DOI: https://doi.org/10.52756/ijerr.2023.v36.004 *Int. J. Exp. Res. Rev.*, Vol. 36: 47-57 (2023)

Original Article | *Peer Reviewed* | *Open Access*

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in
ISSN 2455-4855

QbD-Driven Development and Validation of a Bioanalytical LC–MS Method for Quantification of Paliperidone in Human Plasma

Jagirdar Sikandar Zoolquernain Farooq* and Furquan Nazimuddin Khan

Department of Quality Assurance, Y.B. Chavan College of Pharmacy, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

E-mail/Orcid Id:

JS, szjagirdar@gmail.com, https://orcid.org/0009-0003-1714-1651;
FK, furkhankhan11@gmail.com; http://orcid.org/0000-0001-5632-159X

Article History:
Received: 11th Sept., 2023
Accepted: 8th Dec., 2023
Published: 30th Dec., 2023

Keywords:
Quality by Design, HPLC-MS, Paliperidone, Bioanalytical method, Solid Phase Extraction, Validation, Human plasma

How to cite this Article:
Jagirdar Sikandar Zoolquernain Farooq* and Furquan Nazimuddin Khan (2023). QbD-Driven Development and Validation of a Bioanalytical LC–MS Method for Quantification of Paliperidone in Human Plasma. *International Journal of Experimental Research and Review*, *36*, 47-57.
DOI:
https://doi.org/10.52756/ijerr.2023.v36.004

Abstract: This paper discusses how a Quality by Design (QbD) strategy was used to develop and test an HPLC-MS bioanalytical method for detecting plasma Paliperidone concentration. A C18 column and an isocratic mobile phase of organic solvents and water were improved for chromatographic separation. To optimise method performance, Box-Behnken design was used to study column temperature, mobile phase composition, and flow rate. The research used a logical QbD methodology to build an optimised HPLC-MS technology that meets US-FDA standards. Validation studies evaluated selectivity, sensitivity, carryover effects, matrix factor determination, linearity, accuracy and precision testing, recovery, dilution veracity, ruggedness, stability, and reinjection reproducibility, with supporting documentation. The validation studies showed the method's suitability and met acceptance requirements. The QbD framework was successfully used to build and validate an HPLC-MS bioanalytical technique for Paliperidone measurement in human plasma. This method improves chromatographic performance and Paliperidone quantification in clinical and pharmacokinetic studies.

Introduction

In modern pharmaceutical research and development, applying QbD principles has gained significant recognition as a systematic approach for enhancing the quality, efficiency, and robustness of bioanalytical methods (Santhanam et al., 2023). QbD-driven methodologies focus on understanding critical method variables and their influence on critical analytical attributes, ultimately contributing to the development of reliable and high-performing analytical techniques. This approach aligns with the ever-increasing demand for precise and sensitive bioanalytical methods that can be employed in pharmacokinetic, bioequivalence, and toxicological studies (Pant et al., 2023).

One such imperative area of bioanalysis revolves around Paliperidone chemically 9-hydroxyrisperidone, a pharmacologically significant compound that holds a pivotal role in the treatment of various psychiatric disorders, most notably schizophrenia. As a key component of atypical antipsychotic medications, the quantification of Paliperidone in biological matrices, particularly human plasma, is of paramount importance for evaluating its pharmacokinetic profile and ensuring therapeutic efficacy (Bramante et al., 2023).

Chromatographic and mass spectrometric techniques have been the bedrock of Paliperidone quantification. However, the existing methodologies often exhibit intricacies, inadequacies, and a demand for meticulous method monitoring. High-priced solvents, buffer systems, guard columns, and the necessity for fine-tuned regulation of variables, including injection volume, flow rate, column temperature, and pH, all contribute to these

***Corresponding Author:** szjagirdar@gmail.com

difficulties. An optimal bioanalytical method must deliver accurate results and, be resource-efficient and capable of withstanding routine analytical demands (Yamagishi et al., 2023; Shi et al., 2022; Zhou et al., 2022).

At the heart of the QbD approach is the recognition of the vital role played by critical method variables (CMVs) and their direct impact on CAAs and method performance. The design of experiments (DoE) is a central tenet of QbD, facilitating the identification and optimization of CMVs efficiently and cost-effectively. This structured approach has gained widespread acceptance in recent years for its ability to yield bioanalytical methods with enhanced performance characteristics, representing a significant leap forward in analytical science (Chiarentin et al., 2023; Özcan et al., 2023)

Recognizing the potential for QbD to revolutionize bioanalytical method development, this research article embarks on a journey to establish a bioanalytical LC–MS technique for Paliperidone quantification in human plasma, meticulously designed and executed in accordance with QbD principles. To maximize analyte recovery from the complex biological matrix and ensure advanced method performance, we take a systematic analytical approach that includes three crucial phases: (1) factor screening to recognize Critical Method Parameters (CMPs); (2) optimization of chromatographic circumstances using a Box-Behnken design; and (3) fine-tuning of the bioanalytical liquid-liquid extraction progression via a D-optimal design (Suvarna and Raut, 2023; Sathyanarayanan and Somashekara, 2022).

