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DEVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ENANTIOSEPARATION OF IBUPROFEN

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ABSTRACT

A simple high-performance liquid chromatography (HPLC) method has been developed in this study for enantioseparation and determination of ibuprofen in tablet samples. The HPLC method utilized an α-acid glycoprotein (AGP) as the stationary phase (10 cm × 4.0 mm × 5 µm) with 100 mM phosphate buffer (pH 7) as the mobile phase at a flow rate of 0.7 mL/min, and UV detection at 225 nm. Enantioseparation of ibuprofen has been successfully achieved by the optimized HPLC with a resolution greater than 1.50 and a retention time of less than 9 minutes. The calibration curve was linear for ibuprofen over the concentration range of 25 to 100 mg/L of standards with *r* higher than 0.996. The results show excellent average recoveries (83.20%, *n* = 3) for ibuprofen in the pharmaceutical (tablet) sample.

Keywords: Enantioseparation, HPLC, Ibuprofen, Pharmaceutical Sample.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation in arthritis patients by inhibiting the enzymes cyclooxygenase-1 and 2 (COX-1 and COX-2).[1] It reduces the production of inflammatory mediators such as prostaglandins (PGE2) and prostacyclin (PGI2), causing vasoconstriction, inhibiting prostaglandin production, and affecting sodium retention. According to this mechanism, the use of NSAID stimulates side effects in complications, such as hypertension, edema, impaired kidney function, and gastrointestinal bleeding.[2] Ibuprofen is a propionic acid derivative with one chiral center (Fig.-1). It is a common NSAID[3] patented in 1961 and first used to treat rheumatoid arthritis in the United Kingdom and the United States in 1969 and 1974, respectively.[4] Racemic ibuprofen, which contains equal quantities of R(-)-ibuprofen and S(+)-ibuprofen, has been used as an anti-inflammatory and analgesic agent for over 30 years. Although the S(+)-enantiomer is capable of inhibiting cyclooxygenase (COX) at clinically relevant concentrations, R(-)-ibuprofen is not a COX inhibitor. The two enantiomers of ibuprofen are therefore different in terms of their pharmacological properties.[5] The racemic form of ibuprofen remains available on the market due to the complex process of separating the enantiomers.[6]

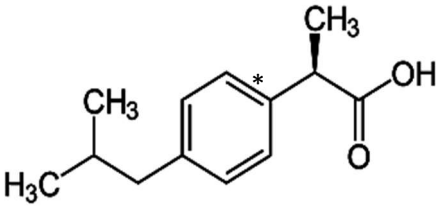

Fig.-1: Chemical Structure of Ibuprofen (*: Chiral Center)

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Several methods have been developed to separate ibuprofen enantiomers, including chromatography,[7] crystallization,[8] electrodialysis,[9] and enzymatic separation.[10] Also, thin-layer chromatography (TLC),[11] capillary electrochromatography (CE),[12] and high-performance liquid chromatography (HPLC)[13,14,15] have been used to separate ibuprofen enantiomers. In particular, the HPLC method for enantiomeric separation of ibuprofen has been reported with a variety of detectors such as diode array detection (DAD),[13] mass spectroscopy (MS),[14] and ultraviolet (UV)[15] detectors, as well as using various chiral columns such as ß-cyclodextrin,[12] and chiral cell OJ-RH columns.[4] Enantiomers are mirror-image molecules with the same chemical formula and structure but different atomic arrangements. This structural difference allows a unique interaction between enantiomers and biological systems, resulting in distinct pharmacokinetics and pharmacodynamics. For example, one enantiomer of a drug may have therapeutic effects, whereas the other may be inactive or cause adverse reactions. Accordingly, enantiomer separation is essential in the pharmaceutical and pharmacological fields for increasing drug efficacy and achieving the desired therapeutic effect while reducing its side effects.[16,17] Developing chiral analytical methods for enantiomer separation is difficult but fascinating; it involves separating identical molecules except for their spatial arrangement. Efforts are underway to quickly and efficiently control the purity of enantiomers at a low cost, improving their appeal to pharmaceutical manufacturers. Chiral analytical methods have revolutionized the pharmaceutical industry by improving drug quality and safety while reducing production costs.[18] The chiral selector is an important component in HPLC methods for separating enantiomers because it determines the selectivity of the process. A good chiral selector should have high-resolution values, excellent stability, and broad applicability to various compounds. Many protein-based chiral selectors have been developed and commercialized over the last few decades. A commonly used stationary phase for separating compounds, including NSAIDs is alpha-acid glycoprotein (AGP).[19,20] The present study developed and optimized the HPLC method for the enantiomeric separation of ibuprofen using the AGP column. Several parameters in the HPLC conditions have been optimized in this research. The optimized HPLC was then applied to determine ibuprofen in pharmaceutical (tablet) samples. In this present study, a simple HPLC method was developed for the chiral separation and determination of ibuprofen in the tablet samples. Several HPLC parameters were optimized in this research. The optimized HPLC method was then applied for the determination of ibuprofen in the pharmaceutical sample (tablet).

