lambda_{\text{max}}) \) of the species formed. A 10 ppm of sample solution was prepared and scanned under UV-visible spectrophotometer in the range of 200 to 400nm against diluent as blank. The wave length selected based on the maximum absorption occurred.

**Selection of column**

Column plays the most important role in achieving the chromatographic separation. The following parameters should be considered while selecting a column.

- Length and diameter of the column
- Packing material
- Size and shape of particles
- Pore size, surface area and end capping
- Percentage of carbon loading

Columns with silica as a packing material is used widely in **Normal phase chromatography**, where the eluent (mobile phase) is non-polar consisting of various organic solvents and the stationary phase is polar. The silanol groups on the surface of the silica give it a polar character.
In reversed phase chromatography a wide variety of columns is available covering a wide range of polarity by cross linking the silanol groups with alkyl chains like C₆, C₈, C₁₈ and nitrile groups (-CN), phenyl groups (-C₆H₆) and amino groups (-NH₂).

**Order of the silica based columns**

I-------Non Polar-------Moderately Polar-------Polar-------I

C₁₈ < C₈ < C₆ < Phenyl < Amino < Cyano < Silica

Experiments are conducted using different columns with different mobile phase to achieve best separation. A column which separates all the impurities and degradants from API peak and which is rugged with mobile phase variation is selected.

**Selection of solvent delivery systems**

Chromatographic separations with single eluent i.e., all the constituents of mobile phase is mixed and pumped as single eluent is called Isocratic Elution and is always preferable. However gradient elution is a powerful tool in achieving separation between closely eluting compounds having different polarities. The importance of gradient elution is that the polarity and ionic strength of the mobile phase can be changed during the run. Gradient elution is of two types

1. Low pressure gradient and
2. High pressure gradient elution

Low pressure gradient is one in which mobile phases are mixed at pre determined ratios and in high pressure gradient mobile phase are pumped at different flow rates to achieve the required composition and mixed in mixing chamber and then introduced to the column. Low pressure gradient is opted when NMT 80% of organic phase has to be pumped. High pressure gradient is opted when more than 80% of organic phase has to be pumped. While optimizing the gradient elution it is important to monitor the following

> The graph is to be monitored so as to ensure that the overall system pressure will not cross 300 bars at any point during the run.
Flow rate is to be physically cross checked by collecting the output from the detector during the run at different time intervals. This avoids pumping problems which may arise due to higher organic phase compositions.

**Selection of flow rate**
Flow rate is selected based on the following factors:

- Retention time
- Column composition
- Separation impurities
- Peak symmetry

Preferably flow rate shall not be more than 2.5 mL/min. A flow rate that gives least retention times, good peak symmetries, least back pressure and better separation of impurities from API peak shall be selected.

**Selection of column temperature**

Ambient temperature is always preferred as column temperature. However if the peak symmetry could not be achieved then the column temperature can be varied between 30° to 80°C. If a column temperature above 80°C is found necessary, packing material which can withstand to that temperature shall be chosen. The increase in column temperature generally will result in reduction in peak asymmetry and peak retentions.

For developing a method, the type of column being used and functionality, structure, pKa and reactivity must be considered. Optimize the temperature and adjust the mobile phase compositions to achieve best separation. Usually the silica column temperatures stable up to 60°C, optimized flow rate achieve the shortest analysis and adequate resolution and efficiency. In Isocratic method performs the analysis at 50°C. Reduce the amount of organic modifier in the mobile phase is necessary to produce the good separation. Some column pre heaters are used to preheat the mobile phase to avoid broad broadening. Also use peltier chip for precise temperature and controls the column effluent. Silica based stationary phases stable at up to 60°C some instances up to 90°C the higher temperature will leads the
shorter column life time. At elevated temperature the solute transfer from mobile phase to the stationary phase is more efficient.

**Selection of diluent and extraction procedure**

Diluent for test preparation is selected initially based on solubility of the drug substance. It is selected in such a way that the drug substance is soluble in which the extraction is complete, due to which there won't be any interference and in which peak symmetry and resolution between impurities and API Peak is found satisfactory. General methods followed for extraction are sonication, rotary shaking or both. In some cases where API is not extracted by above methods then heating is adopted if substance is stable and it should not precipitate upon cooling to room temperature.

Experiments are conducted to optimize the extraction of API in the presence of excipients at different test concentrations using the diluents chosen based on solubility at different time intervals of sonication time or rotary shaking or both and select the test concentrations at which the extraction is most efficient.

**Selection of test concentration and injection volume**

The test concentration is generally chosen based upon the response of API peak at the selected detector wavelength. However test concentration is finalized after it is proved that API is completely extractable at the selected test concentration. Generally an injection volume of 10 to 20μL is recommended for estimation of API. However if the extractions are found to be difficult then the test concentration can be kept low and the injection volume can be increased up to 50μL. But it is to be ensured that at the selected volume the column is not overloaded.

