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To Develop, Validate and Apply a HPLC Method for the Analysis of Sodium Diclofenac

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Abstract: *HPLC is the predominant separation technique in contemporary pharmaceutical and biomedical analysis. A new reverse phase high performance liquid chromatographic method has been developed for the estimation of diclofenac sodium in pharmaceutical dosage forms using RPC-18 column. The mobile phase consisted of methanol and water in the ratio of 50: 50. Losartan potassium was used as internal standard. The detection was carried at 230 nm and the linearity was found to be in the range of 0.1-30 µg. The method was found to be simple, precise and reproducible.*

The objective of this project was to develop and validate a robust, accurate, and precise High-Performance Liquid Chromatography (HPLC) method for the quantitative analysis of [Drug Name or Compound] in [formulation matrix, e.g., pharmaceutical dosage form, biological matrix]. The method was designed to ensure reliable separation and quantification, in accordance with ICH Q2(R1) guidelines for analytical method validation.

The chromatographic separation was achieved using a [Column Type, e.g., C18 reversed-phase column] with a mobile phase consisting of [describe mobile phase composition, e.g., acetonitrile and phosphate buffer] in isocratic/gradient mode. The flow rate was maintained at [e.g., 1.0 mL/min], and detection was carried out at [e.g., 254 nm] using a UV detector.

Method validation parameters including specificity, linearity, accuracy, precision (intra-day and inter-day), limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability were evaluated. The method demonstrated good linearity over the concentration range of [range], with correlation coefficients (R^2) greater than [value, e.g., 0.999]. Recovery studies confirmed the method's accuracy, and %RSD values for precision studies were within acceptable limits, indicating high reproducibility.

This validated HPLC method is suitable for routine quality control analysis and can be applied for the assay and stability testing of [Drug Name or Compound] in bulk and finished dosage forms.

Keywords: Estimation, Diclofenac sodium, Losartan potassium and HPLC

I. INTRODUCTION

High performance liquid chromatography (HPLC) is the primary method used in the contemporary pharmaceutical industry. and essential analytical instrument used throughout the entire drug exploration, creation, and manufacturing.[1] HPLC is the preferred technique for determining a new chemical's peak purity entities, tracking changes in reaction is in synthetic processes or increase, assessing novel formulations and performing quality assurance and control on the finished medication goods.Attempting to isolate and quantify the primary drug, any reaction contaminants, any possible synthetic intermediates, and any degradants are the objectives of the HPLC process. [2]

High Liquid chromatography for performance is currently one of the most effective analytical chemistry tools.[3] It possesses the Capacity to distinguish, recognise, and measure the substances that can dissolve in any sample that contains them liquid. Extensivelyutilized for both qualitative and quantitative examination of the medicinal product and utilized to ascertain the drug product stability. [4] The solution of the sample is the HPLC principle. is introduced into a porous material column (stationary phase), and higher pressure is used to pump the liquid phase (mobile phase). pressure that passes through the column. The separation principle Afterward,towards stationary phase.

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Depending on the analyte's affinity for the present mobile phase, the gradient separates the analyte mixtures. The characteristics of the analyte and stationary phase influence the choice of solvents, additives, and gradient. The qualitative and quantitative makeup of the material being studied is ascertained using analytical chemistry. To comprehend the sample material, both of these elements are required. The two subfields of analytical chemistry are quantitative and qualitative. By determining whether components are present or absent, a qualitative analysis provides us with information about the sample's character. The relative amounts of one or more of these components are expressed numerically in a quantitative analysis. [5]

Chromatography

Chromatography is a technique that uses continuous distribution of the component between two phases to separate the components of a mixture. One phase (mobile phase) continuously passes over the other (stationary phase).The method by which solutes are separated by a differential migration process in a system comprising two or more phases, one of which moves continuously in a specific direction, is known as chromatography, according to USP [6].

chromatographyprinciple:

Adsorption Chromatography: This technique is used when the mobile phase is a liquid or gaseous phase and the stationary phase is a solid. Examples include gas-solid chromatography, thin-layer chromatography, and column chromatography.

Partition Chromatography: This technique is used when the stationary phase and mobile phase are both liquid .Examples include gas-liquid chromatography and paper partition chromatography.[7]

Introduction to chromatography

A process that uses differential movement in a two-phase system to separate and identify the components of a complicated mixture. The movement is influenced by the flow of a liquid or gas (mobile phase) that percolates through an adsorbent (stationary phase) or a second liquid phase. "Chromato" "graphy" is a combination of two words. Since chromo means colour and graphy means to write, colour bands are created throughout the measurement process. Orexamined. The separation of distinct chemicals results in the formation of these colour bands. Chromatography is frequently used in employed technique for identifying, separating, and determining the chemical constituents of complicated mixtures.[8,9]

Chromatography types

- Chromatography in columns
- The thin-layer chromatography method
- Chromatography by gas
- Exchange chromatography of ions
- Two-dimensional chromatography
- High performance (pressure) liquid chromatography
- High pressure thin layer chromatography
- Chromatography on paper

phases of chromatography:

Normal Phase Chromatography: In the Normal Phase mode, the mobile phase is non-polar and the stationary phase is polar. Non-polar chemicals elute first and move more quickly in this method. This is a result of the non-polar molecules' decreased affinity for the stationary phase. Polar compounds have a longer half-life. as they have a stronger affinity for the stationary phase. Consequently, these substances require more time to elute. For this reason, pharmaceutical applications typically do not use the normal phase mode of separation due to the fact the majority of medication molecules are polar and hence elute more slowly. [10]

ReversedPhaseChromatography: In the chemical, biological, pharmaceutical, food, and biomedical sciences, reversed phase chromatography is the most widely used method for analytical and preparatory separations of compounds of interest. In this method, the mobile phase is a polar solvent, and the stationary phase is a non-polar

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hydrophobic packing with an octyl or octa decyl functional group bound to silica gel. In this phase, non-polar molecules are held for a longer period of time while polar compounds elute first. Due to their polar nature, the majority of medications and pharmaceuticals elute more quickly since they are not kept for extended periods of time.Utilising secondary solute chemical equilibrium (ionisation control, ion suppression, ion pairing, and complexation) to regulate retention and selectivity is made possible by an aqueous mobile phase.[11]

Ion Exchange Chromatography: The stationary phase contains ionic groups like NR3+, SO3-which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention. Ion Pair Chromatography: This technique is also referred to as Reversed Phase Ion Pair Chromatography or Soap Chromatography. It may be used for the separation of ionic compounds and this method can also substitute for Ion Exchange Chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs (coulumbic association species formed between two ions of opposite electric charge) with suitable counter ions.

Affinity Chromatography: This method of separation makes advantage of very precise biological interactions. If certain steric and charge-related requirements are met, the sample can be absorbed by particular groups of molecules in the stationary phase. Proteins, enzymes, and antibodies can all be separated from complex mixtures using this method.

