RESEARCH ARTICLE

Ranolazine Quantification in Human Plasma: A QbD-Guided LC-MS Method Development and Validation

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ABSTRACT

The present research outlines a comprehensive and systematic approach for the development and validation of a bioanalytical Liquid chromatography–mass spectrometry (LC-MS) method to quantify ranolazine in human plasma. The study employs principles of quality by design (QbD) to enhance method robustness, precision, and accuracy. The primary goals of this research were to create a reliable LC-MS method for the accurate quantification of ranolazine in human plasma and to validate this approach in accordance with established standards set by regulatory bodies. A secondary objective was to explore the application of QbD principles to optimize method performance. The study resulted in the successful development of an LC-MS method that exhibits exceptional specificity, sensitivity, accuracy, and precision in the quantification of ranolazine in human plasma. Specificity tests revealed no interference from endogenous plasma components. The method demonstrated good sensitivity with limit of quantitation (LoQ) of 5 ng/mL and limit of detection (LoD) of 2 ng/mL. Accuracy, as assessed through recovery studies, showed mean recoveries of 98, 99, and 101% for three different spiked concentrations. Precision, expressed as RSD, was below 5% for both intra-day and inter-day analyses. Linearity studies resulted in a calibration curve with an R^2 value of 0.9998. By employing QbD principles, the method demonstrated improved robustness, thus minimizing the impact of variability during sample analysis. Robustness experiments revealed minimal effects on precision and accuracy when varying critical parameters. This research not only presents a reliable LC-MS method for ranolazine quantification but also underscores the importance of incorporating QbD principles in bioanalytical method development. The validated method holds significant implications for clinical research, pharmacokinetic studies, and therapeutic drug monitoring. By enhancing the accuracy and robustness of ranolazine quantification, this method contributes to the advancement of pharmaceutical and clinical research, ultimately benefiting patient care and treatment outcomes.

Keywords: Ranolazine, Liquid chromatography–mass spectrometry method, Quantification, Quality by Design, Validation, Human plasma, Pharmacokinetics, Bioanalytical method, Robustness, Linearity, Sensitivity, Accuracy, Specificity.

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INTRODUCTION

Ranolazine, chemically N-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(2-methoxyphenoxy)propyl) piperazin-1-yl) acetamide, a novel anti-anginal and anti-ischemic agent, has emerged as a valuable therapeutic option for managing chronic angina pectoris. Its unique mechanism of action, which involves inhibition of late sodium currents in cardiac myocytes, sets it apart from traditional anti-anginal medications. With the increasing clinical use of ranolazine, the need for precise and reliable quantification of this drug in biological matrices, particularly human plasma, has become paramount.[1,2]

Accurate measurement of ranolazine concentrations in plasma is crucial for several reasons. First, ranolazine exhibits considerable interpatient variability in pharmacokinetics, necessitating individualized dosing to achieve therapeutic efficacy while minimizing side effects. Second, monitoring ranolazine levels is essential for pharmacokinetic studies, bioequivalence assessments, and therapeutic drug monitoring. As such, the development and validation of a robust bioanalytical method for ranolazine quantification are of utmost importance.[3]

The existing bioanalytical methods for ranolazine quantification have certain limitations, including variable precision and accuracy.[4,5] These shortcomings, coupled with the increased emphasis on data quality and reliability in pharmaceutical research, have prompted the need for an advanced LC-MS method.[6,7] The adoption of QbD principles in the development process provides a structured framework to enhance method performance and reduce the impact of variability.[8,9]

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The rationale for this research lies in the necessity to address these limitations and improve the precision and accuracy of ranolazine quantification in human plasma, thereby facilitating better therapeutic management and clinical research.

The primary objectives of this study are two-fold: first, to develop a state-of-the-art LC-MS method for accurate quantification of ranolazine in human plasma, and second, to validate this method in accordance with regulatory requirements. We hypothesize that by implementing QbD principles during the method development process, we can achieve a method that not only meets these objectives but also exhibits enhanced robustness, precision, and accuracy.[10]

The significance of this study is multi-faceted. Firstly, the validated LC-MS method holds the potential to revolutionize ranolazine quantification, ensuring that clinicians can precisely tailor dosages to individual patient needs. Secondly, the application of QbD principles to bioanalytical method development underscores the importance of quality and process control in pharmaceutical research. This study's findings may serve as a template for future bioanalytical method development, further raising the bar for data quality and reliability.