**Establishment of stability of the solutions**

The assessment of stability of the solution is carried out by keeping the test solution at room temperature and at 2⁰ to 8⁰c and this test solution is injected along with the freshly prepared standard and % of RSD of the standard solution and test solution is calculated. An acceptance criterion here is that the % of RSD should not be NMT 2%.
Establishment of system suitability

System suitability parameter has to be selected based on the tailing factor, plate count, resolution, and RSD. In general resolution factor for the closely eluting compounds is selected as a system suitability requirement. If the separation of impurities from each other and from API peak is found to be satisfactory, there is no need to keep a resolution factor as system suitability parameter. In such cases only standard reproducibility and symmetry of standard peak can be adopted as a system suitability requirement.

1.9 HPLC METHOD VALIDATION (15-21)

According to ICH and FDA guidelines, validation defined as an act of proving that any procedures, process, equipment, materials, activity or system performs as expected under a given set of conditions. Basically validation is proving that the performance is as intended when extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by some different persons, in same or different laboratories using different reagents, different equipments etc.

According to US FDA defines the term "validation is a process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce meeting, its predetermined specifications and quality attributes".

According to USP, "validation of an analytical method is the process by which it is established by laboratory studies that the performance characteristic of the method meets the requirements for the intended in analytical applications".

Purpose of validation:
1. Enable the scientists to communicate scientifically and effectively on technical matter.
2. Setting the standards of evaluation procedures for checking compliance and taking remedial action.
3. Economic: Reduction in cost associated with process sampling and testing.
4. As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples.

**Types of analytical procedures to be validated**

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures are equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below.

1. Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard.

2. Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

Typical validation characteristics which should be considered are listed below:

- **Accuracy**
Furthermore revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well. Method validation flow chart represented in figure-1.15.

**Fig-1.15: Figure showing Validation parameters.**

**Accuracy:**

It is the closeness of agreement between the actual value of the drug and the measured value. Spike and recovery studies are performed to measure accuracy, a known sample is added to the recipients and the actual drug value is compared to the value found by the assay. Accuracy is expressed as the bias or the % of error between the observed value and the true value (assay value/actual value x 100%).
**Precision:**

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

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

**Repeatability:**

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

**Intermediate precision:**

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

**Reproducibility:**

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

It is expressed as the coefficient of variation (% of CV). CV is the standard deviation of the assay values divided by the concentration of the analyte. Several types of precision can be measured: intra-assay precision (repeatability) is the % of CV of multiple determinations of a single sample in a single test run; inter-assay precision (also called intermediate precision) measures the % of CV for multiple determinations of a single sample, controls and reagents analyzed in several assay runs in the same laboratory.

**Linearity:**

A linearity study verifies that the sample solutions are in concentrations range where analyte response is generally performed by preparing standard
solutions at five concentration levels from 50-150\% of the target analyte concentrations five levels are required to allow detection of curvature in the plotted data.

Validation over a wide range provides confidence that the routine range provides confidence that the routine standard levels are well removed from non linear response concentrations that the method covers a wide enough range to incorporate the limits of content uniformity testing.

Acceptance of linear data is often judged by examining the correlation coefficient $>0.999$ is generally considered as evidence of acceptable fit of the data.

For the evaluation of linearity five different concentrations of standard solutions were prepared (25 to 125 ppm). A graph is plotted to “area” versus amount found”. The co-relation coefficient was found to be 0.999. It is the ability of an assay to obtain test results, which are directly proportional to the concentration of an analyte in the sample. The determination of this parameter will identify the range of the analytical assay. It can be measured as slope of the regression line and its variance or as the coefficient of determination and correlation coefficient (R).

**Range:**

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method.

**Specificity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications.

Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of
impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

**Limit of detection (LOD):**

It is the lowest amount of the analyte in a sample that can be detected but not necessarily be quantitated as an exact concentration or amount. The detection limit of an individual analytical procedure is the lowest amount of an analyte in the sample that can be detected but not necessarily quantitated as an exact value. The limit of LOD value is 3 based on the ICH guidelines.

For determining LOD initially standard deviation and slope of calibration curve was calculated.

\[
\text{LOD} = 3.3 \frac{\text{SD}}{S_1}
\]

Where, \( S_1 = \text{slope of calibration curve} \)

\( \text{SD} = \text{standard deviation of peak response} \)

**Limit of Quantitation (LOQ):**

It is the lowest amount of an analyte that can be measured quantitatively in a sample with acceptable accuracy and precision. The LOQ is a parameter for tests measuring impurities in a drug product.

The quantification limit of an individual procedure is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. The limit of LOQ value is 10.

For determining LOQ initially standard deviation and slope of calibration curve was calculated.

\[
\text{LOQ} = 10 \left( \frac{\text{SD}}{S} \right)
\]

Or

\[
\text{LOQ} = \frac{S}{N}
\]
Robustness:

It is the capacity of an assay to remain unaffected by deliberate changes to various parameters of the method and gives an indication of its reliability during normal assay conditions. The variations could be in room or incubator temperature or humidity, variations in incubation times, minor variations in pH of a reagent, etc.