EXPERIMENTAL

Material and Methods

Ibuprofen (Sigma Aldrich, USA), sodium dihydrogen phosphate (Merck, Germany), purified water (Molsheim, France), and pharmaceutical samples (Gresik, Indonesia) were used as received. A stock solution of ibuprofen (2000 mg/L) was prepared in methanol and stored in the refrigerator at 5 °C. Final dilutions were prepared by diluting the stock solution with methanol. All organic solvents were HPLC grade. A 100 mM phosphate buffer was prepared by dissolving appropriate amounts of sodium phosphate using purified water and pH solution was then adjusted (pH 7) using sodium hydroxide solution. A pharmaceutical (tablet) sample was prepared by dissolving 10 mg of ibuprofen tablet in methanol, sonicating it for 30 minutes, and diluting it to 10 mL. This solution was injected into the HPLC system under the optimized HPLC conditions.

HPLC Instrumentation

An HPLC instrument from the Hitachi L-2000 series HPLC system with an L-2130 pump and L-2420 UV-Vis detector for signal detection and data acquisition processed by D-2000 elite software was used. The chiral separation employed a Chiralpak AGP column (10 cm × 4.0 mm × 5 µm). The mobile phase comprises a 100 mM phosphate buffer (pH 7) at a 0.7 mL/min flow rate. The column temperature was maintained at 25°C. The sample injection volume was 5 µL and UV detection was 225 nm. The HPLC conditions were optimized in this research.

Method Validation

Several validation steps were carried out to determine the performance of the HPLC method. Linearity was tested by injecting standard solutions (25-100 mg/L) in triplicate and plotting the peak area versus concentration. Precision was assessed by injecting a 50 mg/L solution three times and calculating the

relative standard deviation (RSD). The limit of detection and limit of quantification were calculated by signal to noise ($S/N = 3$) and ($S/N = 10$), respectively.

RESULTS AND DISCUSSION

Optimization of the HPLC Method

The HPLC parameters such as phosphate buffer concentration as mobile phase, injection volume, and flow rate were optimized using the AGP column as stationary phase.

Effect of Phosphate Buffer Concentration

This study investigated the impact of mobile phase composition on the separation of ibuprofen enantiomers. Maintaining a stable pH of around 7 is essential because ibuprofen is a weakly acidic molecule. The balance between its neutral and charged forms is crucial for optimal interaction with the stationary phase and achieving a good peak shape in the chromatogram. Deviations from this pH could lead to significant ionization changes, affecting peak shape and resolution potential. Phosphate buffer concentration has evidently played an important role in enantiomeric separation in this study. A higher concentration (100 mM) resulted in better resolution (Rs = 1.53) compared to lower concentrations (50 or 75 mM) due to the buffer's capacity to maintain a constant pH throughout the separation process. Higher buffer capacity minimizes pH fluctuations, ensures consistent retention times for enantiomers, and allows for the exploitation of subtle differences in their interaction with the stationary phase for better separation.

Effect of Injection Volume

The present study optimized the injection volume of ibuprofen using HPLC, varying it between 5 and 15 µL. The results showed that the 5 µL volume provided the highest resolution value for ibuprofen standards, indicating well-separated enantiomer peaks. This volume was selected for accurate quantification and separation of enantiomers as a crucial element in pharmaceutical analysis. A resolution value of 1.5 or higher was considered acceptable. This study has demonstrated that a lower injection volume in the HPLC analysis of ibuprofen results in better resolution and chromatographic performance. This aligns with the general benefits of low injection volume, such as reduced sample dilution, improved mass sensitivity, and reduced peak broadening. Optimized injection volume optimization is an important factor in the HPLC analysis of ibuprofen. Lower injection volume offers an ideal balance between sensitivity and resolution, especially for pharmaceutical analysis that requires accurate quantification and separation of enantiomers.

Effect of Wavelength

The impact of wavelength on the separation and detection of ibuprofen enantiomers in HPLC was investigated in this study. The results showed that 225 nm was the optimal wavelength for ibuprofen analysis. It resulted in the highest resolution (Rs = 1.57). In comparison other wavelengths (215 nm and 235 nm) yielded lower resolution, suggesting that they may not be ideal for effectively separating ibuprofen enantiomers. This could be due to insufficient absorption of ibuprofen or higher background interference at those wavelengths.