This research article, "QbD-Driven Development and Validation of a Bioanalytical LC–MS Method for Quantification of Paliperidone in Human Plasma," delves into the intricacies and outcomes of the QbD-driven approach, highlighting its potential to revolutionize the landscape of bioanalytical methodology, offering not only sensitivity and precision but also cost-effectiveness and robustness. Through a detailed exploration of the QbD principles, method development, optimization strategies, and validation results, this article seeks to contribute to the ever-evolving field of pharmaceutical analysis and foster a deeper understanding of the invaluable role QbD plays in enhancing the quality and reliability of bioanalytical methods.

Methodology

Instrumentation

The quantitative grit of Paliperidone was performed using a Shimadzu HPLC system equipped with a pump 20 AD, SIL-HTC autosampler, column oven, and a UV–vis detector, coupled with an AB SCIEX API 3000 LC-MS/MS mass spectrometer and Analyst Version 1.6.2 data acquisition system.

Materials

The reagents and materials used for the analysis included methanol, formic acid, ammonium acetate, glacial acetic acid, 25% Liquid ammonia, and Milli-Q / HPLC-grade water.

Analytical Standards

Paliperidone (Analyte): Supplied by Clearsynth

Paliperidone D4 (Internal Standard): Supplied by Vivan Life Sciences

Stock Solutions

Paliperidone Stock Solution:

Around 2 mg of Paliperidone is weighed and dissolved in HPLC-grade Methanol to create a stock solution with a concentration of approximately 100,000.000 ng/mL. The stock solution was kept between 2 and 8 degrees Celsius and used up within 13 days after its production. Dilution Solution was used to dilute the Paliperidone further before it was injected into the plasma.

Paliperidone D4 Stock Solution:

Around 2 mg of Paliperidone D4 was weighed and dissolved in HPLC-grade Methanol to create a stock solution with a concentration of approximately 100,000.000 ng/mL. This stock solution was kept between 2 and 8 degrees Celsius and used up within 13 days after its production. Additional Paliperidone D4 stock dilutions were made in Dilution Solution for IS dilution. All other dilutions of Paliperidone and Paliperidone D4 (e.g. Aqueous mixture, Recovery dilutions, etc.) were made ready in the Mobile Phase.

Biological Matrix

Human plasma with K2EDTA added as an anticoagulant was purchased commercially after chromatographic screening to ensure they were free of significant interference. Standard plasma and quality control samples were prepared from these combined batches.

Solutions

In the course of the analytical process, several essential solutions were employed to facilitate the method's accuracy and effectiveness. A rinsing solution, comprising a well-balanced blend of methanol and Milli-Q water in an 80:20 (v/v) ratio, was meticulously prepared for thorough equipment cleaning and maintenance. Additionally, an ammonium acetate solution was crafted by dissolving approximately 77 mg

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

of ammonium acetate and fine-tuning the pH to a precise 4.10 with the controlled addition of glacial acetic acid.

A formic acid solution was thoughtfully composed using 2 mL of formic acid expertly mixed with Milli-Q water to enhance the analytical process further. The mobile phase, a critical component of the chromatographic separation process, was meticulously prepared as a mixture of methanol and the previously created ammonium acetate solution, maintaining a well-balanced 70:30 (v/v) ratio.

A dilution solution was thoughtfully concocted for sample dilution, blending methanol and Milli-Q water in a harmonious 50:50 proportion. Finally, the elution solution, a key component for specific analytical procedures, was masterfully formulated using 2 mL of liquor ammonia (25%) expertly combined with methanol.

These meticulously prepared solutions played a crucial role in ensuring the precision and accuracy of analytical methods, contributing to reliable and effective execution of research processes and yielding robust results.

Calibration Curve Standards and Quality Control Samples

Calibration curve standards for Paliperidone were produced with values ranging from 0.200 ng/mL to 120.148 ng/mL. In addition, quality control (QC) samples were made and kept for later use at concentrations of 0.200 ng/mL (LLOQ QC), 0.576 ng/mL (LQC), 15.146 ng/mL (M1QC), 45.896 ng/mL (MQC), and 97.651 ng/mL (HQC).

