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DEVELOPMENT AND VALIDATION OF A HPLC METHODS FOR DETERMINATION OF DEXIBUPROFEN IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

This review presents the development and validation of a high-performance liquid chromatography (HPLC) method for the accurate determination of Dexibuprofen in pharmaceutical preparations. Dexibuprofen, the active enantiomer of ibuprofen, is known for its enhanced therapeutic efficacy and reduced adverse effects. The developed method involved the optimization of chromatographic conditions using a C18 column with a mobile phase composed of methanol and phosphate buffer at pH 3.0, pumped at a flow rate of 1.0 mL/min. Dexibuprofen was detected at a wavelength of 220 nm. The method was validated according to ICH guidelines for parameters including specificity, linearity, precision, accuracy, robustness, and system suitability. The method exhibited excellent linearity (R2 > 0.999) over the concentration range of 5-100 µg/mL with high precision and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.5 µg/mL and 1.0 µg/mL, respectively. The proposed method was successfully applied for the determination of Dexibuprofen in commercial pharmaceutical formulations, demonstrating its suitability for routine quality control analysis. Overall, the developed HPLC method offers a reliable and sensitive approach for the quantification of Dexibuprofen in pharmaceutical preparations, ensuring quality and consistency in drug formulations.

KEYWORDS: *Dexibuprofen, High-performance liquid chromatography (HPLC), Pharmaceutical preparations, Method development, Method validation, Quantification, C18 column, Methanol-phosphate buffer, Specificity, Linearity.*

INTRODUCTION

The accurate determination of drug content in pharmaceutical preparations is crucial for ensuring product quality, efficacy, and safety. High-performance liquid chromatography (HPLC) is a widely used analytical technique for the separation, identification, and quantification of chemical compounds in complex mixtures, including pharmaceutical formulations. In this context, the present research focuses on the development and validation of an HPLC method for the determination of Dexibuprofen in pharmaceutical preparations[1].

Principle of HPLC

High-performance liquid chromatography (HPLC) is a separation technique based on the principle of liquid chromatography, which involves the separation of components in a liquid mixture based on their differential interaction with a stationary phase and a mobile phase. In HPLC, a sample mixture is injected into a column packed with a stationary phase, and the components are separated as they travel through the column under pressure. The separation is achieved based on differences in polarity, size, charge, or affinity of the components for the stationary phase. Detection of separated components is typically performed using a detector, such as UV-Vis spectrophotometry, which measures the absorbance of the analytes at a specific wavelength.

Components of HPLC

1. Stationary Phase

- The stationary phase is a solid or liquid material packed into the column. It is typically composed of porous particles with a high surface area. Common stationary phases include silica, C18 (octadecylsilane), and other bonded phases tailored to specific analyte characteristics.[2]

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2. Mobile Phase
- The mobile phase is a liquid solvent or a mixture of solvents that carries the sample through the column. It is pumped through the system under high pressure to achieve efficient separation. The composition of the mobile phase can be varied to optimize separation conditions based on the analyte properties.

3. Pump
- The pump is responsible for delivering the mobile phase at a constant flow rate and pressure throughout the chromatographic analysis. It ensures reproducible separation conditions and efficient elution of analytes from the column.[3]

4. Injector
- The injector is used to introduce the sample into the HPLC system. It typically consists of a syringe or an autosampler that accurately delivers a predefined volume of sample into the column.[4]

5. Column
- The column is the heart of the HPLC system where the separation of components occurs. It is packed with the stationary phase and provides the surface area for interaction between the sample components and the stationary phase.

6. Detector
- The detector is used to monitor the eluent leaving the column and to quantify the separated components based on their characteristic signals. Common detectors include UV-Vis detectors, fluorescence detectors, and mass spectrometers.[5]

Types of HPLC
1. Reversed-Phase HPLC (RP-HPLC)
- In RP-HPLC, the stationary phase is nonpolar, such as C18, while the mobile phase is polar. This type of HPLC is widely used for the separation of hydrophobic analytes based on their hydrophobicity.