System suitability:

Tailing factor for the peaks in Standard solution should not be more than 1.5. Theoretical plates for the peaks in Standard solution should not be less than 2500.

1.10 PHARMACEUTICAL DRUG PRODUCTS

1.10.1: DRUG PRODUCT-1

A. Losartan Potassium:

Losartan Potassium is the first member of a new class of non-peptide angiotensin II receptor antagonist. It is used to treat hypertension by suppressing the effects of angiotensin II at its receptors, thereby blocking the renin-angiotensin system. Losartan has been demonstrated to be superior to previous peptide receptor antagonists and angiotensin converting enzyme inhibitors because of its enhanced specificity, selectivity, and tolerability. Losartan potassium is marketed alone or combined with hydrochlorothiazide. Chemical structure of Losartan Potassium represented in figure-1.16.

![Fig-1.16: Structure of Losartan Potassium](image-url)
**Chemical details**

Class: Angiotensin II receptor antagonists  
Chemical name: Losartan potassium  
IUPAC name: 2-Butyl-4-chloro-1-[(2'- (1H-tetrazol-5-yl)[1,1'-biphenyl] -4-yl]-methyl]-1H-imidazole-5-methanol monopotassium salt  
Molecular formula: C_{22}H_{23}ClN_{6}O\cdot K \frac{1}{2}C_{4}H_{4}O_{4}  
Molecular weight: 461.01  
CAS NO.: 124750-99-8

**Therapeutic use**

Pending revision, the material in this section should be considered in light of more recently available information in the MedWatch notification at the beginning of this monograph.

**Preparations:** Excipients in commercially available drug preparations may have clinically important effects in some individuals; consult specific product labeling for details. Available combinations were tabulated in table-1.3.

**Table-1.3: Combination dosage forms**

<table>
<thead>
<tr>
<th>Routes</th>
<th>Dosage forms</th>
<th>Strengths</th>
<th>Brand names</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>tablets, film-coated</td>
<td>50 mg with Hydrochlorothiazide 12.5 mg</td>
<td>Hyzaar</td>
<td>Merck</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mg with Hydrochlorothiazide 12.5 mg</td>
<td>Hyzaar</td>
<td>Merck</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mg with Hydrochlorothiazide 25 mg</td>
<td>Hyzaar</td>
<td>Merck</td>
</tr>
</tbody>
</table>

**B. Hydrochlorothiazide:**

Hydrochlorothiazide (HCTZ) is a thiazide class diuretic drug. This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide
is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. The recommended dose of hydrochlorothiazide for treating high blood pressure is hydrochlorothiazide 25 mg to 50 mg per day. Chemical structure of Hydrochlorothiazide represented in figure-1.18.

![Chemical Structure of Hydrochlorothiazide](image)

**Chemical details:**
- **Class**: Thiazide class of diuretics.
- **Chemical name**: Hydrochlorothiazide
- **IUPAC name**: 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide
- **Molecular formula**: C$_7$H$_8$ClN$_3$O$_4$S$_2$
- **Molecular weight**: 297.74
- **CAS NO.**: 58-93-5

Hydrochlorothiazide is a diuretic drug. It increases the amount of urine passed, which causes the body to lose salt and water. This medicine is used to treat high blood pressure. It is also reduces the swelling and water retention caused by various medical conditions, such as heart, liver or kidney disease.

Hydrochlorothiazide is used to treat excessive fluid accumulation and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure, corticosteroid medications and nephrotic syndrome. It also is used alone or in conjunction with other blood pressure lowering medications to treat high blood pressure. Although hydrochlorothiazide is approved for treating edema in cirrhosis of the liver, it is rarely used because of the availability of more potent diuretics that are more effective. Hydrochlorothiazide can be used to treat calcium-containing
kidney stones because it decreases the amount of calcium excreted by the kidneys in the urine and thus decreases the amount of calcium in urine to form stones.

Used alone or in combination with other anti-hypertensive agents for all stages of hypertension. Thiazides have well-established benefits, can be useful in achieving goal BP alone or combined with other antihypertensive drugs, enhance the antihypertensive efficacy of multidrug regimens, and are more affordable than other agents. Thiazides be used as initial therapy for the treatment of uncomplicated hypertension in most patients, either alone or combined with other classes of antihypertensive drugs with demonstrated benefit (e.g., ACE inhibitors, angiotensin II receptor antagonists, β-blockers, calcium-channel blockers).

C. Enalapril Maleate:

Enalapril maleate is used to treat high blood pressure (hypertension), it works by relaxing blood vessels, causing them to widen. Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems. Enalapril maleate is supplied as 2.5 mg, 5 mg, 10 mg and 20 mg tablets for oral administration. Side effects are light-headed, fainting and urinating more or less than usual, fever, chills, body aches, flu symptoms, pale skin, easy bruising or bleeding. Figure-1.17 represents the chemical structure of Enalapril maleate.