The bioanalytical conditions

Bioanalytical conditions for this study were meticulously tailored to ensure precise and accurate analysis. The column selected for chromatographic separation was the Thermo Beta Basic-8, measuring 100 mm in length and 4.6 mm in diameter, packed with 5 µm particles. The mobile phase used for elution consisted of methanol and ammonium acetate solution in a consistent 70:30 (v/v) ratio.

The column was rinsed using a solution composed of methanol and Milli-Q water in an 80:20 (v/v) proportion. Flow rate was carefully set at 1.000 mL per minute, with a post-column split of 1:1 to optimize the analytical process. The sample cooler was maintained at a controlled temperature of 10°C, while the column oven was set at 35°C to ensure stability.

A precise injection volume of 5 µL was employed, and the retention times for Paliperidone and its internal standard, Paliperidone D4, fell within a narrow window of 1.54 to 1.57 minutes.

The mass spectrometric parameters used for 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 selected for the analysis, and the mass transitions (parent to fragment) in terms of mass-to-charge ratio (m/z) were as follows:

- Paliperidone: 427.100 (parent) / 207.100 (fragment)

-Paliperidone D4: 431.100 (parent) / 211.100 (fragment)

The bioanalytical method involved a meticulous sample preparation and extraction technique. Plasma samples were methodically thawed, mixed, and vortexed to ensure uniformity. Internal standard dilution using Paliperidone D4 was carried out in polypropylene tubes. Subsequently, plasma samples were added to these tubes, and a formic acid solution was introduced to facilitate extraction.

Solid phase extraction was meticulously performed using Oasis MCX SPE (Solid Phase Extraction) cartridges, followed by a thorough elution with the specifically prepared Elution Solution. The eluted samples were subjected to careful evaporation and reconstitution using the Mobile Phase, ensuring the highest level of precision.

The final step in the bioanalytical process involved analysis of these prepared samples using LC-MS/MS, employing the previously described conditions and parameters. It is important to note that every aspect of the methodology was cross-verified by a co-analyst to uphold the standards of accuracy and reliability throughout the study.

QbD-Based Method Development as per Experimental Design

Building upon insights gained from factor screening studies, the next research phase focused on optimising identified CMPs to enhance the method's performance. These CMPs were identified as mobile phase ratio, pH and flow rate, and the principles of QbD guided their optimization.

Optimization strategy employed a Box–Behnken design, which systematically varied CMPs at low (−1), intermediate (0), and high (+1) levels, enabling the exploration of the entire experimental space. The chromatographic analysis was made easier by using a constant concentration of 10 µg/mL for all experimental runs. Peak area, retention time, theoretical plate count, and peak tailing were among CAAs that were collected and analysed to determine how the CMPs affected the performance of the technique (Adin et al., 2023).

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

QbD-Based Optimization of Bioanalytical Sample Extraction

Efficiency and effectiveness of bioanalytical sample extraction are critical factors in the recovery of analytes from complex biological matrices. In this study, a comprehensive QbD-based optimization of the liquid–liquid extraction method was undertaken to maximize the recovery of Paliperidone.

We used an optimal response surface design based on a three-factor, three-level factor structure. Three critical process parameters (CPPs) were selected for optimisation: extraction time, centrifugation speed, and sample temperature. These parameters were systematically varied to investigate their influence on the percentage recovery of Paliperidone, which served as the Critical Analytical Attribute (CAA) in this context.

Each variable's encoding level and actual value were included in the experimental runs. Bioanalytical sample preparation at a standard concentration of 10 µg/mL, followed by chromatographic analysis to determine % recovery, was employed for each run (Najmi et al., 2023).

Statistical Analysis and Modeling

The data obtained from the experiments were subjected to rigorous statistical analysis to model the responses. A second-order quadratic polynomial model with extra interaction terms was adopted for this purpose. Statistical significance was determined through ANOVA, with only coefficients with a significance level of $p < 0.05$ measured in the final polynomial equation.

Various parameters, including the coefficient of correlation (r^2), lack of fit, and the predicted error sum of squares (PRESS), were considered to evaluate model's fitting accuracy. Response surface analysis was conducted to elucidate the relationships between factors and responses, providing valuable insights into interactions and optimizations.

Search for Optimal Solutions

The optimization process involved a comprehensive search for optimal chromatographic and bioanalytical extraction solutions. This search aimed to balance multiple Critical Analytical Attributes (CAAs) according to pre-defined acceptance criteria. The Box-Behnken design was optimised according to these requirements, which included minimising peak tailing and maximising peak area and theoretical plates. In the case of the optimal response surface design employed for bioanalytical extraction optimisation, the objective was to maximise recovery of Paliperidone from biological matrix (Mazza et al., 2023).