Size exclusion chromatography : Using size exclusion chromatography, molecules are separated based on their molecular masses. The smallest molecules elute last, followed by the largest molecules. When a combination contains substances with a molecular mass difference of at least 10%, this approach is typically employed.This technique is widely used for the molecular weight determination of polysaccharides. Ion exchange chromatography: In Ionexchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase.

Bio-affinity chromatography: Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. Proteins bound to a bioaffinity column.

COMPONENTS OF HPLC

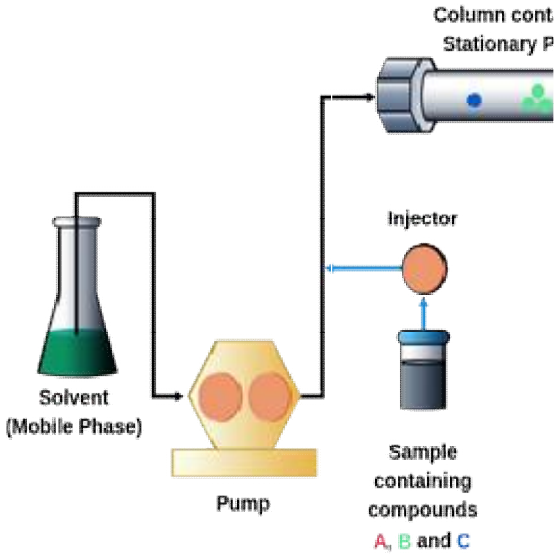

Figure 1. Components of an HPLC instrument

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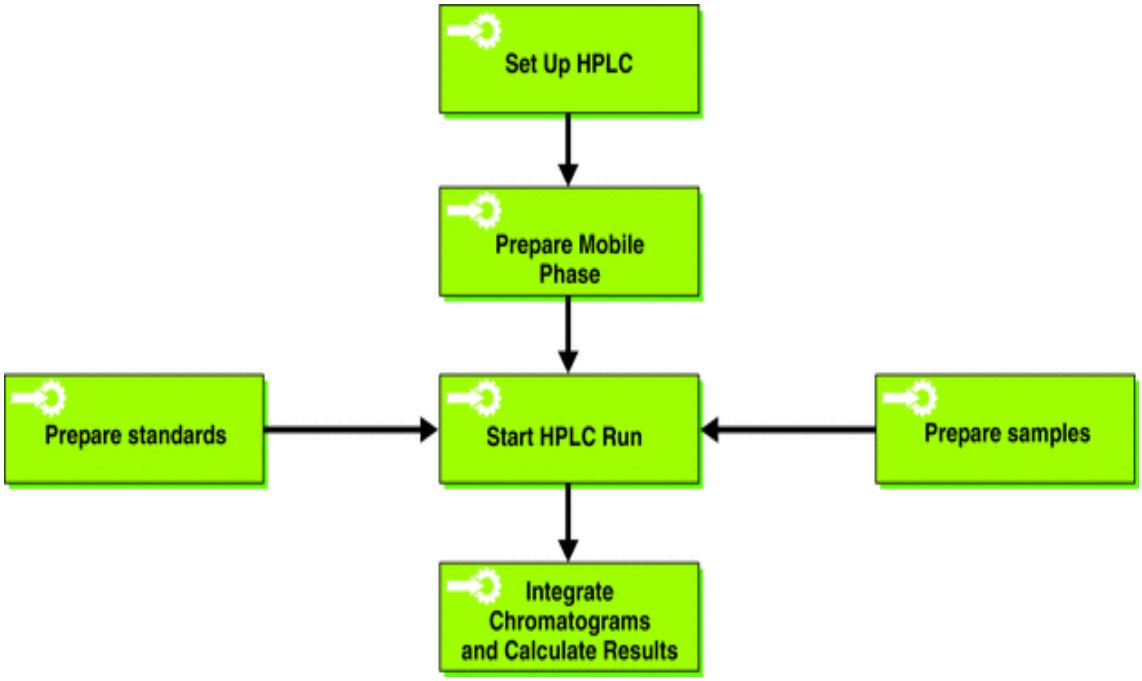

Figure 2. A process flow chart for an HPLC procedure

Components of an HPLC instrument

An infusion pump, a sampler, a chromatographic column, a detector, and a device for recording and processing data make up the majority of the HPLC system. Among these, the detector, chromatographic column, and infusion pump are essential parts. Furthermore, the pre-column or guard column, online degasser, autosampler, gradient elution device, and column temperature controller can all be set up to suit specific needs. For automated instrument control and data processing, modern HPLC devices are equipped with a microprocessor control system. An automatic fraction collecting device is part of the preparative HPLC apparatus.[12]

- Pump
- Mixing unit
- Solvent degassing
- Injector
- Column
- Detectors

The Pump

The development of HPLC led to the development of the pump system.

The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.

High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.

Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces pulses.

Injector

An injector is placed next to the pump.

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The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
The most widely used injection method is based on sampling loops.
The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

The separation is performed inside the column.
The recent columns are often prepared in instead of glass columns.
The packing material generally used is silica or polymer gels compared to calcium carbonate.
The eluent used for LC varies from acidic to basic solvents.
Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents

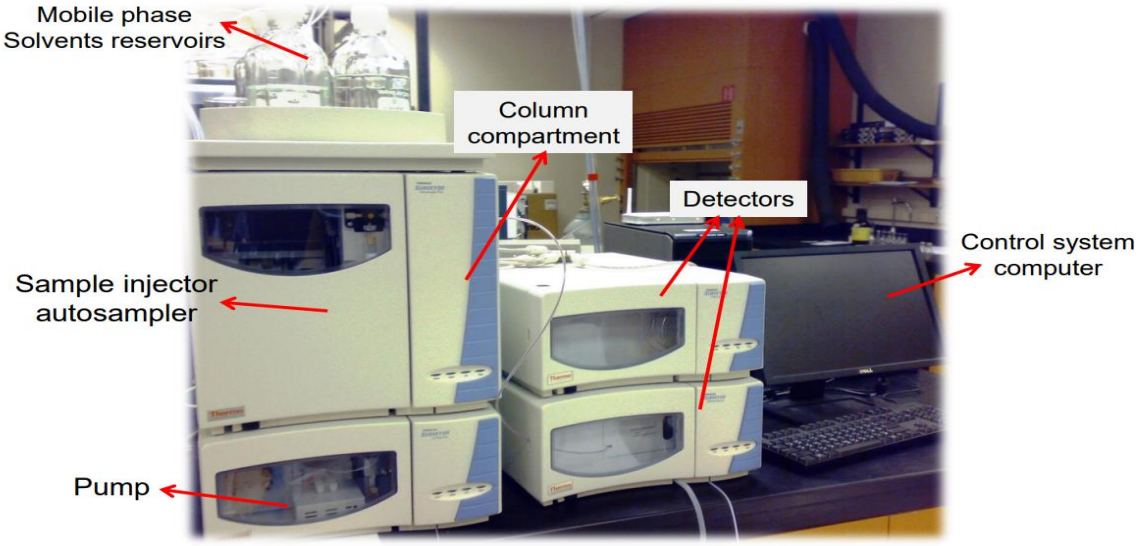

Fig.3 A HPLC Instrumentation

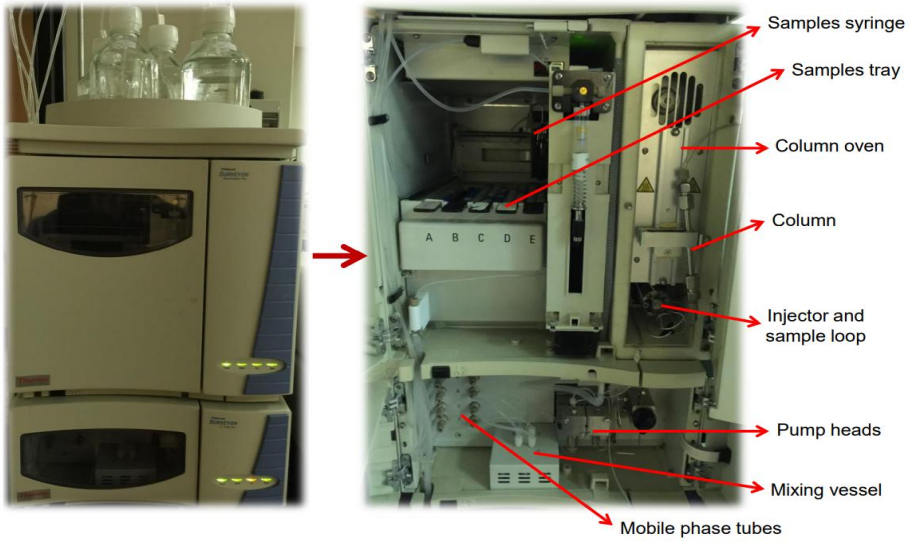

Fig. 3 B HPLC Instrumentation

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Column

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The recent columns are often prepared in instead of glass columns.
The packing material generally used is silica or polymer gels compared to calcium carbonate.
The eluent used for LC varies from acidic to basic solvents.
Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation. The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
This difference is monitored as a form of an electronic signal. There are different types of detectors available[13]

Sr.No.	Detector	Sample Size
1	UV/Vis detector	Response is due to the presence of light absorbing functional group in the analyte. In some cases the analyte can be derivatized to give a response.
2	Photo Diode Array Detector	This detects an entire spectrum simultaneously; Hence response can be checked for the most suitable wavelength without repeating the analysis. This detector is generally used for impurity detection or while conducting degradation studies
3	Fluorescence detector	As the name suggests is used for sample which are Fluorescent, Derivatization can be done in case of non Fluorescent analyte. The advantage of the method is its high sensitivity for selective groups of compounds at ~fg level
4	Chemilumniscence	Detector is similar to the Fluorescence detector however in this case the excitation of the analyte is initiated by a chemical reaction. This detector is even more sensitive than the Fluorescence detector
5	Optical Rotation detector	This detector is specific for the optical isomer measurement. The column can separate R- and L- type optical isomers and the detector can distinguish the same
6	Mass Spectroscopic Detector	Mass spectroscopy offers very high sensitivity and selectivity. Detection is based on fragmentation of molecules by electric fields and separation on basis of mass to charge ratios of fragmented molecules.
7	Refractive Index Detector	The principle of detection is measuring the change in RI due to the presence of the analyte. However, gradient programming is not possible while using the RI detector as there would be changes in refractive index of mobile phase with change in composition.
8	Electrochemical Detector	There are different types based on principles of amperometry,polarography,coulometry,and conductrometry .Like the RI detector gradient analysis is not possible with this detector. They are very selective and sensitive.
9	Evaporative Light Scattering Detectors	Light scattering detectors are useful for detection of high molecular weight molecules. It provides good sensitivity for non volatile analytes and can be used for gradient analysis unlike the RI

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		detector.
10	Multi-Angle Light Scattering Detector	For the SEC analysis, MW of analyte is estimated from the calibration curve drown using a set of known standards.
11	ICP detector	ICP-MS detection with reversed-phase (HPLC) is used for the detection of transition metals in pharmaceutical compounds..

Tab.No.1 Types Of Detectors

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation.The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.This difference is monitored as a form of an electronic signal. There are different types of detectors available[14].

Recorder

The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.When gas is present in the eluent, this is detected as noise and causes an unstable baseline.Degasser uses special polymer membrane tubing to remove gases.The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.

METHOD VALIDATION

The process by which laboratory tests demonstrate that an analytical procedure's performance characteristics satisfy the specifications for its intended usage is known as validation. The applicant's deliberate and methodical gathering of the validation data to support the analytical procedure is the first step in the methods validation process for analytical procedures [14–15]. Validation is required for all analytical techniques meant to be applied to the analysis of clinical samples. Analytical methods are validated in accordance with ICH criteria.Method validation components [15]

Typical analytical performance traits that could be examined during techniques validation include the following:

- Precision
- Repeatability
- Intermediate precision
- Linearity
- Detection limit
- Quantitation limit
- Specificity
- Range
- Robustness
- System suitability determination
- Forced degradation studies
- Solution stability studies

Accuracy: The degree to which a measured value resembles the true or recognised value is known as accuracy. The difference between the actual value and the mean value that was discovered is known as accuracy. It is ascertained by using the procedure on samples that have been treated with known concentrations of analyte. To make sure there is nointerference, these should be compared to blank and standard solutions. The accuracy is then computed as a

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percentage of the analyte recovered by the assay based on the test findings. The recovery by assay of known, additional amounts of analyte is a common way to express it [16].

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method [17]. It consists of two components: repeatability and intermediate precision. Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts [18-19] The precision is then expressed as the relative standard deviation. Accuracy and precision are not the same. A method can have good precision and yet not be accurate.

Repeatability : Variability experienced by a single analyst on a single instrument is known as repeatability. It does not differentiate between variance resulting from the sample preparation procedure and variation resulting from the instrument or system alone. Analysing several duplicates of an assay composite sample using the analytical approach ensures repeatability throughout validation.

Linearity: It computes the recovery value. The capacity of an analytical process to produce a response that is exactly proportionate to the analyte concentration (amount) in the sample is known as linearity. The test results are directly or through a precise mathematical transformation proportionate to the analyte concentration in samples within a specified range if the procedure is linear. The confidence interval surrounding the regression line's slope is typically used to express linearity.

Detection Limit: The lowest concentration of analyte in a sample that can be identified but not always quantified as an exact value is known as the detection limit (DL) or limit of detection (LOD) of a particular process. The signal-to-noise (S/N) ratio (3:1), which is typically represented as the analyte concentration in the sample, can serve as the basis for the LOD in analytical methods that display baseline noise.(textbook) The following formula determines the signal-to-noise ratio: s = H/h where H is the component's equivalent peak height. h is the absolute value of the biggest noise variation from the blank solution's chromatogram baseline.