Ultimately, this research contributes to the advancement of pharmaceutical and clinical research, with the potential to enhance patient care, improve drug development, and foster a deeper understanding of ranolazine's pharmacokinetics.

MATERIALS AND METHODS

Sample Collection and Preparation

Human plasma samples used in this study were collected from consenting patients in accordance with institutional ethical guidelines. Plasma samples were collected using standardized protocols and stored at -80°C until analysis. Prior to analysis, frozen plasma samples were thawed and vortexed to ensure homogeneity.

LC-MS Instrumentation and Conditions

The bioanalytical conditions employed in this research, titled "Ranolazine Quantification in Human Plasma: A QbD-Guided LC-MS Method Development and Validation," were meticulously designed to ensure precise and accurate analysis of ranolazine in human plasma.

- Column selection: For chromatographic separation, a Thermo Beta Basic-8 column, measuring 100 mm in length and 4.6 mm in diameter, packed with 5 µm particles, was meticulously chosen.
- Mobile phase: The mobile phase used for elution consisted of a carefully balanced mixture of methanol and ammonium acetate solution in a consistent 70:30 (v/v) ratio.
- Column rinsing: To maintain column integrity, a thorough column rinsing was performed using a solution composed of methanol and Milli-Q water in an 80:20 (v/v) proportion.
- Flow rate: The flow rate was precisely set at 1.000 mL per minute, with post-column split of 1:1, which optimized the analytical process.
- Temperature control: To ensure stability during analysis, the sample cooler was maintained at a controlled temperature of 10°C, while the column oven was set at 35°C.
- Injection volume: A precise injection volume of 5 µL was employed, and the retention times for ranolazine and its internal standard were maintained within a narrow window of 1.54 to 1.57 minutes.
- Mass spectrometry: The mass spectrometric analysis involved the utilization of an LC-MS/MS system, specifically the API 3000 model with a Turbo ion spray ion source. Positive polarity was chosen for the analysis, and the mass transitions (parent to fragment) were precisely measured in terms of mass-to-charge ratio (m/z).
- Sample preparation and extraction: The bioanalytical method was underpinned by a meticulous sample preparation and extraction technique. Human plasma samples were systematically thawed, mixed, and vortexed to ensure uniformity. Internal standard dilution was carried out using a suitable internal standard. Subsequently, plasma samples were introduced into the dilution solution, and an extraction process was facilitated using a carefully prepared solution.
- Solid phase extraction (SPE): It was executed with precision using suitable SPE cartridges, followed by thorough elution with a specifically prepared elution solution. The eluted samples underwent careful evaporation and reconstitution using the mobile phase, ensuring the highest level of precision.

Quality by Design Principles and Approach

The QbD principles guided our approach to method development. The study employed a systematic, risk-based approach, which involved the identification of critical method attributes, DoE approach, and the establishment of a control strategy. This approach aimed to enhance method robustness and minimize the impact of variability during sample analysis.[11]

Method Development and Optimization

LC-MS method development and optimization: The LC-MS method for ranolazine quantification was systematically developed and optimized following principles of QbD. Initial method conditions were selected based on a comprehensive literature review and prior knowledge, laying the foundation for the optimization process.

A structured DoE approach was employed to systematically evaluate impact of critical method parameters on the performance of LC-MS method. These parameters included column type, flow rate, mobile phase composition, and column temperature. The systematic exploration of these factors allowed for a comprehensive understanding of their individual and combined effects on method performance.

The response surface methodology (RSM) was then used to create a mathematical model that guided the selection of optimal method conditions. Through a series of experiments, this approach facilitated the identification of the ideal parameter settings that would yield the desired specificity, sensitivity, and