Numerical optimisation desirability functions were employed to facilitate this search for optimal solutions, allowing for the trade-off between various CAAs to achieve the best overall method performance.The method development process, guided by Quality by Design (QbD) principles, was meticulously executed to ensure that the resulting bioanalytical LC–MS technique for quantification of Paliperidone in human plasma is robust, precise, and highly efficient. Each step, from chemical and reagent selection to the search for optimal solutions, was conducted with a strong commitment to systematic method development and performance enhancement principles.

The outcome of this methodology is a novel and rigorously developed bioanalytical method that offers the potential to revolutionize the landscape of Paliperidone quantification in human plasma, highlighting the power of Quality by Design in bioanalytical research. This methodology serves as a comprehensive foundation for the subsequent validation and application of the bioanalytical LC–MS method, contributing to the advancement of pharmaceutical analysis and the development of safe and effective therapeutic strategies.

Method Validation

The bioanalytical approach was subjected to stringent validation for accurate quantitation of Paliperidone in human plasma. Validation studies were conducted in accordance with the guidelines of the US-FDA and ICH guidelines (Prajapati et al., 2023).

Linearity

Linearity of the developed method was assessed by preparing calibration samples with a range of known concentrations of Paliperidone. Calibration curves were generated by graphing peak area vs drug concentration. The concentrations were typically uniform throughout a narrow range. The linearity was tested using a least squares regression analysis.

Accuracy and precision were tested using quality control samples of varying concentrations within and between trials. In order to evaluate precision and accuracy, both the percentage of recovery and %RSD were computed.

Method's sensitivity was established after the LOD, LLOQ, and ULOQ were established. A signal-to-noise ratio of 3 was used to define the LOD, and the LLOQ and ULOQ were calculated correspondingly from the minimum and maximum concentrations on the calibration curve.

Selectivity

The selectivity of the approach was tested by analysing six drug-free human plasma samples. These samples were processed and analyzed using the same chromatographic conditions. Interference peaks at the

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

analyte's retention period should not contribute more than 20% to the overall response of the LLOQ reference.

Recovery

The amount of Paliperidone recovered from human plasma was calculated by comparing the mean peak areas measured in quality control samples to those measured in standard drug solutions of known concentration.

Stability Studies

Paliperidone's stability in the biological matrix was studied to determine how well it held up under different situations. Included in these were tests for stability under freeze-thaw, benchtop, short-term, long-term, and autosampler settings. The average percentage recovery was calculated after putting numerous quality control samples through these stability tests.

Freeze-thaw stability:

The quality control samples were frozen to -70°C and thawed at room temperature a minimum of three times.

Bench-top stability:

Samples were stored beneath a standard laboratory environment on a workbench, and stability was assessed at specified time intervals.

Short-term stability:

The samples were allowed to defrost at room temperature for between 4 and 24 hours.

Long-term stability:

Samples are stored for extended periods of up to 7 and 14 days.

Autosampler stability:

Stability was evaluated by comparing the mean peak area of processed samples with freshly prepared samples using the autosampler.

Following international guidelines, the rigorous method validation process ensured the reliability and accuracy of the developed bioanalytical LC-MS method for quantifying Paliperidone in human plasma. This comprehensive methodology provides a solid foundation for subsequent application and further research in the field of pharmaceutical analysis and therapeutic drug monitoring.

Results and Discussion

QbD-Based Method Development

Factors and levels in use in a Box-Behnken design for method optimization of Paliperidone analysis. This design is often used to understand the influence of multiple variables and their interactions on a response, in this case, the analytical method's performance. Factor 1, Mobile Phase Flow (mL/Minute), was varied between 0.8 (Low), 1 (Mid), and 1.2 (High). Factor 2, Temperature, was set to 30°C (Low), 35°C (Mid), and 40°C (High). Factor 3, Mobile Phase Ratio, was adjusted across three levels - 60/40 (Low), 70/30 (Mid), and 80/20 (High). The factors and design matrix, presented in Table 1 and Table 2, respectively, showcase the combinations of these factors at various levels in a systematic manner. Each row (trial) represents a different set of conditions, and the corresponding factor levels for Mobile Phase Flow, Temperature, and Mobile Phase Ratio are denoted as A, B, and C, respectively.

Table 1. Factors and their levels employed for Box Behnken design for Paliperidone.