![Structure of Enalapril Maleate](image-url)
Chemical details:
Class: Anangiotensin converting enzyme (ACE) inhibitor
Chemical name: Enalapril Maleate
IUPAC name: (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1)
Molecular formula: C20H28N2Os-C4H404,
Molecular weight: 492.53
CAS NO.: 76095-16-4

Pharmacokinetics
Absorption: Bioavailability is approximately 60%. T_max is within 1 h (enalapril); 3 to 4h
Distribution: Enalapril crosses the blood-brain barrier poorly, if at all. Enalapril does not cross the blood-brain barrier.
Metabolism: Enalapril is a prodrug and is hydrolyzed to Enalapril maleate (more potent than enalapril).
Elimination: Intact enalapril and approximately 40% of the dose is excreted in the urine. Approximately 94% is recovered in the urine and feces. The t_1/2 is 1.3h (enalapril).

Special populations
Renal function impairment: In those with glomerular filtration rate 30 mL/min or less, the peak and trough enalaprilat levels increase, T_max increases, and time to steady state may be delayed. Dosage adjustment recommended.

Dosage and administration
Heart failure (Adults): PO Initial dose: 2.5 mg twice daily. Usual dose: 2.5 to 20 mg/day in 2 divided doses (max, 40 mg/day). Titrate doses upward as tolerated over a period of a few days or weeks. The max daily dose is 40 mg in divided doses.
High-risk patients (Adults): Hypertensive patients at risk (eg, those with heart failure, hyponatremia, high-dose diuretic therapy, recent intensive diureses or increase in diuretic dose, renal dialysis or severe volume or salt depletion of any etiology) have potential for extremely hypotensive response. Initiate therapy under very close medical supervision. The starting dose should be 0.625 mg or less administered IV over a period of 5min or more and preferably longer (up to 1h).
**Hypertension (Adults):** PO Initial dose: 2.5 to 5mg/day. Titrate to desired BP control. Usual maintenance dose: 10 to 40mg/day in single or twice daily doses. For patients with Ccr of 30 mL/min or less, the dose is 0.625 mg. Dose may be repeated if after 1h the clinical response is inadequate. Additional doses of 1.25 mg may be administered at 6h intervals. For dialysis patients, the initial dose is 0.625 mg or less given over 5 min or preferably longer.

**Children:** PO initial dose: 0.08 mg/kg (up to 5 mg) every day. Adjust dose according to BP response. Doses above 0.58 mg/kg (or in excess of 40 mg) have not been studied in pediatric patients.

### 1.10.2. DRUG PRODUCT-2

**A. Metformin hydrochloride:**

Metformin is an oral medication that lowers blood glucose (sugar) and is used for treating type-II diabetes. Insulin is a hormone produced by the pancreas that controls glucose levels in blood by reducing the amount of glucose made by the liver and by increasing the removal of glucose from the blood by muscle and fat tissues. As a result, blood glucose levels fall. Diabetes caused by a decrease in production of insulin that causes increased production of glucose by the liver and reduced uptake (and effects) of insulin on fat and muscle tissues. Metformin acts by increasing the sensitivity of liver, muscle, fat and other tissues to the uptake and effects of insulin. These actions lower the level of sugar in the blood.

Metformin is an oral diabetes medicine that helps control blood sugar levels. Metformin is for people with type-II (non-insulin-dependent) diabetes. Metformin is sometimes used in combination with insulin or other medications, but it is not for treating type-1 diabetes. Metformin chemical structure represented in figure-1.19.

![Fig-1.19: Structure of Metformin](image-url)
Chemical details:

Class: Anti-diabetic drug in the biguanide
Chemical name: Metformin
IUPAC name: 1-carbamimidamido-N,N-dimethylmethanimidamide
Molecular formula: C₄H₁₁N₅
Molecular weight: 129.16
CAS NO.: 657-24-9

Dosage:

For treating type-II diabetes in adults, Metformin (immediate release) usually is begun at a dose of 500 mg twice a day or 850 mg once daily. The dose is gradually increased by 500 mg weekly or 850 mg every two weeks as tolerated and based on the response of the levels of glucose in the blood. The maximum daily dose is 2550 mg given in three divided doses. If extended tablets are used, the starting dose is 500 mg or 1000 mg daily with the evening meal. The dose can be increased by 500 mg weekly up to a maximum dose of 2000 mg (2500 mg of Fortamet) once daily or in two divided doses. Glumetza tablets are given once daily. Metformin should be taken with meals.

For pediatric patients 10-16 years of age, the starting dose is 500 mg twice a day. The dose can be increased by 500 mg weekly up to a maximum dose of 2000 mg. Glucophage XR has not been studied in children.

B. Glipizide:

Glipizide is an oral medium-to-long acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. Mechanism of action is produced by blocking potassium channels in the β-cells of the islets of Langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each β-cell.