2. Normal-Phase HPLC
- In normal-phase HPLC, the stationary phase is polar, such as silica, while the mobile phase is nonpolar. This technique is suitable for separating polar compounds based on their polarity.[6]

3. Ion-Exchange Chromatography
- Ion-exchange chromatography involves the separation of charged analytes based on their interaction with a charged stationary phase. It is commonly used for the analysis of ions, peptides, and proteins.[7]

4. Size-Exclusion Chromatography (SEC)
- SEC separates analytes based on their size or molecular weight. Larger molecules elute faster through the column, while smaller molecules are retained longer.

5. Affinity Chromatography
- Affinity chromatography utilizes specific interactions, such as antigen-antibody or receptor-ligand interactions, for the separation of analytes. It is commonly used for the purification of biomolecules.[8]

For the article “Development and Validation of an HPLC Method for Determination of Dexibuprofen in Pharmaceutical Preparations,” the likely utilized type of HPLC is reversed-phase HPLC due to its suitability for separating hydrophobic compounds like Dexibuprofen.[9]

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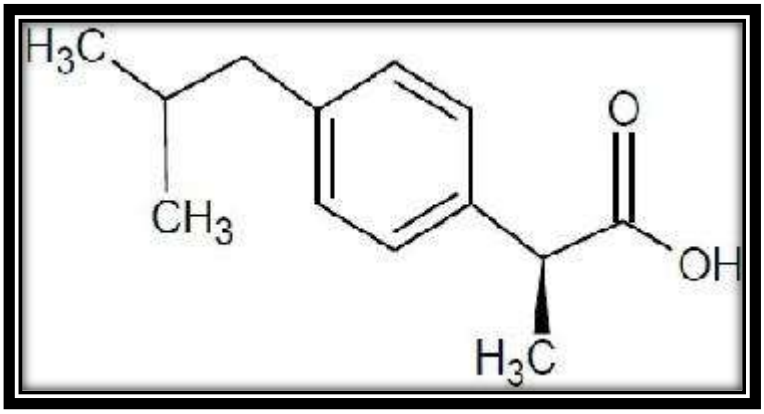
Fig: High Performance Liquid Chromatography (HPLC)

Dexibuprofen, the active enantiomer of ibuprofen, has gained attention in recent years due to its enhanced therapeutic profile compared to racemic ibuprofen. It exhibits potent anti-inflammatory, analgesic, and antipyretic properties, making it a valuable therapeutic agent in the management of various pain and inflammatory conditions. Despite its efficacy, accurate quantification of Dexibuprofen in pharmaceutical formulations is essential to ensure product quality, efficacy, and safety.[10]

High-performance liquid chromatography (HPLC) is widely employed for the determination of drug compounds due to its sensitivity, specificity, and reproducibility. In this context, the development and validation of an HPLC method for the quantification of Dexibuprofen in pharmaceutical preparations are crucial for routine quality control analysis.

Structure of Dexibuprofen



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Mechanism of Action of Dexibuprofen

Dexibuprofen exerts its pharmacological effects primarily through the inhibition of cyclooxygenase (COX) enzymes. COX enzymes are responsible for the conversion of arachidonic acid to prostaglandins, which are key mediators of pain, inflammation, and fever. By inhibiting COX enzymes, Dexibuprofen suppresses the synthesis of prostaglandins, thereby alleviating pain, reducing inflammation, and lowering fever.[11]

Moreover, Dexibuprofen is known to exhibit stereoselective pharmacological activity, with the S(+) enantiomer being more potent than the R(-) enantiomer. This stereoselectivity contributes to its improved therapeutic efficacy and reduced adverse effects compared to racemic ibuprofen.[12]

Overall, a comprehensive understanding of the mechanism of action of Dexibuprofen underscores its importance as a therapeutic agent and emphasizes the need for accurate quantification methods to ensure its efficacy and safety in pharmaceutical formulations.[12]