Methods Validation

Linearity

Validation relies on linearity to ascertain that the results were proportional to ibuprofen concentration (25-100 mg/L) under optimized HPLC conditions (225 nm, 5 µL, 100 mM phosphate buffer). Both peaks had high correlation coefficients ($R > 0.99$), demonstrating good linearity and suitability for routine analysis. This was consistent with the established principle of R near 1, indicative of a strong linear relationship. Successful validation of linearity, evidenced by high R values, provided confidence that this optimized HPLC method was suitable for routine analysis of ibuprofen enantiomers. The HPLC method included a calibration curve to measure ibuprofen concentrations in unknown samples accurately.

The Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ were calculated based on the statistical parameters of the calibration curve, associated with the ibuprofen concentration to the detector response (peak height or area). The curve's slope represents the method's sensitivity, while the residual standard deviation reflects the random error in the measurement. Well-established formulas use these parameters and target values ($S/N = 3$ for LOD and $S/N = 10$ for LOQ)

to calculate the limits. Validation results showed LOD and LOQ in the range of 5.0-5.2 mg/L and 17.0-17.4 mg/L, respectively, for both ibuprofen peaks. A lower LOD value indicated higher sensitivity for detecting a particular enantiomer, while the LOQ provided a practical working range for accurate quantification.

Precision

The precision test measures the closeness of results obtained from repeated measurements on the same homogeneous sample under identical conditions.[20] This test uses the repeatability principle, where equipment, solvents, and reagents remain constant within a short timeframe. Standard solutions of ibuprofen (50 mg/L) were injected six times under optimized conditions. Relative standard deviation (% RSD) values of 0.26% and 0.58%, respectively (Table-1). A low % RSD value indicated satisfactory precision for the proposed method, which aligns with the established criteria of a % RSD below 1% for high accuracy.[21]

Table-1: Analytical Performance of the HPLC Method

Parameter	Peak 1*	Peak 2*
Linearity range (mg/L)**	25-100	25-100
Regression equation***	$y = 22.98x - 3488$	$y = 151.71x - 2878.5$
r	0.9991	0.9968
LOD (mg/L)	5.00	5.20
LOQ (mg/L)	17.00	17.40
Accuracy	106.6%	100.94%
%RSD ($n = 3$)	0.26%	0.58%

*Peak notation as in Fig.-2
** Linearity range: 25 - 100 mg/L (in methanol);
*** y = peak area; x = concentration (mg/L)

Accuracy

The accuracy test evaluates how closely the measured concentration matches the known amount of analyte in the sample. A sample with a defined concentration was used for this assessment. This study determined accuracy by analyzing the standard solution of ibuprofen (50 mg/L) under optimal conditions (Table-1). High accuracy in analytical methods is crucial for ensuring the effectiveness and safety of drugs. The recovery values (106.6% and 100.94%) indicate the method's reliability and ability to produce accurate results within the acceptable range (90-107%).[21] This makes it suitable for routine analysis and quality control of ibuprofen-containing drugs.

Determination of Ibuprofen in Tablet Sample

Quantitative analysis of drug samples is critical for ensuring the presence of the advertised amount of active ingredient. This study employed HPLC to determine ibuprofen concentration in tablet preparations. Samples were prepared at 50 mg/L to fall within the calibration curve range. The percentage recovery of ibuprofen in the tablet sample (83.2%) which was determined from the peak height in the Fig.-2 fell within the calibration curve range, indicating the method's accuracy and precision in quantifying ibuprofen in various drug samples. This demonstrates the reliability of the HPLC method for routine analysis of ibuprofen.

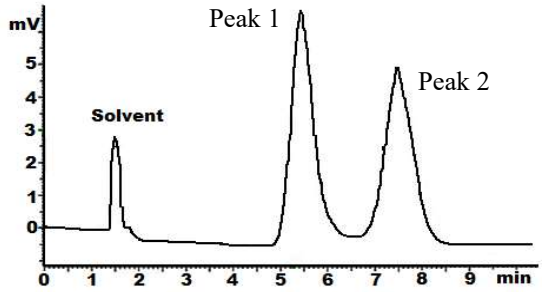

Fig.-2: Typical Chromatogram of Ibuprofen Enantiomers (Peak 1 and Peak 2) in the Tablet Sample. HPLC Conditions: α-acid glycoprotein (AGP) Column as Stationary Phase (10 cm × 4.0 mm × 5 µm), 100 mM Phosphate Buffer (pH 7) as Mobile Phase, Flow Rate of 0.7 mL/min, and UV Detection at 225 nm.

CONCLUSION

This study has described the development and validation of the HPLC method for separating ibuprofen enantiomers. The HPLC method employs an α-acid glycoprotein (AGP) column with a phosphate buffer as a mobile phase, resulting in rapid analysis times within 9 minutes. The proposed method could be used for routine analysis of ibuprofen enantiomer in the pharmaceutical samples.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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