Quantitation Limit: The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the limit of quantitation (LOQ) or the quantitation limit of a certain analytical process. The LOQ is often estimated from a determination of the S/N ratio (10:1) for analytical processes like HPLC that exhibit baseline noise. This is typically confirmed by injecting standards that provide this S/N ratio and also have an acceptable percent relative standard deviation.

Specificity : The ability to clearly evaluate the analyte in the presence of potentially present components, such as excipients, degradation products, and contaminants, is known as specificity. Separation is not always necessary; specificity measures only the desired component without interference from other species that may be present.

Range: is the distance between the sample's highest and lowest analyte concentrations for which it has been shown that the analytical process has an adequate degree of linearity, precision,,accuracy.

robustness : The capacity of an analytical technique to withstand minor but intentional changes in method parameters (such as pH, mobile phase composition, temperature, and instrumental settings) is known as robustness, and it gives an indication of how reliable the method is under typical operating conditions. The methodical process of changing a parameter and assessing the impact on the approach through sample analysis and/or system appropriateness monitoring is known as robustness determination.

System Adequacy Determination : is the assessment of an analytical system's constituent parts to demonstrate that the system's performance satisfies the requirements set forth by a method [19]. A quantitative system suitability test report can be produced by experimentally calculating these parameters: Resolution, tailing, separation (relative retention), capacity factor, and number of theoretical plates (efficiency)

Studies on Forced Degradation: Studies on stress or forced degradation are thought to purposefully break down the sample. Through the generation of probable degradation products, these experiments have been used to assess an analytical method's capacity to measure an active ingredient and its degradation products without interference. The drug substance was exposed to acid, base, heat, light, and oxidising agents during method validation, resulting in a 10% to 30% degradation of the active ingredient. Information regarding potential degradation products and mechanisms during storage may also be obtained from the studies. In order to increase the stability of drug products, these studies may also

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aid in formulation development, manufacturing, and packaging. Forced degradation studies are conducted for a variety of reasons, such as identifying drug degradation pathways and developing and validating stability-indicating methodologies.

Solution Stability Studies : During validation the stability of standards and samples is established under normal conditions, normal storage conditions, and sometimes in the instrument to determine if special storage conditions are necessary, for instance, refrigeration or protection from light [21-22].

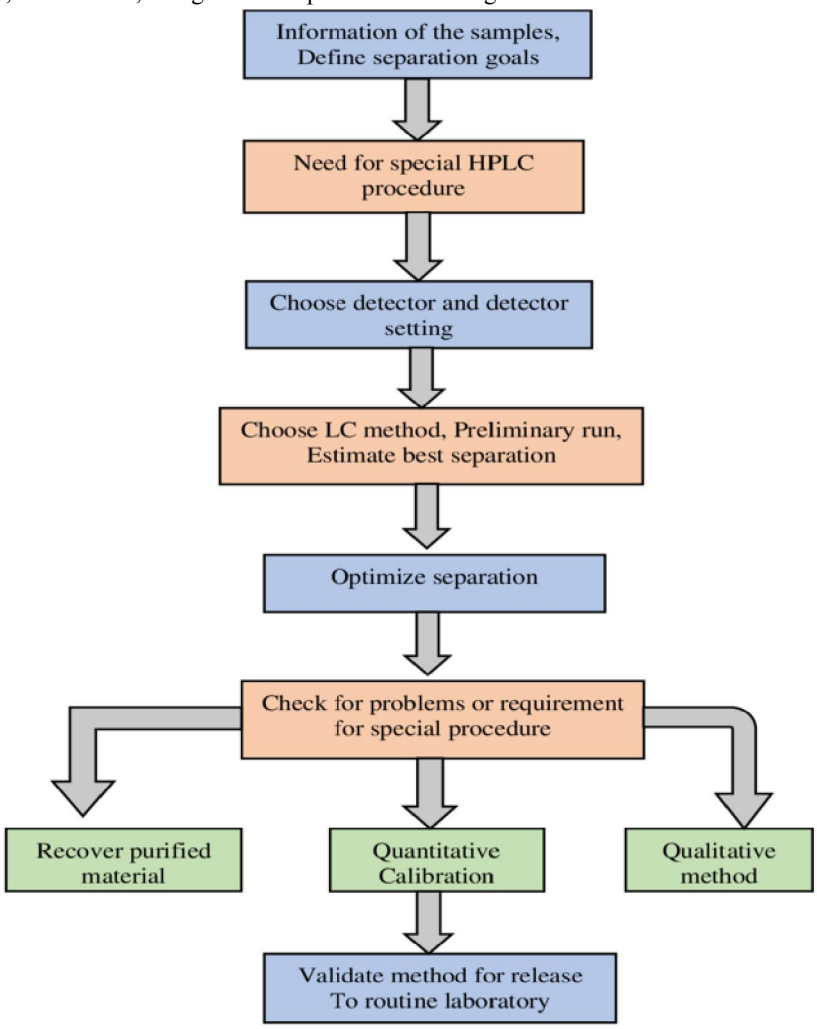

Fig.3 Steps involved in HPLC method development

Drug physicochemical characteristics: When developing a method, a drug molecule's physicochemical characteristics are crucial. Prior to developing a method, one must examine the drug molecule's physical characteristics, including its solubility, polarity, pKa, and pH. A compound's polarity is one of its physical characteristics. It helps an analyst identify the mobile phase and solvent. composition. In a non-polar covalent connection, two atoms share an equal number of electrons. A covalent bond that is polar is characterised by one atom's greater attraction to electrons than of the other. Molecule polarity can be utilised to clarify solubility of molecules. Non-polar solvents like benzene and polar solvents like water don't mix. Generally speaking, The fact that like dissolves like indicates that ingredients.

Chromatographic condition selection: A set of beginning conditions (column, mobile phase, and detector) is chosen at the initial stages of technique development.These are typically based on re-versed-phase separations on a UV-

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detection C18 column. At this stage, a choice should be taken about the development of an isocratic or gradient approach.[18]Its Includes :

Selection of chromatographic conditions: A set of starting conditions (column, mobile phase, and detector) is chosen during the first stages of technique development. Typically, these rely on re-versed-phase separations on a UV-detected C18 column. This is the time to decide whether to design a gradient approach or an isocratic method.(2 Column selection)The core of an HPLC system is the column. The biggest influence on analyte resolution during method development will come from altering a column. Good chromatographic separation and accurate, dependable analysis can be obtained with the right column selection. Confusion, challenges, and inadequate separations can often emerge from the improper use of a column, which can provide results that are difficult to understand or invalid. Silica is the most often used matrix for HPLC columns. Silica matrices come in standard sphere sizes, are robust, and are easily derivatised.[23, 24]

Choosing the Chromatographic Mode The analyte's subatomic weight and extremity determine the chromatographic modes. Every contextual study will concentrate on the most widely utilised technique for tiny natural atoms, reversed-phase chromatography (RPC). RPC frequently isolates ionisable mixtures (bases and acids) utilising ion-pairing reagents or portable stages (to maintain the analytes in a nonionized state).[25]

Choosing a buffer : The intended pH determines the choice of buffer. On silica-based packing, the pH range for the reversed phase is normally 2 to 8. Since buffers regulate pH best at their own pH, it is crucial that the buffer's pKa is near the target pH. pKa. Generally speaking, you should choose a buffer whose pKa value is less than two units higher than the pH of the intended mobile phase.[26]

General factors to take into account while choosing a buffer:

Methanol and water dissolve phosphate more readily than acetonitrile or THF do.