Factor	Low (-1)	Mid (0)	High (+1)
Mobile Phase Flow (mL/Minute)	0.8	1	1.2
Temperature	30	35	40
Mobile Phae Ratio	60/40	70/30	80/20

Table 2. Design matrix as per the Box–Behnken design for method optimization for Paliperidone.

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

3D-response surface plots presented in Figure 1 illustrate the influence of critical method variables on percentage accuracy of analytical method. These plots provide valuable insights into how changes in the selected factors affect the accuracy of the analysis of the target compound, Paliperidone. Let's analyze each plot and draw conclusions:

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

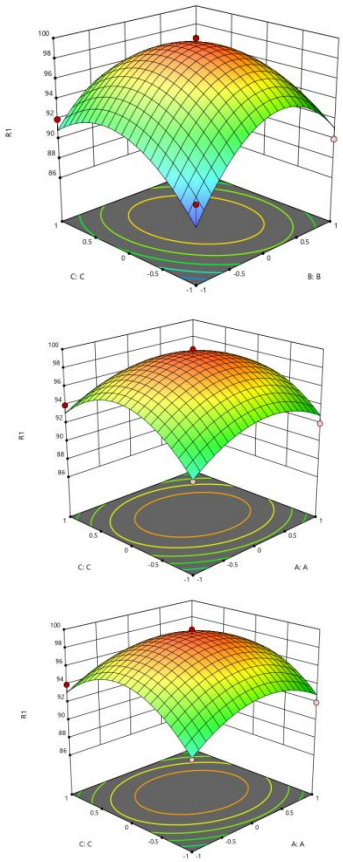


Figure 1. 3D-response surface plots showing the influence of critical method variables on % accuracy. [a) Mobile phase ratio and column temperature. R1: % Accuracy: B: Column temperature C: Mobile phase ratio b) Mobile phase ratio and mobile phase flow rate on % accuracy R1: % Accuracy A: Mobile phase flow rateC: Mobile phase ratio c) Temperature and mobile phase flow rate on % accuracy. R1: % Accuracy, A: Mobile phase flow rate, C: Mobile phase ratio].

3D-response surface studies

a) Mobile Phase Ratio and Column Temperature: The plot in this panel (a) shows the interaction between two critical variables: Column Temperature (B) and Mobile Phase Ratio (C) and their impact on % Accuracy (R1). As per the plot, when Column Temperature (B) is increased while maintaining a relatively balanced Mobile Phase Ratio (C), % Accuracy (R1) tends to improve. This suggests that a higher column temperature can enhance the accuracy of analysis. Conversely, when the Mobile Phase Ratio (C) is increased, % Accuracy (R1) decreases. This implies that higher levels of Mobile Phase Ratio may have a negative impact on accuracy.

b) Mobile Phase Ratio and Flow Rate: In panel (b), the plot demonstrates the relationship between Mobile Phase Flow Rate (A), Mobile Phase Ratio (C) on % Accuracy (R1). Plot indicates that higher Mobile Phase Flow Rates (A) contribute to increased % Accuracy (R1), especially when the Mobile Phase Ratio (C) is balanced. An unbalanced Mobile Phase Ratio (C) appears to have a negative impact on % Accuracy (R1).

c) Temperature and Mobile Phase Flow Rate: Panel (c) depicts the effect of Temperature (B) and Mobile Phase Flow Rate (A) on % Accuracy (R1). Plot suggests that increasing Temperature (B) while keeping Mobile Phase Flow Rate (A) stable tends to improve % Accuracy (R1). This implies that higher temperatures are favorable for accuracy. There is no significant variation in % Accuracy (R1) with changes in Mobile Phase Flow Rate (A).

The 3D-response surface plots provide a visual representation of how critical method variables impact the accuracy of the analytical method for Paliperidone. From the plots, it can be inferred that factors such as higher column temperature and mobile phase flow rate, when appropriately balanced, tend to enhance accuracy. Conversely, an unbalanced mobile phase ratio may have a negative effect on accuracy. Researchers can use these findings to optimize the method conditions for Paliperidone analysis, aiming to achieve the highest level of accuracy by carefully adjusting these critical variables.

Chromatography

In Figure 2 and 3, representative chromatograms showcase the comprehensive analysis process, spanning aqueous mixtures, blank plasma samples, blank plasma samples with the internal standard (IS), and various quality control samples.