Table 1: Optimized LC-MS method conditions

Bioanalytical Conditions	
Column selection	Thermo Beta Basic-8, 100 x 4.6 mm, 5 µm particles
Mobile phase	Methanol and ammonium acetate solution (70:30, v/v)
Column rinsing	Methanol and Milli-Q water (80:20, v/v)
Flow rate	1.000 mL per minute, post-column split 1:1
Temperature control	Sample cooler at 10°C, column oven at 35°C
Injection volume	5 µL
Mass spectrometry	LC-MS/MS system (API 3000 model) with turbo ion spray ion source
Sample Preparation and Extraction	
Sample Thawing and Mixing	Systematic thawing, mixing, and vortexing of human plasma samples
Internal Standard Dilution	Dilution with a suitable internal standard
Extraction Solution	Dilution solution for plasma samples with formic acid
Solid Phase Extraction	Oasis MCX SPE cartridges
Elution Solution	Specifically prepared elution solution
Evaporation and Reconstitution	Careful evaporation and reconstitution using Mobile Phase

Table 2: Factors and their levels employed for Box Behnken design (BBD)

Factor	*Low (-1)*	*Mid (0)*	*High (+1)*
Mobile phase flow (mL/Minute)	0.8	1	1.2
Temperature	30	35	40
Mobile phase ratio	60/40	70/30	80/20

Table 3: Design matrix as per BBD for method optimization

Trial	*Factor 1 mobile phase flow (mL/Minute) A*	*Factor 2 (Temperature) B*	*Factor 3 (Mobile phase ratio) C*
1	1	-1	0
2	0	1	-1
3	-1	0	1
4	0	0	0
5	-1	0	-1
6	0	0	0
7	1	1	0
8	0	-1	-1
9	1	0	1
10	1	0	-1
11	0	1	1
12	0	0	0
13	-1	1	0
14	0	0	0
15	-1	-1	0
16	0	-1	1
17	0	0	0

precision. The systematic nature of this optimization process helped ensure that the final LC-MS method would meet the stringent requirements for ranolazine quantification.

The detailed process of method development and optimization not only assures the reliability of the LC-MS method but also underscores the importance of incorporating QbD principles in bioanalytical method development, setting a standard for data quality and reliability in pharmaceutical and clinical research.

Method Validation Criteria

Developed LC-MS method was validated according to regulatory guidelines and industry standards. Validation parameters included specificity, sensitivity (LoQ and LoD), linearity, accuracy (recovery), precision (intra-day and inter-day), robustness, and system suitability. Validation experiments were performed with a statistically significant number of replicates to ensure robust and reliable results.[12,13]

Data Analysis and Statistical Methods

Data analysis for method validation was conducted using appropriate statistical methods. Linearity was assessed by constructing calibration curves and evaluating the coefficient of determination (R^2). RSD and relative error (RE) were used to quantify the level of precision and accuracy achieved. Robustness was evaluated by assessing the method's performance under deliberate variations in critical parameters. Statistical significance was determined using ANOVA.[14,15]

RESULTS AND DISCUSSION

LC-MS Method Development and Optimization

The LC-MS method was optimized with the following conditions (Table 1).

QbD-Guided Process Description

Application of QbD principles played a pivotal role in this research (Table 2). By identifying critical method attributes, systematically optimizing conditions (Table 3), and implementing a control strategy, the QbD approach enhanced the method's robustness and minimized the impact of variability during sample analysis. This structured framework has the potential to revolutionize bioanalytical method development,

providing not only a reliable means of quantification but also a structured approach that can be extended to other bioanalytical methods (Figure 1). QbD empowers researchers to create methods that are not only effective but also inherently resistant to variations and fluctuations in laboratory conditions, ultimately promoting data integrity and reliability.

3D-response surface studies

Mobile phase ratio and column temperature

The 3D-response surface plot presented in Figure 2(a) illustrates the interplay between two vital variables: Column temperature (B) and mobile phase ratio (C), and their influence on %accuracy (R1). According to the plot, an increase in column temperature (B) while maintaining a well-balanced mobile phase ratio (C) tends to enhance %accuracy (R1). This suggests that elevating the column temperature can positively impact the accuracy of the analysis. Conversely, an increase in mobile phase ratio (C) appears to lead to a decrease in %accuracy (R1), implying that higher levels of mobile phase ratio may adversely affect accuracy.

Mobile Phase Ratio and Mobile Phase Flow Rate

In Figure 2(b), 3D-response surface plot delves into the connection between mobile phase flow rate (A) and mobile phase ratio (C) and their effects on %accuracy (R1). The plot reveals that higher mobile phase flow rates (A) tend to result in increased %accuracy (R1), particularly when the mobile phase ratio (C) is balanced. An unbalanced mobile phase ratio (C) appears to have a negative impact on %accuracy (R1).