Certain salt buffers exhibit hygroscopicity. This could result in chromatographic alterations (increased tailing of basic substances, and even variations in selectivity).

In general, ammonium salts dissolve better in Water and organic mobile phases TFA is volatile, absorbed at low levels, and can deteriorate with time. UV radiation. In buffered mobile environments, microbial growth can happen rapidly. stages with minimal or nonexistent organic modifiers. This On column inlets, growth will build up and may cause damage.

At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7 .

The concentration of buffer : A buffer value of 10–50 mM is typically adequate for tiny compounds. A buffer shouldn't contain more almost half organic content. The particular buffer and its concentration will determine this. The most common buffer systems for reversed-phase Phosphoric acid and its potassium or sodium salts are known as HPLC. Whentesting organophosphate chemicals, sulfonate buffers can be used instead of phosphonate buffers.[27]

Choosing the mobile phase : The choice of the mobile-phase and gradient conditions is influenced by the analyte's ionogenicity and the mixture's analytes' hydrophobicity, respectively. Acidic analytes won't change in buffers that are appropriately low pH, which enhances retention. Neutral basic substances are better preserved at higher pH values, whereas ionised acidic ones elute more quickly. When a chemical's pKa is comparable to that of the buffer and the analyte, peak splitting may happen. Both a charged and an uncharged species are eluted. The retention of non-ionizable substances is not significantly affected by a buffer's pH. components of the sample. Tetrahydrofuran (THF), methanol (MeOH), andacetonitrile(ACN) are often utilised solvents in Low UV cut-offs for RP-HPLC are 190, 205, and 212 nm, respectively. These These solvents are miscible with water. Mixture of acetonitrile and water is the best initial choice for the mobile phase during method development.[28, 29]

Choosing a detector : One crucial part of HPLC is the detector. The choice of detector is influenced by the chemical makeup of the analysts, possible interference, the necessary detection limit, and the availability and/or cost of the detector. The A dual-wavelength absorbance detector for HPLC is the UV-Visible detector. The high sensitivity is provided by this detector. essential for common UV-based uses like the detection and measurement of impurities. Array of Photodiodes (PDA) For Waters analytical HPLC, preparative HPLC, or LC/MS system solutions, the detector provides sophisticated optical detection. Its cutting-edge optics and software offer excellent chromatographic and

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spectral sensitivity. The index of refraction (RI) Because of its high sensitivity, stability, and reproducibility, the detector is a good choice for analysing parts with minimal or nonexistent UV absorption. [30].

Creating the analysis methodology : Selecting different chromatographic parameters, including the mobile phase, column, mobile phase flow rate, and mobile phase pH, is the first step in creating an RP-HPLC analytical procedure. Each of these elements is established by testing and thereafter contrasted with the parameters of system appropriateness. A retention period longer than 5 minutes, more than 2000 theoretical plates, less than two tailing factors, more than five resolutions, and a Typical systems have an analyte peak area R.S.D. of no more than 2.0 percent in standard chromatograms. qualities of appropriateness. The detection wavelength used for estimating two components at once is usually an [31], isosbestic point

Preparing the sample: In HPLC analysis, sample preparation is essential because it guarantees that the solution is homogeneous and reproducible enough to be placed on the column. The goal of sample preparation is to create an aliquot of the sample that is compatible with the intended HPLC technique, free of interferences, and won't damage the column, which suggests that neither sample retention nor resolution will be impacted by the sample solvent dissolving in the mobile phase. An example Sample collection is the first step in preparation, while sample injection onto the HPLC column is the last step [32]

Mobile phase :

Type of solvent: Type of solvent (tetrahydrofuran, acetonitrile, and methanol) will impact the selectivity. The decision to use methanol orThe solubility of the analyte may affect acetonitrile. together with the buffer that was used. The least polar is tetrahydrofuran. of these three solvents, frequently accountable for significantSelectivity shifts and is incompatible with the The majority of pharmaceuticals require low-wavelength detection. substances. The mobile phase has an impact on selectivity, resolution, and effectiveness. In chromatography in reverse phase, the mobile phase includes a non-UV active ingredient and an aqueous buffer. organic solvent that is miscible with water.

The analysis of the drug molecule will be impacted by the effects of the organic and aqueous phases as well as the ratios in which they are combined. Choosing the gradient and mobile-phase conditions are reliant on the The analyte's ionogenic properties and its hydrophobicity the mixture'sanalytes, accordingly. The water-based buffer fulfils a number of functions. When the pH is low, the mobile phase lowers the peak by protonating free silanols on the column. dragging. Basic analytes are protonated at low enough pH levels. The analyte will elute faster when ionised, but with enhanced peak form. Analytes that are acidic in buffers of A pH that is low enough will not change, rising retention. On the other hand, neutral basic substances at higher pH will be better maintained, and acidic substances will be ionised.

If the analyte elutes as both a charged and an uncharged species and the compound's pKa is comparable to the buffer's pKa, peak splitting may be seen. The pH of a buffer will not significantly impact non-ionizable sample elements. A 10–50 mM aqueous buffer solution is usually used. H3PO4 is the aqueous phase most frequently utilised in phosphate buffer, or water. Phosphate buffers have a pH of simple to modify using mono-, di-, or tribasic phosphate Salts. However, the solution changes when phosphate salts are added. should use a 0.22µm filter to get rid of insoluble particles. filter paper. Other acids and bases that are not UV active could likewise be utilised to alter peak retention and form.

mobile phase's pH There is no separation when the material is eluted using a 100% organic mobile phase because it is eluted in the volume of vacuum. The reason for this is that the sample is not kept, rather retention is seen when the solvent strength of the mobile phase is reduced to enable the solute's equilibrium competition. molecules separating the mobile phase from the bound phase .

When there is a complicated separation, meaning that several When the solvent is ready, components must be separated. strength is reduced, and no agreement has been reached between Another organic solvent of a different kind with two close peaks It can be necessary to use polarity or perhaps a combination of two organics. attempted to create isolation. Further more, the mobile phase When combined with bonded phase optimisation (i.e., replacing C18/C8 with cyano or phenyl), optimisation can be improved. A target for a solute's band spacing (K') should have a runtime of roughly fifteen minutes and be between four and nine. Alternatively, for the most common product rollout, 20 minutes at most, stability.