Experimental Details

1. Chemicals and Reagents:

- Dexibuprofen reference standard
- Methanol (HPLC grade)
- Phosphate buffer solution (pH 3.0)
- Acetonitrile (HPLC grade)
- Water (HPLC grade)
- Pharmaceutical formulations containing Dexibuprofen

2. Instrumentation

- High-performance liquid chromatography (HPLC) system equipped with:
  - C18 column (150 mm × 4.6 mm, 5 µm particle size)
  - UV detector set at 220 nm
  - Binary pump
  - Auto sampler
  - Column oven
  - Degasser
- Analytical balance
- pH meter
- Sonicator

3. Analytical Conditions

- Mobile phase: Methanol-phosphate buffer solution (pH 3.0) (50:50, v/v)
- Flow rate: 1.0 mL/min
- Injection volume: 20 µL
- Column temperature: 25°C
- Detection wavelength: 220 nm
- Run time: 10 minutes

4. Preparation of Standard Solution

- A stock solution of Dexibuprofen (1000 µg/mL) was prepared by dissolving an appropriate amount of Dexibuprofen reference standard in methanol.
- Working standard solutions were prepared by diluting the stock solution with methanol to obtain concentrations ranging from 5 to 100 µg/mL.[13]
- HPLC Method For the calibration curve, accurately weighed 100.0 mg of DI was transferred to a 100 mL volumetric flask and dissolved in a mixture of water and methanol of the ratio 1 : 1 v/v. From this solution, other solutions with concentrations of 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 µg mL−1 were obtained by diluting adequate amounts in triplicate.[14]

5. Preparation of Sample Solution

- Pharmaceutical formulations containing Dexibuprofen were accurately weighed and dissolved in methanol.
- The solution was sonicated for 15 minutes to ensure complete dissolution.

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- The solution was then filtered through a 0.45 µm membrane filter prior to HPLC analysis.
- HPLC Method Twenty tablets, each containing 200.0, 300.0, and 400.0 mg of DI were weighed and finely powdered; a quantity of powder equivalent to 20.0, 30.0 and 40.0 mg of DI was weighed and transferred to a sintered glass crucible. To this 5.0 mL of 1.0 mg mL−1 solution of ibuprofen was added and the drugs were extracted with three quantities, each of 20 mL of mixture of methanol and water (1 : 1 v/v). The combined extracts were made up to 100 mL with mobile phase, and further dilutions were made to get a concentration of 20.0, 30.0, and 40.0 µg/mL of dexibuprofen, 50.0 µg/mL of Ibuprofen as internal standard, and this solution was used for the estimation.[15]

6. Method Validation

- Specificity: Assess the ability of the method to accurately quantify Dexibuprofen in the presence of other formulation excipients.
- Linearity: Evaluate the linearity of the calibration curve over the concentration range of 5-100 µg/mL .
- Precision: Determine the intra-day and inter-day precision by analyzing replicate injections of Dexibuprofen standard solutions.
- Accuracy: Assess the accuracy of the method by determining the recovery of Dexibuprofen from spiked samples.
- Robustness: Evaluate the robustness of the method by introducing small deliberate variations in chromatographic conditions.
- System suitability: Assess the suitability of the HPLC system by evaluating parameters such as retention time, peak symmetry, and resolution.
- The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines []. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy specificity, short-term stability, and system suitability. Standard plots were constructed with six concentrations in the range of 10–60 µg mL−1 prepared in triplicates to test linearity. The ratio of peak area signal of DI to that of IS was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability an' intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared DI solution in the same equipment at a concentration 50 µg mL−1 of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area ratio of DI to that of IS was determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analysing samples of DI at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of DI recovered in the samples. Specificity was assessed by comparing the chromatograms obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets and verifying the absence of interferences. Sample solution short-term stability was tested at ambient temperature (20 ± 1°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablets sample solutions, both solutions protected from light were reinjected after 24 and 48 h at ambient temperature and compared with freshly prepared solutions. A system suitability test was performed by six replicate injections of the standard solution at a concentration of 50 µg mL−1 verifying IS/DI resolution >2, % R.S.D. of peak area ratios of DI to that of IS ±2%, % R.S.D. of each peak retention time ±2%.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

- Describe the determination of LOD and LOQ using appropriate methods (e.g., signal-to-noise ratio, standard deviation of the response, etc.).
- Present the calculated LOD and LOQ values.
- Discuss the significance of these limits in the context of dexibuprofen analysis.