Glipizide is used along with diet and exercise and sometimes with other medications, to treat type-II diabetes (condition in which the body does not use
insulin normally and therefore, cannot control the amount of sugar in the blood). Glipizide is in a class of medications called sulfonylureas. Glipizide lowers blood sugar by causing the pancreas to produce insulin (a natural substance that is needed to break down sugar in the body) and helping the body use insulin efficiently. This medication will only help lower blood sugar in people whose bodies produce insulin naturally. Glipizide is not used to treat type-I diabetes (condition in which the body does not produce insulin and therefore, cannot control the amount of sugar in the blood) or diabetic ketoacidosis (a serious condition that may occur if high blood sugar is not treated) the structural formula is shown below figure-1.20.

**Fig-1.20: Structure of Glipizide**

**Chemical details:**

- **Class**: Anti-diabetic drug from the sulfonylurea
- **Chemical name**: Glipizide
- **IUPAC name**: N-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide
- **Molecular formula**: C_{21}H_{27}N_{5}O_{4}S
- **Formula weight**: 445.54
- **CAS NO.**: 29094-61-9

**Drug Interactions:**

Alcohol may prolong the action of Glipizide by delaying the absorption and elimination of Glipizide. Patients taking Glipizide should keep alcohol consumption to a minimum. Cholestyramine may reduce the absorption and consequently the effect of Glipizide. Therefore, Glipizide should be administered 1-2 hours before
cholestyramine is administered. Fluconazole may increase the absorption and therefore increase the effect of Glipizide.

Many drugs can potentially increase or decrease glucose levels thus increasing or decreasing the effect of Glipizide. Drug interactions which cause low blood glucose (hypoglycemia) can occur with nonsteroidal anti-inflammatory drugs (eg: Ibuprofen), sulfa drugs, warfarin, miconazole, and β-blockers (eg: propranolol). Drug interactions which cause high blood glucose (Hyperglycemia) can occur with thiazide diuretics, corticosteroids, thyroid medicines, estrogens, niacin, phenytoin, and calcium channel blocking drugs (eg: Diltiazem). Patients should be monitored closely for loss of glucose control when such drugs are administered.

**Glipizide Dosing Information**

**Usual adult Glipizide dose for diabetes mellitus Type-II:**

Initial dose: 5 mg (immediate or sustained-release) orally once a day, 30 minutes before breakfast. Maintenance dose: 2.5-30 mg (immediate-release) orally in 1 or 2 divided doses or 5 to 20 mg (sustained-release) orally in 1 or 2 divided doses.

**Usual geriatric Glipizide dose for diabetes mellitus type-II:**

Initial dose: 2.5 to 5 mg orally once a day. Doses may be increased in 1 or 2 week intervals in 2.5 to 5 mg/day increments.

**Patients receiving insulin**

As with other sulfonylurea-class hypoglycemics, many stable non-insulin-dependent diabetic patients receiving insulin may be safely placed on Glipizide. When transferring patients from insulin to Glipizide, the following general guidelines should be considered.

For patients whose daily insulin requirement is 20 units or less, insulin may be discontinued and Glipizide therapy may begin at usual dosages. Several days should elapse between Glipizide titration steps.

For patients whose daily insulin requirement is greater than 20 units, the insulin dose should be reduced by 50% and Glipizide therapy may begin at usual
dosages. Subsequent reductions in insulin dosage should depend on individual patient response. Several days should elapse between Glipizide titration steps.

During the insulin withdrawal period, the patient should test urine samples for sugar and ketone bodies at least three times daily. Patients should be instructed to contact the prescriber immediately if these tests are abnormal. In some cases, especially when patient has been receiving greater than 40 units of insulin daily, it may be advisable to consider hospitalization during the transition period.

**Patients receiving other oral hypoglycemic agents**

As with other sulfonylurea-class hypoglycemics, no transition period is necessary when transferring patients to Glipizide. Patients should be observed carefully (1-2 weeks) for hypoglycemia when being transferred from longer half-life sulfonylureas (eg: chlorpropamide) to Glipizide due to potential overlapping of drug effect.

Glipizide comes as tablets and extended-release (long-acting) tablets to take by mouth. The regular tablet is usually taken one or more times a day, 30 minutes before breakfast or meals. The extended-release tablet is usually taken once a day with breakfast. To help you remember to take Glipizide, take it around the same time(s) every day. Follow the directions on your prescription label carefully, and ask your doctor or pharmacist to explain any part you do not understand. Take Glipizide exactly as directed.

**C. Repaglinide:**

Repaglinide is for the treatment of type-II diabetes. Repaglinide belongs to the meglitinide class of blood glucose-lowering drugs. Repaglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the β-cells. Repaglinidie chemical structure represented in figure-1.21.
Chemical details:

Class: Type-II diabetic
Chemical name: Repaglinide
IUPAC name: \((S)-(\pm)-2\text{-ethoxy}-4\text{-}[2\text{-}(3\text{-methyl-1\text{-}[2\text{-}(\text{piperidin-1-yl) phenyl}] \text{butylamino})\text{-2\text{-oxoethyl}}] \text{benzoic acid}\)
Molecular weight: 452.59
Molecular formula: \(C_{27}H_{36}N_{2}O_{4}\)
CAS NO.: 135062-02-1

Drug class and mechanism:

Repaglinide is an oral medication for lowering blood sugar (glucose) in individuals with diabetes. It is in a class of drugs for treating diabetes type-II called meglitinides and is chemically unlike other anti-diabetic medication.