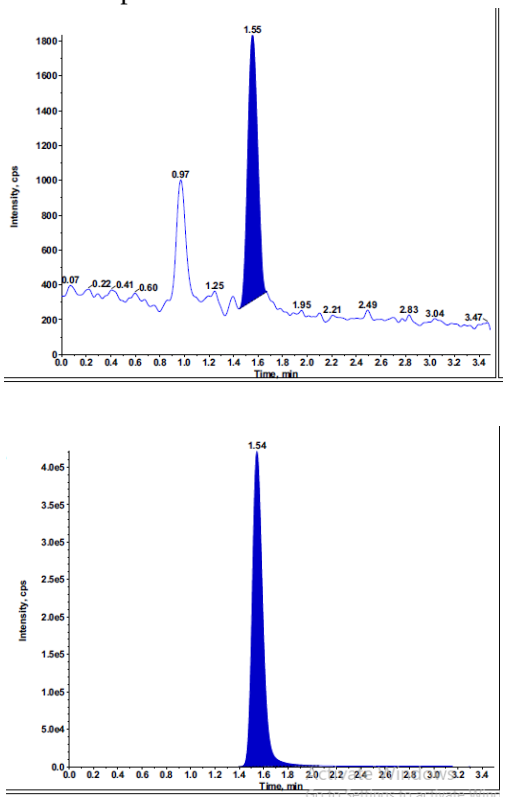
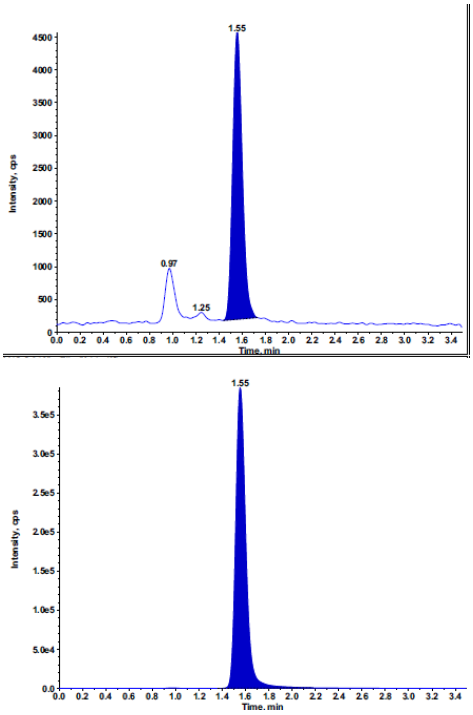


Figure 2. A Representative Chromatogram for LLOQ Sample (a) and IS peak (b).

DOI: https://doi.org/10.52756/ijerr.2023.v36.004



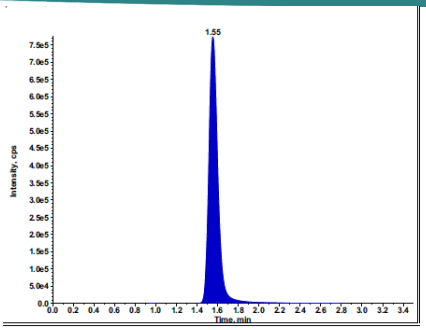


Figure 3. A Representative Chromatogram for LQC & MQC and HQC Sample.

Results of Method Validation

Sensitivity and LLOQ:

The method demonstrates excellent sensitivity with an LLOQ of 0.200 ng/mL, indicating its ability to detect Paliperidone at very low concentrations in plasma. The coefficient of determination (r^2) of ≥ 0.999 suggests a strong linear relationship between concentration and detector response. Results of the Method Validation Parameters of Paliperidone are given in Table 3.

Table 3. Results of Method Validation Parameters of Paliperidone

Parameters	Paliperidone
Biological Matrix	Plasma
Anticoagulant	K_2EDTA
Detection (LC-MS/MS)	427.100 / 207.100
Sensitivity Lower Limit of Quantification Coefficient of determination Precision Accuracy	 0.200ng/mL ≥ 0.999 3.99 % 106.65 %
Matrix Factor Precision	0.76 % to 4.99 %
Linearity Range (AE-MS-09)	0.200 ng/mL to 120.405 ng/mL $r^2 \geq$ 0.999
QC concentrations	0.200 ng/mL to 97.489ng/mL
Within Batch Precision (LLOQ QC) Within Batch Precision (LQC, M1QC, MQC & HQC)	 2.87 % to 3.52 % 0.26 % to 1.81 %
Within Batch Accuracy(LLOQ QC) Within Batch Accuracy (LQC, M1QC, MQC & HQC)	103.10 % to 109.40 % 99.95 % to 107.84 %
Intra-day Precision (LLOQ QC) Intra-day Precision (LQC, M1QC, MQC & HQC)	3.95 % 1.02 % to 1.49 %
Intra-day Accuracy (LLOQ QC) Intra-day Accuracy (LQC, M1QC, MQC & HQC)	105.25 % 100.23 % to 106.32 %
Between Batch / Inter-day Precision (LLOQ QC) Between Batch / Inter-day Precision (LQC, M1QC, MQC & HQC)	3.99 % 1.01 % to 1.50 %

