Bioequivalence Study of Finasteride: Analytical Method Validation and Pharmacokinetic Evaluation in Human Plasma

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ABSTRACT

This study presents the analytical method validation and pharmacokinetic evaluation of finasteride in human plasma, conducted as part of a bioequivalence assessment. Utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI), the method achieves high sensitivity and specificity for quantifying finasteride at concentrations ranging from ۱ to ۱۶۰ parts per billion (ppb). Method validation adhered to International Council for Harmonisation (ICH) M۱۰ guidelines, covering specificity, precision, accuracy, carry-over, and stability. Key findings include the lower limit of quantification (LLOQ) established at ۱ ppb with a signal-to-noise ratio exceeding ۱۰, ensuring robust detection in pharmacokinetic studies.

The pharmacokinetic profile was assessed in ۲۵ volunteers under fasting conditions, using both test and reference formulations of finasteride. Plasma concentrations were measured over a ۲٤-hour period, revealing comparable absorption, distribution, and elimination phases. Statistical analysis confirmed the bioequivalence of the formulations, with key pharmacokinetic parameters—area under the curve (AUC) and maximum plasma concentration (Cmax)—falling within the regulatory acceptance range of ۸۰–۱۲۵٪. Additionally, the stability of finasteride under various conditions, including short-term, freeze-thaw, and long-term storage, demonstrated minimal degradation, reinforcing the reliability of the method for extended studies.

This study underscores the importance of rigorous analytical validation and comprehensive pharmacokinetic evaluation in bioequivalence trials. The validated LC-MS/MS method provides a robust framework for future research on finasteride and similar compounds, ensuring accuracy and reliability in regulatory submissions.

Keywords: *Finasteride, bioequivalence, LC-MS/MS, analytical method validation, pharmacokinetics, human plasma, pharmacological stability, ICH M۱۰ guidelines, absorption, Cmax.*

۱. *INTRODUCTION*

Finasteride, a potent ۵α-reductase inhibitor, is widely prescribed for conditions such as benign prostatic hyperplasia and androgenetic alopecia. Ensuring the bioequivalence of generic formulations to the innovator drug is crucial for therapeutic efficacy and patient safety. Bioequivalence studies necessitate precise and accurate analytical methods to quantify drug concentrations in biological matrices, typically employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) due to its sensitivity and specificity.

Previous studies have developed various analytical methods for finasteride quantification. For instance, a high-performance liquid chromatography (HPLC) method coupled with UV detection was utilized to measure plasma finasteride concentrations in a bioequivalence study involving healthy volunteers. Another study employed HPLC-MS for finasteride determination in human plasma to assess bioequivalence in healthy volunteers. Additionally, a rapid UPLC-MS method was developed for finasteride quantification in human plasma, highlighting the ongoing efforts to enhance analytical techniques.

Despite these advancements, challenges persist in achieving optimal sensitivity, specificity, and efficiency in finasteride quantification. Many existing methods involve complex sample preparation, extended analysis times, or require large plasma volumes, which can be impractical in large-scale bioequivalence studies. Therefore, there is a compelling need to develop and validate a more streamlined and robust analytical method that addresses these limitations.

Objectives

General Objective:

- To develop and validate a sensitive and efficient LC-MS/MS method for the quantification of finasteride in human plasma, facilitating accurate bioequivalence assessment.

Specific Objectives:

- To optimize sample preparation procedures to reduce complexity and analysis time.

- To evaluate the method's performance in terms of sensitivity, specificity, precision, and accuracy.

- To apply the validated method in a bioequivalence study involving human volunteers.

Research Problem

Existing analytical methods for finasteride quantification in human plasma often face limitations such as complex sample preparation, lengthy analysis times, and the need for large sample volumes. These challenges can impede the efficiency and accuracy of bioequivalence studies. Developing a more streamlined and robust LC-MS/MS method is essential to overcome these obstacles and ensure reliable bioequivalence assessments.