Approximately 90% of patients with diabetes have type-II or non-insulin dependent diabetes mellitus. (Type-II diabetes usually occurs in adulthood and is associated with obesity and a strong family history of diabetes.) Glucose intolerance in diabetes type-II is caused by reduced insulin secretion from the pancreas after meals and resistance of the body's cells to insulin's effect which is to stimulate the cells to remove glucose from the blood. This leads to high levels of blood glucose.

Like Sulfonylureas, for example, glyburide (Glynase; Micronase), Glipizide, Glimepiride, Tolbutamide and Tolazamide, Repaglinide stimulates cells in the pancreas to produce insulin. Glyburide may be more potent than Repaglinide at
increasing insulin release in persons with low or high blood glucose levels, whereas Repaglinide may be more potent in persons with moderate blood glucose levels. Repaglinide is unusual in that it has a rapid onset of action and a short duration of action. When taken just prior to meals, it promotes the release of insulin that normally occurs with meals and is responsible for preventing blood glucose levels from becoming high. It has been shown to lower hemoglobin A1c levels by 1.6% to 1.9%. (Hemoglobin A1c is a blood test which measures the effectiveness of a drug in controlling high blood glucose levels. The lower the hemoglobin A1c, the better the control.) Repaglinide was approved by the FDA in 1997. It can be used alone (monotherapy) or combined with Metformin.

**Dosage:**

Repaglinide is taken immediately before a meal or 15 to 30 minutes before a meal. It should be taken with every meal up to 4 times a day. Doses are adjusted by the physician to achieve the best effect.

### 1.10.3. DRUG PRODUCT-3:

**A. Ornidazole:**

Ornidazole is a type of concomitant administration of oral anticoagulants may increase the risk of hemorrhage due to diminished hepatic metabolism. Ornidazole belongs to category of concomitant administration of oral anticoagulants may increase the risk of hemorrhage due to diminished hepatic metabolism, drugs. It is a 5 nitroimidazole derivative. Their antimicrobial action is similar to metronidazole and is used similarly in the treatment of susceptible protozoal infections and prophylaxis of anaerobic bacterial infections. It has been investigated for use in Crohn's disease after bowel resection. This drug is commonly sold as Brand Name Dazolic. Ornidazole have antimicrobial activity is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, inhibiting further synthesis and causing degradation of existing DNA. Chemical structure of Ornidazole represented in figure-1.22.
Chemical details:
Class: Nitroimidazole derivatives antiprotozoals
Chemical Name: Ornidazole
IUPAC name: \( \alpha\)-[Chloromethyl]-2-methyl-5-nitroimidazole-1-ethanol-1-Chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol \)
Molecular Formula: \( \text{C}_7\text{H}_{10}\text{ClN}_3\text{O}_3 \)
Formula Weight: 219.63
CAS NO.: 16773-42-5

Dosage and prescribing information for ornidazole
Amoebiasis: Adults: 500mg twice a day orally for 5 days.
Childrens: 10-25 mg/kg body weight in two divided doses.

Giardiasis: Adults: 1.5 g, once daily for 12 days.
Childrens: 40mg/kg body weight for 2 days.

Trichomoniasis: 1.5 gm once orally or 500mg twice a day for 5 days.

Bacterial vaginosis: 3 tablets of 500rng each as a single dose or one tablet of 500mg once daily for 5-7 days.

B. Ofloxacin:
Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be second-generation fluoroquinolone. [http://en.wikipedia.org/wiki/Ofloxacin](http://en.wikipedia.org/wiki/Ofloxacin). The original brand, Floxin, has been discontinued by the manufacturer in the United States on 18 June 2009, though generic equivalents continue to be available. Chemical structure of Ofloxacin represented in figure-1.23.
Chemical details:

Class: Synthetic chemotherapeutic antibiotic of the fluoroquinolone

Chemical name: Ofloxacin

IUPAC name: 9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid or Fluoro Dihydro Methyl (4-methyl 1-piperazinyl) 7-oxo 7H-pyrido 1,4 Benzoxazine 6-Carboxylic acid

Molecular formula: C₁₈H₂₀FN₃O₄

Molecular weight: 361.37

CAS NO.: 82419-36-1

Medical uses

Oral and I.V. Floxin is not licensed by the FDA for use in children due to the risk of serious reversible and irreversible injury to the musculoskeletal system. Other fluoroquinolones do have a limited licensed uses in children but are generally not recommended due to safety concerns. Ofloxacin (and its derivatives) has also been associated with a few isolated reports of unexplained pediatric fatalities. Children (those under 18) are also at an increased risk of bone, joint, or tendon toxicities. Prescribing ofloxacin in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of the development of severe adverse drug reactions.