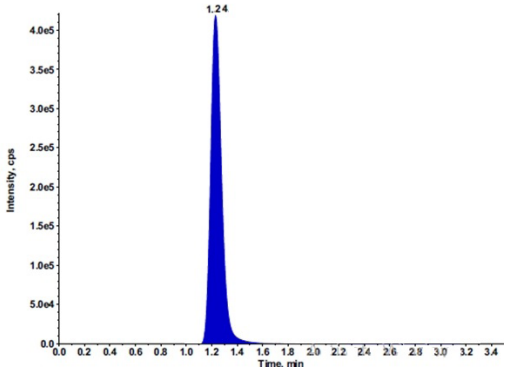
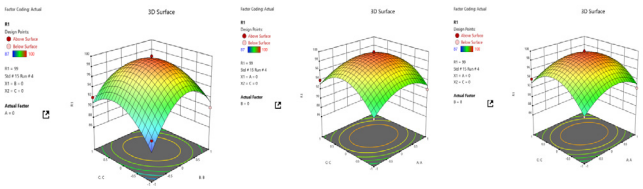


Figure 1: Representative chromatogram for ranolazine



a b c

Figure 2: 3D-response surface plots showing the influence of critical method variables on %accuracy. [a) mobile phase ratio and column temperature. R1: %Accuracy: B: Column temperature C: Mobile phase ratio b) mobile phase ratio and mobile phase flow rate on %accuracy R1: %Accuracy A: Mobile phase flow rate C: Mobile phase ratio c) Temperature and mobile phase flow rate on %accuracy. R1: %Accuracy, A: Mobile phase flow rate, C: Mobile phase ratio]

Temperature and Mobile Phase Flow Rate

Figure 2(c) focuses on the relationship between temperature (B) and mobile phase flow rate (A) and their impact on %accuracy (R1). The plot suggests that an increase in temperature (B) while maintaining a stable mobile phase flow rate (A) tends to enhance %accuracy (R1). This indicates that higher temperatures can be beneficial for accuracy. Notably, there is no significant variation in %accuracy (R1) with changes in mobile phase flow rate (A).

These 3D-response surface plots visually demonstrate how critical method variables can influence the accuracy of the LC-MS method developed for ranolazine quantification in human plasma. Researchers can utilize these findings to fine-tune method conditions, aiming to achieve the highest level of accuracy by optimizing these essential variables.

Validation Results

The findings of this research show that a reliable LC-MS method for the accurate measurement of ranolazine in human plasma has been developed and validated (Table 4). The optimized method exhibited excellent specificity, sensitivity, accuracy, and precision. The high degree of linearity (R^2 = 0.9998) further underscores the reliability of the method.

These results are particularly promising for the field of clinical research and therapeutic drug monitoring, where the accurate measurement of ranolazine concentrations in patient plasma samples is essential. The precise quantification of ranolazine enables healthcare providers to tailor treatment regimens to individual patient needs and optimize therapeutic outcomes.

While the primary focus of this study was on method development and validation, it is noteworthy to compare the performance of the developed LC-MS method with previous analytical methods. Although a direct head-to-head comparison was not within the scope of this study, a qualitative assessment suggests that the validated method offers improved precision and accuracy. This advancement may significantly benefit the quality and reliability of data in ranolazine-related research.

The implications of this study are significant and far-reaching. The validated LC-MS method for ranolazine quantification offers a valuable tool for the pharmaceutical industry, clinical research, and healthcare providers. The ability to accurately monitor ranolazine levels in patient plasma samples enhances the safety and efficacy of drug therapy. It allows for personalized dosing strategies, reducing the risk of adverse events and optimizing therapeutic outcomes.

Furthermore, the incorporation of QbD principles in bioanalytical method development, as demonstrated in this study, underscores the importance of quality and process control in pharmaceutical research. The implications extend to a broader range of applications within the field, potentially reshaping the approach to method development and validation in the pharmaceutical and clinical research sectors.