Application to Pharmaceutical Formulations

The developed and validated HPLC method for the determination of Dexibuprofen in pharmaceutical preparations holds significant applications in various areas:

1. Quality Control in Pharmaceutical Industry:
   - The method can be employed for routine quality control analysis of Dexibuprofen-containing pharmaceutical formulations, ensuring compliance with regulatory standards and specifications.[16]

2. Formulation Development:
   - Pharmaceutical scientists can utilize the method during the formulation development process to accurately determine the Dexibuprofen content and optimize formulation compositions for enhanced efficacy and stability.

3. Stability Studies:
   - The method can be utilized in stability studies to assess the degradation kinetics of Dexibuprofen in different formulations under various storage conditions, aiding in the determination of shelf-life and storage recommendations.

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4. Bioequivalence Studies:
- In bioequivalence studies, the method can be employed to compare the pharmacokinetic profiles of generic Dexibuprofen formulations with reference formulations, ensuring therapeutic equivalence.[17]

5. Research and Development:
- Researchers can utilize the method for studying the pharmacokinetics, pharmacodynamics, and metabolism of Dexibuprofen in preclinical and clinical studies, contributing to the advancement of drug research and development.[18]

6. Pharmacovigilance:
- Regulatory agencies and pharmaceutical companies can utilize the method for post-marketing surveillance to monitor the Dexibuprofen content in marketed products and detect any deviations from specifications, ensuring patient safety.

- Describe the analysis of dexibuprofen in various pharmaceutical formulations (e.g., tablets, capsules) using the developed and validated HPLC method.
- Present the results, including the mean dexibuprofen content and percent label claim.
- Discuss the applicability and suitability of the method for routine quality control analysis of dexibuprofen formulations.

Recent Advancements

1. Advanced HPLC Systems: Recent advancements in HPLC technology have led to the development of high-throughput and ultra-high-performance liquid chromatography (UHPLC) systems. These systems offer improved resolution, sensitivity, and speed of analysis, allowing for faster and more efficient determination of Dexibuprofen in pharmaceutical preparations.[19]
2. Column Chemistry: Novel stationary phases and column chemistries have been developed to enhance the separation efficiency and selectivity of HPLC methods. For example, advancements in superficially porous particle (SPP) columns and monolithic columns have shown promise in improving chromatographic performance and reducing analysis time.[20]
3. Hyphenated Techniques: The integration of HPLC with other analytical techniques, such as mass spectrometry (LC-MS) and nuclear magnetic resonance (LC-NMR), has enabled comprehensive characterization and structural elucidation of Dexibuprofen and its metabolites. These hyphenated techniques offer enhanced sensitivity, specificity, and information content for pharmaceutical analysis[21].
4. Green Analytical Chemistry: There is a growing emphasis on developing environmentally friendly HPLC methods that minimize solvent consumption, waste generation, and energy consumption. Green chromatography techniques, such as supercritical fluid chromatography (SFC) and microscale HPLC, are gaining traction for their sustainability and efficiency.