In the adult population ofloxacin is limited to the treatment of proven serious and life threatening bacterial infections such as:
Acute bacterial exacerbations of chronic bronchitis
Community-acquired pneumonia
Uncomplicated skin and skin structure infections
Nongonococal urethritis and cervicitis
Mixed Infections of the urethra and cervix
Acute pelvic inflammatory disease
Uncomplicated cystitis
Complicated urinary tract infections
Prostatitis
Acute, uncomplicated urethral and cervical gonorrhea.

Ofloxacin has not been shown to be effective in the treatment of syphilis. Ofloxacin is now considered to be contraindicated for the treatment of certain sexually transmitted diseases by some experts due to bacterial resistance. Ofloxacin administered together with theophylline can lead to elevated blood levels of theophylline. Theophylline is used to open airways in the treatment of asthma. If concurrent use of ofloxacin and theophylline cannot be avoided, frequent blood tests to monitor theophylline blood levels should be performed. Ofloxacin should be used with caution in patients with central nervous system diseases such as seizures, because rare seizures have been reported in patients receiving this medication. Ofloxacin should be avoided in children and adolescents less than 18 years old, as safe use in these patients have not been established.

Many antibiotics, including ofloxacin, can alter the normal bacteria in the colon and encourage overgrowth of bacteria responsible for the development of inflammation of the colon (pseudomembranous colitis). Pseudomembranous colitis can cause fever, abdominal pain, diarrhea, and sometimes even shock. Patients taking ofloxacin can develop sensitivity of the skin to direct sunlight. Ofloxacin can enhance the action of the anticoagulant warfarin (Coumadin) and increase the risk of bleeding. Both high and low blood sugars have been reported in patients with
diabetes taking ofloxacin together with insulin and other medications used to lower the blood sugar. Careful monitoring of blood sugars is, therefore, recommended when these drugs are concurrently used.

**Available forms**

Ofloxacin for systemic use is available as tablets (multiple strengths), oral solution (250 mg/ml) and injectable solution (multiple strengths). It is also used as eye drops (trade name Exocin, known as Ocuflox in the United States) and ear drops. Ofloxacin is also used in animals. Its veterinary formulation is sold as Marfloxacin (not to be confused with marbofloxacin, another veterinary-use fluoroquinolone).

**C. Cefixime:**

Cefixime is used to treat a wide variety of bacterial infections. This medication is known as a cephalosporin antibiotic. It works by stopping the growth of bacteria. This antibiotic treats only bacterial infections. It will not work for viral infections (e.g., common cold, flu). Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness.

Take this medication by mouth with or without food, usually once a day. The dosage is based on your medical condition and response to therapy. Antibiotics work best when the amount of medicine in your body is kept at a constant level. Therefore, take this drug at evenly spaced intervals. Continue to use this medication until the full prescribed amount is finished, even if symptoms disappear after a few days. Stopping the medication too early may allow bacteria to continue to grow, which may result in a relapse of the infection.

Cefixime is an oral third generation cephalosporin antibiotic. Cefixime is a cephalosporin antibiotic used to treat infections caused by bacteria such as pneumonia; bronchitis; gonorrhea; and ear, lung, throat, and urinary tract infections. It was sold under the trade name Suprax in the USA until 2003 when it was taken off the market by drug manufacturer Wyeth after its patent expired. The oral suspension form of "Suprax" was re-launched by Lupin in the USA.

Cefixime API is manufactured by many pharmaceutical companies the world over. The major manufacturers are based in India.
API There is many API producers in world for this molecule and few are based in India. Nectar Lifesciences is one the leading manufacturer of this API and FDF in India. Neclife is cGMP compliant company and the products are manufactured in USFDA, EU GMP approved API facilities and finished products are also available in EU GMP Audited Facilities. Neclife claims to have one the biggest capacities of manufacturing of Cephalosporins specifically Intermediates, API, Finished dosage Forms. Chemical structure of Cefixime was shown in figure-1.24.

Fig-1.24: structure of Cefixime

Chemical details:

Class : Third generation cephalosporin antibiotic.
Chemical name : Cefixime
IUPAC name : (6R, 7R)-7-[2-(2-amino-1,3-thiazol-4-yl)-2-\n\[\text{(carboxymethoxy)imino}\]acetamido]-3-ethenyl-8-oxo-5-thia-\n1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Molecular formula : C_{16}H_{15}N_{5}O_{7}S_{2}
Formula weight : 453.45
CAS NO. : 79350-37-1

Cefixime is a cephalosporin antibiotic used to treat infections caused by bacteria such as pneumonia; bronchitis; gonorrhea and ear, lung, throat and urinary tract infections. Antibiotics will not work for colds, flu, or other viral infections. This medication is sometimes prescribed for other uses; ask your doctor or pharmacist for more information.