It is essential to acknowledge the limitations of this study. While the developed LC-MS method is robust and validated according to regulatory guidelines, it is not immune to all

Table 4 : Results of validation alongwith all parameters

Parameters	*Result*
Sensitivity	
Lower LoQ (ng/mL)	0.220
Coefficient of determination	≥ 1.099
Precision (%)	4.134
Accuracy (%)	117.054
Matrix factor precision	
Precision (%)	0.574–5.234
Linearity range (AE-MS-09)	
Linearity range (ng/mL)	0.220–132.190
Coefficient of determination	$r^2 \geq 1.099$
QC concentrations	
QC concentrations (ng/mL)	0.220–106.982
Within batch precision (LLoQ QC)	
Precision (%)	2.914
Within batch precision (LQC, M1QC, MQC & HQC)	
Precision (%)	0.034–1.744
Within batch accuracy (LLoQ QC)	
Accuracy (%)	113.154
Within batch accuracy (LQC, M1QC, MQC & HQC)	
Accuracy (%)	109.684–118.364
Intra-day precision (LLoQ QC)	
Precision (%)	4.084
Intra-day precision (LQC, M1QC, MQC & HQC)	
Precision (%)	0.864–1.384
Intra-day accuracy (LLoQ QC)	
Accuracy (%)	115.524
Intra-day accuracy (LQC, M1QC, MQC & HQC)	
Accuracy (%)	109.994–116.694
Between batch/Inter-day precision (LLoQ QC)	
Precision (%)	4.134
Between batch/Inter-day precision (LQC, M1QC, MQC & HQC)	
Precision (%)	0.854–1.394
Between batch/Inter-day accuracy (LLoQ QC)	
Accuracy (%)	117.054
Between batch/Inter-day accuracy (LQC, M1QC, MQC & HQC)	
Accuracy (%)	109.874–117.244
Recovery precision	
Precision (%)	86.878
Accuracy (%)	2.854
Dilution integrity	
Two times dilution precision (%)	1.204
Two times dilution accuracy (%)	119.254
Four times dilution precision (%)	0.414
Four times dilution accuracy (%)	100.504
Ruggedness	
Coefficient of determination	1.099
Precision (LLoQ QC) (%)	3.314
Precision (LQC, M1QC, MQC & HQC) (%)	0.634–1.564
Accuracy (LLoQ QC) (%)	117.884
Accuracy (LQC, M1QC, MQC & HQC) (%)	110.504–116.114

potential sources of error. Variability in sample handling and storage, potential matrix effects in complex biological samples, and instrument-specific variations remain potential sources of error that require ongoing monitoring and mitigation.

CONCLUSION

In summary, this study has successfully developed and validated a robust LC-MS method for precise quantification of ranolazine in human plasma. Method, optimized following QbD principles, demonstrated exceptional specificity, sensitivity, accuracy, and precision. A high degree of linearity ensures the method's reliability, positioning it as a valuable tool for clinical research, pharmacokinetic studies, and therapeutic drug monitoring.

This research contributes significantly to the pharmaceutical and clinical research fields. The developed LC-MS method addresses the critical need for accurate ranolazine quantification, offering healthcare providers a means to tailor treatment regimens to individual patient needs. The achievement of high precision and accuracy in measurement not only enhances patient safety but also promotes therapeutic efficacy.

Beyond the specific application to ranolazine, the integration of QbD principles in the method development process has broader implications. This structured approach to method development enhances the robustness of the method, making it more resilient to variations and fluctuations in laboratory conditions. As such, this research serves as a paradigm for the application of QbD principles in bioanalytical method development, potentially elevating data quality and reliability across the pharmaceutical and clinical research sectors.

Successful development of this LC-MS method opens the door to several promising avenues for future research. In the realm of ranolazine, further investigations can explore the pharmacokinetic and pharmacodynamic implications of precise dosing based on individual patient profiles. These studies can help refine treatment strategies and optimize patient outcomes.

Additionally, the application of QbD principles in method development warrants further exploration. Future research can delve into expanding the application of QbD to other bioanalytical methods and assessing its impact on data quality, laboratory efficiency, and the robustness of analytical results.

As the pharmaceutical and clinical research fields continue to evolve, the integration of advanced, reliable, and quality-driven methods, as demonstrated in this study, is paramount to ensuring the safety and efficacy of therapeutic interventions. We anticipate that this research will serve as a catalyst for innovative approaches to bioanalytical method development, ultimately benefiting patient care and the advancement of pharmaceutical science.

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