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Isocration Separations : Constant eluent composition and isocratic separations denotes the column's equilibrium conditions and the actual Compounds travelling through the column at a certain velocity include constant; analyte-stationary-phase and analyte-eluent interactions are also persistent durithe complete run. This increases the predictability of isocratic separations, while the separation power, or thquantity of substances that might be resolved) isn't particularly high. There has a limited peak capacity, and the longerThis makes the use of a gradient necessary. The chromatographic selectivity can be varied by using either shallow or high gradients. This would be explained by the retention's varying slopes. vs the mixture's organic makeup for every analyte. When using a gradient approach, the column needs to be permitted to reach equilibrium under the initial mobile-phase conditions before the beginning of the subsequent gradient and the subsequent sample injection Run. Choosing an isocratic or gradient mode is contingent upon the number of ongoing issues that need to be fixed or divided. An first gradient run is used to determine whether a gradient is necessary or if isocratic mode would suffice. conducted, as well as the proportion between the overall gradient duration and The gradient time difference between the initial and final Components are calculated

Introduction of Diclofenac Sodium

Diclofenacsodium [43] is a non-steroidal anti-inflammatory, analgesic
and antipyretic agent with minimal side effects. Chemically it is sodium [2-(2, 6-dichloro anilino) phenyl]-acetate. It is commonly prescribed for the treatment of inflammatory condi-tions associated with rheumatoid arthritis. So far two HPCL methods [44-45] one GLC method [46] and few spectrophotometric methods [47-48] have been reported for the estimation of diclofenac sodium alone. The present study is aimed at developing a sensitive, specific, precise and accurate HPLC method for the estimation of diclofenac sodium in pharamaceutical tablet dosage forms using losartan potassium as an internal standard

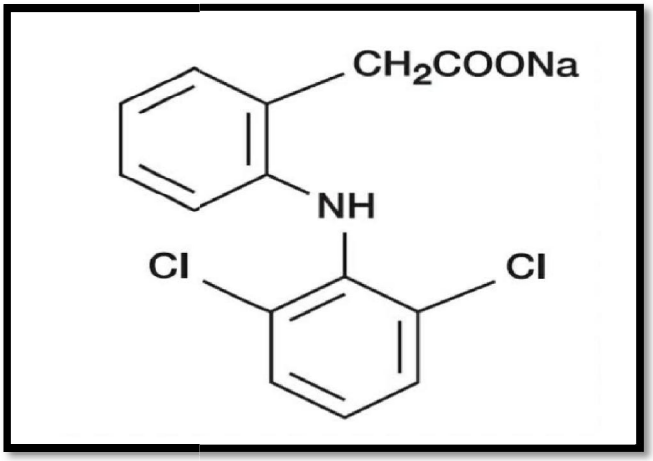

Fig. Structure of Diclofenac Sodium

Aim and objectives

Aim:

To develop, validate, and apply a HPLC method for the analysis of sodium diclofenac.

Objectives

Development a robust and reliable HPLC method for quantifying sodium diclofenac in pharmaceutical formulations.

Validate the HPLC method according to regulatory guidelines to ensure its accuracy, precision, specificity, and sensitivity.

Evaluate the method's performance in terms of linearity, range, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy.

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Demonstrate the method's suitability for routine quality control and assurance of sodium diclofenac products.

LITERATURE SURVEY

1.Mustafa Çelebier, Tuba Reçber, RP-HPLC method development and validation for estimation of rivaroxaban in pharmaceutical dosage forms, Brazilian Journal of Pharmaceutical Sciences 49, 359-366, 2013

Abstract: Rivaroxaban, an anti-clotting medication, acts at a crucial point in the blood-clotting process and stops the formation of blood clots. In this study, RP-HPLC method was developed for the determination of rivaroxaban in tablets (Xarelto® (10 mg)). Phenomenex Luna 5 µm C18 100 Å LC Column (250 x 4.6 mm) was used at 40 ºC. Isocratic elution was performed with ACN:Water (55:45 v/v) mixture. The flow rate was 1.2 mL min-1 and UV detection was at 249 nm. Internal standard (Caffeine) and rivaroxaban were eluted within 2.21 and 3.37 minutes, respectively. The developed method was validated according to the ICH guidelines and found to be linear within the range 0.005 - 40.0 µg mL-1. The method was accurate, precise, robust and rapid. Thus, it was applied successfully for the quality control assay of rivaroxaban in tablet dosage form.

2. V Ganjiwale, AP Dewani, AV Chandewar, A comprehensive overview of HPLC method development and validationJ Pharm Sci 2, 1, 2024

Abstract: -Performance Liquid Chromatography (HPLC) is an analytical technique employed for the separation of solutes based on their differential rates of elution within a chromatographic column. This separation method hinges on the distribution of solutes between the mobile phase and the stationary phase. The HPLC instrumentation comprises eight fundamental components: a mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing, and a computer, integrator, or recorder [1]. Achieving successful outcomes with HPLC involves optimizing various operating conditions, including the type of column packing and mobile phase, column length and diameter, mobile phase flow rate, column temperature, and sample size

3.Pasha Latha E.and Sailaja B.Ranjit singh, Bioanalytical Method Development and Validation by HPLC: A Review,2014

Abstract: The development of selective, sensitive and reliable bioanalytical methods for the quantitative evaluation of drugs and their metabolites in biological matrices is crucial for the successful drug development. The data obtained from these methods is required in the pharmacokinetic and toxicokinetic studies of investigational new drug applications (INDs), new drug applications (NDAs)and abbreviated new drug applications (ANDAs). The results of animal toxicokinetic studies and of human clinical trials, including bioavailability and bioequivalence studies requiring pharmacokinetic evaluation are used to make critical decisions supporting the safety and efficacy of a drug. Therefore, it is of paramount importance that the developed bioanalytical methods are well designed, adequately validated and documented to a satisfactory standard to apply in drug analysis in order to obtain reliable results. High pressure liquid chromatography is a versatile analytical tool useful in identification and quantitative estimation of low concentration of drugs and metabolites in biological matrices. So it is advantageous to develop and validate bioanalytical HPLC method for low dose drugs. This article reviews current progress in HPLC based bioanalytical method development and validation of different drugs. So, far drugs like Anti malarials, Omeprazole, Clofarabine, Palonosetron HCl, etc have been analyzed bioanalytically.

4.HPLC method development and validation-an overview, Journal of Pharmaceutical Education & Research 4 (1), 2013

Abstract: HPLC method development and validation play important role in the discovery, development and manufacture of pharmaceutical products. This article mainly focuses on the optimization of HPLC conditions and other important perspectives during method development and validation. Various critical steps related to analytical method development and validation is discussed. A sequence of events required for method development and analytical validation are described. The steps involved in developing a stability-indicating HPLC method influences the analysis of degradation products/impurities in stability study and its validation demonstrate the suitability for its intended purpose.