Cefixime comes as a tablet and liquid to take by mouth. It is usually taken once a day or every 12 hours (twice a day) for 5-14 days. Gonorrhea may be treated in 1-10 days. Follow the directions on your prescription label carefully.
1.10.4. DRUG PRODUCT-4

A. Cinnarizine:

Cinnarizine is an antihistamine which is mainly used for the control of nausea and vomiting due to motion sickness. It acts by interfering with the signal transmission between vestibular apparatus of the inner ear and the vomiting centre of the hypothalamus. The disparity of signal processing between inner ear motion receptors and the visual senses is abolished, so that the confusion of brain whether the individual is moving or standing is reduced. Vomiting in motion sickness is actually a physiological compensatory mechanism of the brain to keep the individual from moving so that it can adjust to the signal perception.

Cinnarizine could be also viewed as a nootropic drug because of its vasorelaxating abilities (due to calcium channel blockage), which happen mostly in brain. It is also effectively combined with other nootropics, primarily Piracetam; in such combination each drug potentiates the other in boosting brain oxygen supply. Chemical structure of cinnarizine represented in figure-1.25.

Chemical structure of cinnarizine represented in figure-1.25.

**Fig-1.25**: structure of Cinnarizine

### Chemical details:
- **Class**: Anti histamine
- **Chemical name**: Cinnarizine
- **IUPAC name**: 1-benzhydryl-4-cinnamyl-piperazine
- **Molecular formula**: C₂₆H₂₈N₂
- **Molecular weight**: 368.51
- **CAS No**: 298-57-7

### Pharmacodynamics:
Cinnarizine is an antihistamine and a calcium channel blocker. Histamines mediate a number of activities such as contraction of smooth muscle of the airways and gastrointestinal tract, vasodilatation, cardiac stimulation, secretion of gastric...
Competitive antagonists at histamine H1 receptors may be divided into first (sedating) and second (non-sedating) generation agents. Some, such as Cinnarizine also block muscarinic acetylcholine receptors and are used as anti-emetic agents. Cinnarizine through its calcium channel blocking ability also inhibits stimulation of the vestibular system.

**Mechanism of action:**

Cinnarizine inhibits contractions of vascular smooth muscle cells by blocking L-type and T-type voltage gated calcium channels. Cinnarizine has also been implicated in binding to dopamine D2 receptors, histamine H1 receptors and muscarinic acetylcholine receptors.

Cinnarizine is not free of central activity over the usual therapeutic dose range of 15 to 30 mg. It is contraindicated for motion sickness in aircrew involved in the control of aircraft. Caution should be exercised in the use of the drug by other aircrew who may be involved in tasks which demand continued attention.

**B. Dimenhydrinate:**

Dimenhydrinate is used to prevent and treat nausea, vomiting, dizziness, and vertigo associated with motion sickness. Dimenhydrinate is an over-the-counter drug used to prevent nausea and motion sickness. It is most commonly used as pills, although it is also available in liquid form and in suppositories. Chemically, dimenhydrinate is a salt of two drugs: diphenhydramine, and 8-chlorotheophylline, a chlorinated derivative of theophylline. Chemical structures of three ingredients were represented in figures-1.26 to 1.28.

![Structure of 8-Chlorotheophylline](Fig-1.26)
Dimenhydrinate has been reported to be abused for non-medicinal purposes. Street drug users abuse DMH for the acute effects of euphoric sensations and hallucinations, while psychiatric patients abuse DMH for its anxiolytic or anticholinergic effects. Further support comes from reports of acute and chronic abuse of DMH by humans. Collectively, results confirm the abuse liability of DMH.

**Prescription:**

Dimenhydrinate is used to prevent and treat nausea, vomiting and dizziness caused by motion sickness. Dimenhydrinate is in a class of medications called antihistamines. It works by preventing problems with body balance.

Dimenhydrinate comes as a tablet and chewable tablet to take by mouth with or without food. To prevent motion sickness, the first dose should be taken 30 minutes to 1 hour before you travel or begin motion activity. Adults and children older than age 12 may usually take dimenhydrinate every 4 to 6 hours as needed to prevent or treat motion sickness. Children under age 12 may usually be given dimenhydrinate every 6 to 8 hours as needed to prevent or treat motion sickness.
Do not take more or less of it or take it more often than directed by the package labels.

**Other uses**

Dimenhydrinate is also sometimes used to treat Meniere's disease (condition of the inner ear which causes extreme dizziness, loss of balance, ringing in the ears, and hearing loss) and other inner ear problems.

**1.11 OBJECTIVE**

The main objective of this study is to develop the methods for pharmaceutical combination drug products by using high performance liquid chromatography (HPLC). Some of products were selected for this research work, the selected drug products are,

1. Losartan Potassium, Enalapril maleate and Hydrochlorothiazide combinations.
2. Metformin hydrochloride, Glipizide and Repaglinide pharmaceutical dosage forms.
3. Ornidazole, Ofloxacin and Cefixime combinations.
1.12 REFERENCES


15. The International Conference on Harmonization (ICH)-Validation of Analytical Procedures: Methodology (Q2B), Food and Drug Administration, USA, Nov. 1996.