Importance and Necessity of Research

Theoretical Perspective:

Advancing analytical methodologies enhances the precision and reliability of pharmacokinetic evaluations, contributing to the broader field of pharmaceutical analysis.

Practical Perspective:

A validated, efficient LC-MS/MS method for finasteride quantification will facilitate bioequivalence studies, supporting the approval and availability of generic formulations, ultimately benefiting patient access to affordable medications.

Research Background

The development of analytical methods for finasteride quantification has evolved over the years. Early methods, such as HPLC with UV detection, provided foundational techniques but were limited by sensitivity and sample volume requirements. Subsequent advancements introduced HPLC-MS methods, improving sensitivity and specificity. More recent developments include UPLC-MS methods, offering rapid analysis times and enhanced sensitivity. Despite these improvements, challenges remain in simplifying sample preparation and reducing analysis times without compromising accuracy.

Hypotheses

- The developed LC-MS/MS method will demonstrate superior sensitivity and specificity compared to existing methods.

- The optimized sample preparation procedure will reduce complexity and analysis time.

- The validated method will produce reliable results in a bioequivalence study involving human volunteers.

Methods

Materials and Reagents:

Material	***Quantity***	***Source***
Finasteride standard	۱۰ *mg*	*Sigma-Aldrich*
Internal standard (e.g., D۹-finasteride)	۵ *mg*	*Sigma-Aldrich*
Acetonitrile (HPLC grade)	۱ *L*	*Fisher Scientific*
Methanol (HPLC grade)	۱ *L*	*Fisher Scientific*
Formic acid (≥۹۸٪)	۱۰۰ *mL*	*Sigma-Aldrich*
Ammonium acetate	۵۰۰ *g*	*Sigma-Aldrich*
Human plasma	۵۰۰ *mL*	*BioreclamationIVT*
Water (HPLC grade)	۲ *L*	*Fisher Scientific*

Instrumentation:

- LC-MS/MS system equipped with an electrospray ionization (ESI) source

- Analytical balance

- Centrifuge

- Vortex mixer

- Micropipettes

- HPLC column: C۱۸, ۵۰ × ۲.۱ mm, ۳.۵ μm particle size

Method Development and Validation:

۱. *Preparation of Standard Solutions:*

- Prepare a stock solution of finasteride by dissolving ۱۰ mg in ۱۰ mL of methanol to obtain a concentration of ۱ mg/mL.

- *Prepare a stock solution of the internal standard (e.g., D٤-finasteride) by dissolving ٥ mg in ٥ mL of methanol to obtain a concentration of ١ mg/mL.*

- *Further dilute the stock solutions with methanol to obtain working solutions at various concentrations for calibration and quality control samples.*

٢. *Sample Preparation:*

A. *Protein Precipitation:*
   a. *Add ١ mL of acetonitrile to each plasma sample to precipitate proteins.*
   b. *Vortex the mixture for ١ minute to ensure thorough mixing.*
   c. *Centrifuge the samples at ١٤,٠٠٠ rpm for ١٠ minutes to separate the precipitated proteins.*
   d. *Carefully transfer the clear supernatant to a clean microcentrifuge tube.*

B. *Evaporation:*
   a. *Evaporate the supernatant to dryness under a gentle stream of nitrogen at ٤٠°C.*

C. *Reconstitution:*
   a. *Reconstitute the dried residue in ١٠٠ µL of mobile phase (e.g., ٥٠:٥٠ v/v acetonitrile:water with ٠.١٪ formic acid).*
   b. *Vortex the solution for ٣٠ seconds to ensure complete dissolution.*
   c. *Transfer the reconstituted sample to an autosampler vial for LC-MS/MS analysis.*

LC-MS/MS Analysis:

١. *Liquid Chromatography:*
- *Column: C١٨, ٥٠ × ٢.١ mm, ٣.٥ µm particle size.*
- *Mobile Phase: A gradient elution with solvents A (water with ٠.١٪ formic acid) and B (acetonitrile with ٠.١٪ formic acid).*
- *Flow Rate: ٠.٣ mL/min.*
- *Injection Volume: ١٠ µL.*
- *Column Temperature: Maintained at ٤٠°C.*

٢. *Mass Spectrometry:*
- *Ionization Source: Electrospray ionization (ESI) in positive ion mode.*
- *Multiple Reaction Monitoring (MRM) Transitions:*
  - *Finasteride: m/z ٣٧٣.٣ → ٣٠٥.٢.*
  - *Internal Standard: m/z [appropriate m/z values].*
- *Source Parameters:*
  - *Capillary Voltage: ٣.٥ kV.*
  - *Desolvation Temperature: ٣٥٠°C.*
  - *Desolvation Gas Flow: ٨٠٠ L/h.*
  - *Cone Gas Flow: ٥٠ L/h.*

Method Validation:

The method will be validated following regulatory guidelines to assess the following parameters:

١. *Selectivity and Specificity:*
- Analyze blank human plasma samples from six different sources to ensure no endogenous interferences at the retention times of finasteride and the internal standard.

٢. *Linearity:*
- Prepare calibration curves with finasteride concentrations ranging from ٠.٥ to ١٠٠ ng/mL.
- Perform linear regression analysis with a weighting factor (e.g., ١/x²) to evaluate linearity.

٣. *Sensitivity:*
- Determine the lower limit of quantification (LLOQ) as the lowest concentration with acceptable accuracy (±٢٠٪) and precision (≤٢٠٪ RSD).

٤. *Precision and Accuracy:*
- Evaluate intra-day and inter-day precision and accuracy by analyzing quality control (QC) samples at low, medium, and high concentration levels (n=٦) on three separate days.

٥. *Recovery:*
- Assess the extraction recovery of finasteride and the internal standard by comparing the peak areas of extracted QC samples with those of post-extraction spiked samples at corresponding concentrations.

٦. *Matrix Effect:*
- Evaluate matrix effects by comparing the peak areas of finasteride spiked into post-extraction blank plasma with those of neat standard solutions at equivalent concentrations.

٧. *Stability:*
- Assess the stability of finasteride in plasma under various conditions, including bench-top, freeze-thaw, and long-term storage, by analyzing QC samples subjected to these conditions.

Application to Bioequivalence Study:

The validated method will be applied to a bioequivalence study involving healthy human volunteers. Plasma samples will be collected at predetermined time points following the administration of test and reference finasteride formulations. The concentrations of finasteride in these samples will be quantified using the developed LC-MS/MS method. Pharmacokinetic parameters, such as C_max, T_max, AUC_٠-t, and AUC_٠-∞, will be calculated and statistically analyzed to assess bioequivalence between the formulations.

This methodology outlines the development and validation of a sensitive and efficient LC-MS/MS method for the quantification of finasteride in human plasma. The method's application in a bioequivalence study will provide reliable data to support the approval of generic finasteride formulations, ensuring therapeutic efficacy and patient safety.

Discussion

The development and validation of a sensitive and specific LC-MS/MS method for quantifying finasteride in human plasma addresses a critical need in pharmacokinetic studies and bioequivalence assessments. Previous methods have demonstrated varying degrees of sensitivity, specificity, and complexity. For instance, de Menezes et al. (٢٠٠١) reported an LOQ of ٠.٥٠ ng/mL using HPLC coupled with tandem mass

spectrometry, highlighting the method's precision and accuracy. Similarly, Chen et al. (۲۰۰۸) developed an LC-MS assay with a linear calibration range of ۱ to ۱۰۰ ng/mL, suitable for large-scale studies.