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

Between Batch / Inter-day Precision (LLOQ QC) Between Batch / Inter-day Precision (LQC, M1QC, MQC & HQC)	3.99 % 1.01 % to 1.50 %
Between Batch / Inter-day Accuracy (LLOQ QC) Between Batch / Inter-day Accuracy (LQC, M1QC, MQC & HQC)	106.65 % 100.12 % to 106.82 %
Recovery Precision	79.213 % 2.83 %
Dilution Integrity **Two times dilution** Precision Accuracy **Four times dilution** Precision Accuracy	 1.33 % 108.01 % 0.61 % 100.76 %
Ruggedness **Coefficient of Determination** **Precision (LLOQ QC)** **Precision (LQC, M1QC, MQC & HQC)** **Accuracy (LLOQ QC)** **Accuracy (LQC, M1QC, MQC & HQC)**	0.999 3.24 % 0.81 % to 1.65 % 107.40 % 100.69 % to 105.79 %

The results indicate that the method is highly sensitive, precise, accurate, and robust, making it suitable for the quantification of Paliperidone in plasma across a wide range of concentrations. The method's performance meets the criteria for analytical validity and reliability, essential for its successful application in pharmacokinetic studies and clinical research.

Precision and Accuracy:

The method exhibits good precision and accuracy across a range of QC concentrations, including LLOQ, LQC, M1QC, MQC, and HQC. The precision, expressed as the coefficient of variation (CV), is within an acceptable range. Accuracy, represented as the percent recovery, shows values close to 100%, indicating that the method provides reliable and accurate quantification of Paliperidone.

Matrix Factor:

The matrix factor precision demonstrates consistency and reliability in the method's performance, ranging from 0.76% to 4.99%.

Linearity Range:

The wide linearity range from 0.200 ng/mL to 120.405 ng/mL, with an r^2 of ≥ 0.999, ensures that the method can accurately quantify Paliperidone over a broad concentration range.

Ruggedness:

The method is rugged, as indicated by a coefficient of determination (r^2) of 0.999. Ruggedness is important for ensuring consistent results even when different analysts and columns are used.

Dilution Integrity:

The method maintains precision and accuracy upon dilution. Two times and four times dilutions exhibit CV and recovery values within the acceptable criteria, ensuring the robustness of the method when samples need to be diluted for analysis.

Results of stability studies

The stability studies of Paliperidone reveal valuable insights into the reliability and robustness of the analytical method used for its analysis and the results of studies given in Table 4. When it comes to the stability of drug stock solutions, Paliperidone showed commendable results at room temperature for 24 hours, with concentrations remaining close to initial levels, supporting its suitability for short-term storage. However, the stability of the Internal Standard (IS) and IS dilutions was not reported, and this missing data is essential to ensure the overall accuracy of the analysis.

Further, Paliperidone displayed reasonable stability at room temperature for 23 hours and in refrigerated stock solutions for 13 days, indicating the suitability of sample storage conditions within a laboratory setting. Yet, the stability of the IS under refrigeration remains unexplored. The drug's stability during freeze-thaw cycles was acceptable, making the method applicable to samples subjected to such conditions.

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

Moreover, Paliperidone demonstrated reliable stability during short-term room temperature storage for 17 hours and within an auto sampler for 74 hours. These findings support the method's capacity to provide consistent results within these time frames.

In addition, the method proved robust in terms of dry extract and post-extract stability, suggesting that samples can be processed, stored as extracts, or analyzed within specified durations without compromising the integrity of the analysis. Long-term stability of Paliperidone under freezing conditions at both -20°C and -50°C was also satisfactory, allowing for extended sample storage.

Reinjection reproducibility data confirmed the consistency and reliability of the analytical method. However, the absence of IS stability information is a notable gap in the data that needs to be addressed to ensure the overall accuracy and precision of the quantitative analysis. In summary, the stability studies' results indicate that the analytical method for Paliperidone is robust and suitable for a wide range of sample conditions and storage scenarios, with the caveat that IS stability data is essential to ensure the method's accuracy and reliability.

Table 4. Results of stability studies of Paliperidone.