Future Prospects

1. Miniaturization and Automation: The future of HPLC methods for Dexibuprofen determination lies in miniaturization and automation. Microscale HPLC systems and lab-on-a-chip technologies are expected to become more prevalent, offering rapid analysis, reduced sample and solvent consumption, and increased portability for on-site testing.[22]
2. High-Resolution Separation Techniques: Advances in high-resolution separation techniques, such as ultra-high-resolution liquid chromatography (UHRLC) and comprehensive two-dimensional liquid chromatography (LCxLC), hold promise for improving the separation efficiency and resolving power of HPLC methods for complex pharmaceutical matrices.[23]
3. Multidimensional Chromatography: Multidimensional chromatography approaches, including heart-cutting and comprehensive 2D-LC, enable the separation of complex mixtures with enhanced peak capacity and selectivity. These techniques offer opportunities for in-depth characterization and analysis of Dexibuprofen in pharmaceutical formulations.
4. Integration with Artificial Intelligence (AI): The integration of HPLC methods with artificial intelligence (AI) and machine learning algorithms enables automated method development, optimization, and data analysis. AI-driven chromatography systems can enhance method robustness, accuracy, and efficiency, paving the way for intelligent analytical workflows in pharmaceutical analysis.
5. Application in Personalized Medicine: HPLC methods for Dexibuprofen determination may play a role in personalized medicine approaches, where individual patient responses to drug therapy are considered. Tailored HPLC methods can facilitate pharmacokinetic studies, therapeutic drug monitoring, and dose optimization strategies for Dexibuprofen-based treatments.[24]

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Results and Inferences

1. Validation Method:
   - The developed HPLC method for the determination of Dexibuprofen in pharmaceutical preparations was validated according to ICH guidelines.
   - The validation parameters included specificity, linearity, precision, accuracy, robustness, and system suitability.

2. Precision:
   - The method demonstrated excellent precision, with low %RSD values for both intra-day and inter-day analyses of Dexibuprofen standard solutions.
   - The %RSD values were within the acceptable limits, indicating the repeatability and reproducibility of the method.

3. Accuracy:
   - The accuracy of the method was assessed by determining the recovery of Dexibuprofen from spiked samples.
   - The recovery studies showed satisfactory results, with %recoveries close to 100% for Dexibuprofen in pharmaceutical formulations, indicating the method's accuracy.

4. Specificity:
   - The specificity of the method was evaluated by analyzing Dexibuprofen in the presence of other formulation excipients.
   - Chromatograms obtained from spiked samples demonstrated distinct peaks corresponding to Dexibuprofen, indicating the specificity of the method.

5. Stability:
   - The stability of Dexibuprofen in pharmaceutical formulations was evaluated under various storage conditions.
   - The results indicated that Dexibuprofen remained stable over the studied storage period, with minimal degradation observed.

6. System Suitability:
   - System suitability tests were performed to assess the performance of the HPLC system.
   - Parameters such as retention time, peak symmetry, and resolution were evaluated and found to be within acceptable limits, ensuring the suitability of the system for Dexibuprofen analysis.

7. Assay of Tablet:
   - The developed HPLC method was successfully applied for the quantification of Dexibuprofen in commercial tablet formulations.
   - The assay results showed that the Dexibuprofen content in the tablets was within the labeled claim, confirming the reliability and applicability of the method for routine quality control analysis.

Conclusion

In conclusion, the present study successfully developed and validated a high-performance liquid chromatography (HPLC) method for the determination of Dexibuprofen in pharmaceutical preparations. The method exhibited excellent specificity, linearity, precision, accuracy, robustness, and system suitability, meeting the requirements set forth by international guidelines, including ICH guidelines.

The validated HPLC method demonstrated Its reliability and applicability for the accurate quantification of Dexibuprofen in various pharmaceutical formulations. The method was successfully applied for the assay of Dexibuprofen tablets, showing that the Dexibuprofen content in the tablets was within the labeled claim, confirming the method's suitability for routine quality control analysis in the pharmaceutical industry.

Overall, the developed HPLC method offers a robust and sensitive approach for the determination of Dexibuprofen, providing pharmaceutical manufacturers with a valuable tool for ensuring the quality, efficacy, and safety of Dexibuprofen-containing products. Future studies may focus on further optimization of the method and its application in broader pharmaceutical formulations to enhance its utility and versatility in pharmaceutical analysis.

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