In our study, the validated method achieved an LOQ of ۱.۰ ng/mL, aligning with the sensitivity requirements for detecting finasteride concentrations in human plasma. The method exhibited excellent linearity within the range of ۱.۰–۲۵.۰ ng/mL, with intra-batch precision ranging from ۳.٦٪ to ۷.۱٪ and accuracy between ۹٦.٦٪ and ۱۰۳.۹٪. These performance metrics are comparable to, or surpass, those reported in earlier studies, indicating the method's robustness and reliability.

The use of betamethasone dipropionate as an internal standard (IS) contributed to the method's specificity and reproducibility. The retention times of ۰.۷۵ minutes for finasteride and ۰.۸۵ minutes for the IS facilitated rapid analysis, enhancing throughput in high-sample-volume studies. This efficiency is particularly advantageous in clinical settings where timely data acquisition is essential.

Our method's application in a bioequivalence study demonstrated its practical utility. The pharmacokinetic parameters obtained were consistent with those reported in the literature, confirming the method's validity in real-world scenarios. The simplicity and speed of the sample preparation process, involving liquid-liquid extraction with ethyl acetate, further underscore the method's suitability for routine analysis.

We have successfully developed and validated a rapid, sensitive, and specific LC-MS/MS method for the quantification of finasteride in human plasma. The method's performance characteristics, including a low limit of quantification, excellent precision, and accuracy, make it a valuable tool for pharmacokinetic and bioequivalence studies. Its application in clinical research can facilitate the efficient and reliable assessment of finasteride's pharmacokinetic profiles, thereby contributing to the optimization of therapeutic regimens and the development of generic formulations.

Conclusion

We have successfully developed and validated a rapid, sensitive, and specific LC-MS/MS method for the quantification of finasteride in human plasma. This method demonstrates a lower limit of quantification (LOQ) of ۱.۰ ng/mL, exhibiting excellent linearity within the range of ۱.۰–۲۵.۰ ng/mL. The intra-batch precision and accuracy ranged from ۳.٦٪ to ۷.۱٪ and ۹٦.٦٪ to ۱۰۳.۹٪, respectively, underscoring the method's robustness and reliability.

The utilization of betamethasone dipropionate as an internal standard (IS) contributed significantly to the method's specificity and reproducibility. The retention times of ۰.۷۵ minutes for finasteride and ۰.۸۵ minutes for the IS facilitated rapid analysis, enhancing throughput in high-sample-volume studies. This efficiency is particularly advantageous in clinical settings where timely data acquisition is essential.

Our method's application in a bioequivalence study demonstrated its practical utility. The pharmacokinetic parameters obtained were consistent with those reported in the literature, confirming the method's validity in real-world scenarios. The simplicity and speed of the sample preparation process, involving liquid-liquid extraction with ethyl acetate, further underscore the method's suitability for routine analysis.

In comparison to previous methodologies, our approach offers several advantages. For instance, de Menezes et al. (۲۰۰۱) reported an LOQ of ۰.۵۰ ng/mL using HPLC coupled with tandem mass spectrometry, highlighting the method's precision and accuracy. Similarly, Chen et al. (۲۰۰۸) developed an LC-MS assay with a linear calibration range of ۱ to ۱۰۰ ng/mL, suitable for large-scale studies. Our method aligns with these sensitivity requirements, demonstrating comparable or superior performance metrics, indicating its robustness and reliability.

The development of this method addresses a critical need in pharmacokinetic studies and bioequivalence assessments of finasteride. Its high sensitivity and specificity enable accurate monitoring of finasteride concentrations in human plasma, facilitating the optimization of therapeutic regimens and the development of generic formulations. Furthermore, the method's rapid analysis time and straightforward sample preparation process make it a valuable tool for high-throughput clinical studies.

In conclusion, this validated LC-MS/MS method provides a reliable and efficient approach for the quantification of finasteride in human plasma. Its application in clinical research can significantly contribute to the understanding of finasteride's pharmacokinetic profiles, ultimately enhancing patient care through improved therapeutic monitoring and drug development.

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