5.N A espthein , validation of HPLC technique for pharmaceutical analysis, 14, Vol. 38, No. 4, 2014

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Abstract: Validation (evaluation of suitability) of an analyticaltechnique is a procedure aimed at obtaining experimentallyjustified evidence of the ability of this technique to give re-sults characterized by the required accuracy and All analytical techniques used for the developmentof pharmaceuticals and for the determination of their qualitycharacteristics have to be validated. In the case of usingmethods stipulated and described in the State Pharmaco-poeia, it is not necessary to evaluate their suitability, pro-vided that the analyses are conducted with strict observation of the text of each particular article. In most other cases, es-pecially in cases of modification of the drug composition, thescheme of synthesis, or the analytical procedure, it is neces-sary to re-evaluate the suitability of the analytical techniques.

6.SL Patwekar, RS Sakhare, NN Nalbalwar, HPLC method development and validation-A general Concept,International Journal of Chemical and Pharmaceutical Sciences 6 (1), 8-14, 2015

Abstract: Chromatography, although primarily a separation technique, is mostly employed in chemical analysis. In which High-performance liquid chromatography (HPLC) is an extremely versatile technique where analytes are separated by passage through a column packed with micrometer-sized particles. Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass. This article involves the strategies and the issues for designing HPLC method development and validation. The method development often follows the well established steps, like selection of buffer, selection mobile phase, selection of column. The method so developed should be as simple as possible, the best strategy being some blend of theoretical and empirical approach.

7.**pawan Tiwari, BK Singh, HPLC: a modern approach of development and validationWorld J Pharm Res 5, 1616-31, 2016**

Abstract : High performance liquid chromatography is the dominant analytical technique which is Proficient to separate, detect and quantify various drugs and its related degradants. HPLC methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs and various other studies related to humans and animals. Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. A number of chromatographic parameters were evaluated in order to optimize the method.

8.Vidushi, Bharkatiya Meenakshi, M Bharkatiya, A review on HPLC method development and validation,*Res J Life Sci, Bioinform, Pharm Chem Sci 2 (6), 178, 2017*

Abstract: is the dominant separation technique to detect, separate and quantify the drug. A number of chromatographic parameters were analyzed to optimize the method like sample pretreatment, choosing mobile phase, column, detector selection. The objective of this article is to review the method development, optimization and validation. HPLC method development depends on chemical structure of the molecules, synthetic route, solubility, polarity, pH and pKa values, and functional groups activity etc. Validation of HPLC method as per ICH Guidelines gives information regarding various stages and knowing characteristics like Accuracy, specificity, linearity limit of detection, limit of quantificationmar

9.Nageswara Rao Ramisetti,,An overview of experimental designs in HPLC methoddevelopment and validation, *Journal of pharmaceutical and biomedical analysis 147, 590-611, 2018*

Abstract : Chemometric approaches have been increasingly viewed as precious complements to high performance liquid chromatographic practices, since a large number of variables can be simultaneously controlled to achieve the desired separations. Moreover, their applications may efficiently identify and optimize the significant factors to accomplish competent results through limited experimental trials. The present manuscript discusses usefulness of various chemometric approaches in high and ultra performance liquid chromatography for (i) methods development from dissolution studies and sample preparation to detection, considering the progressive substitution of traditional detectors with tandem mass spectrometry instruments and the importance of stability indicating assays (ii) method validation through screening and optimization designs.

10. Mohan Varma, Ashok Thulluru, KT Sunil Kumar, HPLC method development andvalidation: A review, *World J Pharm Res 10, 405-426, 2021*

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Abstract : High performance liquid chromatography the most precise techniques commonly used for the qualitative and quantitative study of drug substance. Drug research, drug production, and pharmaceutical manufacturing all depend on the development and evaluation of analytical methods. It involves evaluating a drug substance's purity and toxicity. To refine the methods in the study of system production in HPLC, a variety of chromatographic parameters were evaluated. We create an effective mobile phase, column, column temperature, wavelength, and gradient.

PLAN OF WORK

1: Literature Review and Method Development

1. Conduct a literature review of existing HPLC methods for sodium diclofenac analysis.
2. Develop a new HPLC method or modify an existing one based on the literature review.
3. Optimize the chromatographic conditions (e.g., column, mobile phase, temperature).

Phase 2: Method Validation

1. Validate the HPLC method according to regulatory guidelines (e.g., ICH, USP).
2. Evaluate the method's specificity, linearity, accuracy, precision, and sensitivity.
3. Determine the method's limit of detection (LOD) and limit of quantification (LOQ).

Phase 3: Method Application

1. Apply the validated HPLC method to analyze sodium diclofenac in pharmaceutical formulations.
2. Evaluate the method's performance in real-world samples.
3. Compare the results with existing methods (if applicable).

Phase 4: Documentation and Reporting

1. Document the method development, validation, and application procedures.
2. Prepare a detailed report of the results and findings.
3. Discuss the implications of the results and potential future work.

EXPERIENTIAL PART

Equipment-

1. HPLC instrument: A high-performance liquid chromatograph with a suitable detector (e.g., UV-Vis, PDA, or MS).
2. Column: A suitable HPLC column (e.g., C18, C8) with a compatible stationary phase.
3. Autosampler: An autosampler for injecting samples into the HPLC instrument.
4. Detector: A detector suitable for sodium diclofenac analysis (e.g., UV-Vis detector).

Additional Equipment

1. Balance: An analytical balance for weighing samples and standards.
2. Pipettes: Pipettes for accurately measuring and transferring small volumes of liquids.
3. Volumetric flasks: Volumetric flasks for preparing standards and sample solutions.

Chemicals

1. Sodium diclofenac standard- High-purity sodium diclofenac standard for method validation and calibration.
2. HPLC-grade solvents+High-quality solvents suitable for HPLC analysis, such as:
   - Acetonitrile
   - Methanol
   - Water
3. Mobile phase buffers- Buffers for preparing the mobile phase, such as:
   - Phosphate buffer (e.g., potassium dihydrogen phosphate, disodium hydrogen phosphate)
   - Acetate buffer (e.g., acetic acid, sodium acetate)
4. pH adjusters-Chemicals for adjusting the pH of the mobile phase, such as:
   - Orthophosphoric acid
   - Sodium hydroxide
5. Sample preparation reagents: Reagents for preparing samples, such as:
   - Solvents (e.g., methanol, acetonitrile)

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- Dilution buffers (e.g., phosphate buffer)

Additional Chemicals

1. Internal standard- A suitable internal standard for method validation and quantification (if required).
2. Quality control samples- Quality control samples containing known concentrations of sodium diclofenac.

Chromatographic conditions:

The optimized chromatographic conditions were as follows:
Mobile phase- Methanol: Water (50:50)
Column- ODS C-18 (4.6 mm I.D. x 25 cm length)
Flow rate- 0.7 mL/min
Detection- UV set at 230 nm
Injection Volume-20 µL
Temperature- Ambient
Retention time
of drug – 7.4
of L.S.- 5.57
Run time -10 min

METHODS:

About 100 mg of diclofenac sodium was accurately weighed and dissolved in 100 mL of T.D. water so as to give 1 mg/mL solution. Subsequent dilutions of the solution were made after addition of losartan potassium as an internal standard to get concentrations of 0.1 to 30 µg/mL of diclofenac sodium and 1 µg/mL of internal standard in each dilution. The standard solutions prepared above were injected three times into the column at a flow rate of 0.7 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentration was calculated. The regression equation of drug concentration over the ratio of drug peak area to that of internal standard was obtained.

Diclofenac sodium solutions containing 0.1 to 30 µg/mL were subjected to the proposed HPLC analysis for finding out the intra and inter day variations. The recovery studies were carried out by adding known amounts (10 µg and 20 µg) of the drug to the preanalysed samples and subjecting them to the proposed HPLC method.

Estimation of diclofenac sodium in tablet dosage forms: Contents of twenty tablets
containing diclofenac sodium were pooled, powder equivalent to 25 mg of diclofenac sodium was accurately weighed and placed in a 25 mL volumetric flask. Diclofenac sodium was extracted in to methanol and the volume was adjusted to 25 mL, mixed and filtered through a 0.45 µ filter. From the filtrate, 0.1 mL was pipetted in to a 10 mL graduated test tube and spiked with the required aliquot of internal standard solution and then the volume was adjusted to 10 mL with the mobile phase such that the concentration of internal standard in each sample was 1.0 µg/mL and this solution (20 µL) was injected five times into HPLC column to obtain the chromatogram. The mean concentration of diclofenac sodium corresponding to the ratio of AUC of diclofenac sodium to the AUC of internal standard was calculated from the standard graph. The same procedure was followed for the estimation of diclofenac sodium in three different brar.ds of tablet dosage forms.

II. RESULTS AND DISCUSSION:

The present investigation was carried out to develop aspecific, sensitive, precise and accurate HPLC method for the analysis of diclofenac sodium. The column pressure varied from 135 to 145 Kgf/cm². The retention time for drug (diclofenac sodium) and internal standard (losartan potassium) were 7.60 and 4.57 min respectively. Each of the samples was injected three times and almost the same retention times were observed in all the cases. The ratio of peak area of drug to that of internal standard for different concentrations set up as above were calculated and the average values for such determinations are shown in Table 1. The peak areas of both drug and internal standard were reproducible as indicated by low coefficient of variation (3.22%). A good linear relationship (r=09978) was observed between the concentration of drug and the respective ratio of peak areas. The calibration graph was found to be Y=0.0919+0.618X (where Y ratio of peak area of drug to that of internal standard, X is the concentration of drug in the

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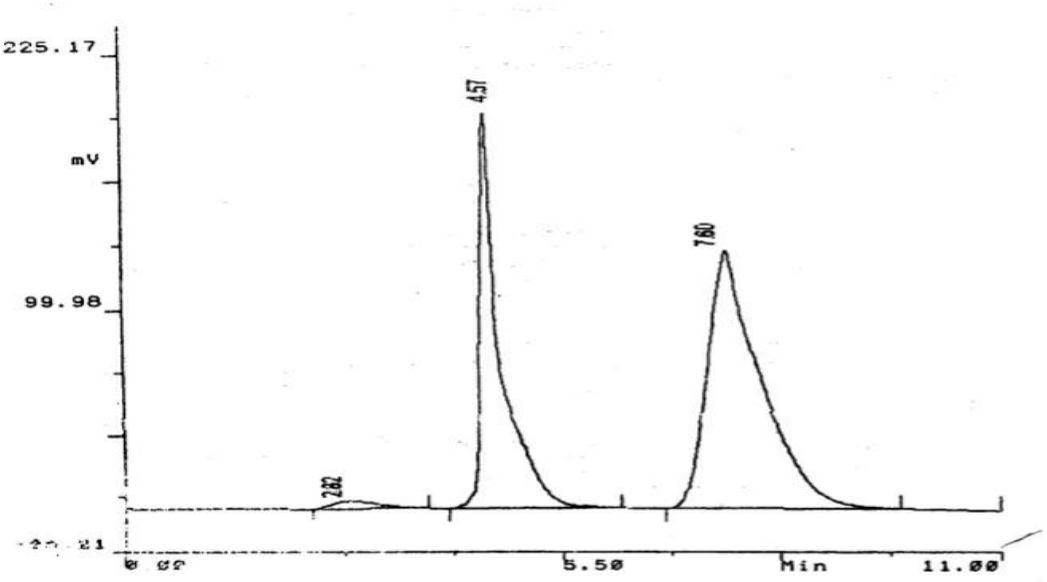
range of 0.1 to 30 µg/mL). When diclofenac sodium solutions containing 10 µg/mL and 30 µg/mL were analyzed by the proposed HPLC method for finding out intra and inter day variations a low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The amounts of diclofenac from the preanalyzed samples containing known amounts of drug are shown in Table 3. About 99.96% of diclofenac sodium could be recovered from the preanalyzed samples indicating high accu-racy of proposed method.



Chromatogram of Diclofenac Sodium

Table no- 2 Calibration of the HPLC method for the estimation of diclofenac sodium.

Concentration of diclofenac sodium (mg/ml)	Mean (s.d.) of AUC of drug to internal standard (n=5)	% CV
0	0	0
0.1	0.165	2.31
0.2	0.32	1.27
0.4	0.68	1.7
0.8	1.21	1.69
1	1.18	2.60
2	3.57	1.97
4	5.81	1.47
8	11.94	3.22
20	29.75	2.19
30	45.78	3.19

The drug content in the tablet was quantified using the proposed analytical method. The mean amount of diclofenac sodium in three different brands of tablet dosage forms is shown in Table 3

Table no 3 Assay of different brands of diclofenac sodium tablets.

Brand	Labelled amount of drug (mg)	Mean (s.d.) amount (mg) found (5)	Mean (s.d.)% labelled amount (n=5)	% CV
1	50	49.89±0.21	99.78±0.08	1.89
2	50	49.76 ± 0.19	99.52 ± 0.12	1.36

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3	50	50.09 ± 0.05	100.18±0.21	1.92

The tablets were found to contain 99.52 to 100.18% of the labelled amount. The low percent of CV indicates the reproducibility of the assay of diclofenac sodium in the tablet dosage forms. The proposed method was found to be simple, precise, highly accurate, specific, eco-nomical and less time-consuming. Hence this method can be employed to estimate diclofenac sodium in tablet dosage forms effectively.

III. CONCLUSION

A robust and reliable HPLC method was successfully developed and validated for the analysis of sodium diclofenac.

The method demonstrated excellent specificity, linearity, accuracy, precision, and sensitivity, making it suitable for routine quality control and assurance of sodium diclofenac products. The method's applicability was demonstrated by analyzing sodium diclofenac in various pharmaceutical formulations.

The results of this study indicate that the developed HPLC method is a valuable tool for ensuring the quality and efficacy of sodium diclofenac products

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