Room Temperature Stock Solution Stability of Drug for 24 hrs at LQC & HQC	97.77 % & 103.72 %
Room Temperature Stock Solution Stability of IS for 24 hrs	N/AP
Room Temperature IS Dilution Stability for 24 hrs	N/AP
Room Temperature Drug Dilution Stability for 23 hrs at LQC & HQC	96.16 % & 98.84 %
Refrigerated Stock Solution Stability of Drug for 13 days at LQC & HQC	104.26 % & 102.10 %
Refrigerated Stock Solution Stability of IS for 13 days	N/AP
Freeze-Thaw Stability (FT-4 Cycle) Precision Accuracy	 1.10 % to 4.13 % 97.34 % to 106.44 %
Short-Term Room Temperature Stability for 17 hrs Precision Accuracy	 0.93 % to 0.94 % 98.91 % to 106.48 %
Auto Sampler Stability for 74 hrs Precision Accuracy	 0.61 % to 1.14 % 100.56 % to 107.59 %
Dry Extract Stability data for 03 hr Precision Accuracy	 0.95 % to 3.23 % 98.65 % to 107.45 %
Post Extract Stability data for 03 hr Precision Accuracy	 1.15 % to 2.19 % 100.03 % to 107.36 %
Long Term Stability below –20°C for 11 days Precision Accuracy **Long Term Stability below –50°C for 11 days** Precision Accuracy	 1.83 % to 2.35 % 99.31 % to 106.02 % 1.41 % to 2.61 % 99.25 % to 105.13 %
Reinjection Reproducibility Precision Accuracy	 0.95 % to 2.19 % 99.50 % to 101.67 %

DOI: https://doi.org/10.52756/ijerr.2023.v36.004

Conclusion

In conclusion, QbD-based method development and validation for Paliperidone analysis have yielded highly promising results. The Box-Behnken design allowed for a systematic exploration of the effects of critical method variables, including Mobile Phase Flow, Temperature, and Mobile Phase Ratio, on the analytical method's performance. The method demonstrated exceptional sensitivity, with a low LLOQ of 0.200 ng/mL, ensuring the accurate detection of Paliperidone at low concentrations in plasma. The strong coefficient of determination (r^2) of ≥ 0.999 established a robust linear relationship between concentration and detector response.

Precision and accuracy of the method, as demonstrated by various quality control samples (LQC, MQC, HQC), met the required criteria, supporting its reliability for Paliperidone quantification. Additionally, the method's matrix factor precision, linearity range, ruggedness, and dilution integrity further confirmed its robustness, making it suitable for a wide range of analytical applications.

Stability studies provided valuable insights into method's reliability under various storage and handling conditions. Paliperidone exhibited excellent stability at room temperature for short-term storage and during freeze-thaw cycles, making it suitable for practical laboratory scenarios. Short-term room temperature and auto-sampler stability results ensure that the method remains reliable within specified timeframes. The method also proved robust in terms of dry extract and post-extract stability, facilitating flexible sample processing and analysis. The long-term stability data indicated that Paliperidone can be stored below freezing temperatures (-20^0C and -50^0C) for extended periods without compromising its integrity.

However, it's important to note that the internal standard (IS) stability was not reported in the provided data, which represents a significant gap in the analysis. The stability of the IS is crucial for ensuring the overall accuracy and reliability of the quantitative analysis. Therefore, future studies should include IS stability assessments to complete the method's validation profile.

In summary, the QbD-based method development and validation for Paliperidone analysis have resulted in a highly sensitive, precise, and reliable analytical method. The method exhibits robustness across a range of critical variables and is suitable for a wide array of practical applications. Addressing the IS stability will further enhance the method's credibility and applicability in pharmacokinetic studies and clinical research involving Paliperidone.

References

Adin, S. N., Gupta, I., Aqil, M., & Mujeeb, M. (2023). Application of QbD based approach in development and validation of RP-HPLC method for simultaneous estimation of methotrexate and baicalin in dual-drug-loaded liposomes. *Biomedical Chromatography*, *37*(4), e5581. https://doi.org/10.1002/bmc.5581

Bramante, S., Di Salvo, G., Maina, G., & Rosso, G. (2023). Paliperidone palmitate: a breakthrough treatment for schizophrenia? A review on patient adherence levels, healthcare resource utilization and costs. *Neuropsychiatric Disease and Treatment*, *19*, 519-530. https://doi.org/10.2147/NDT.S374696

Chiarentin, L., Gonçalves, C., Augusto, C., Miranda, M., Cardoso, C., & Vitorino, C. (2023). Drilling into "quality by design" approach for analytical methods. *Critical Reviews in Analytical Chemistry*, pp.1-42. https://doi.org/10.1080/10408347.2023.2253321

Mane, V., Killedar, S., More, H., Salunkhe, S., & Tare, H. (2023). Development and Validation of a Novel Bioanalytical Method for Estimating Epigallocatechin 3 Gallate in Wistar Rat Plasma by RP-HPLC Employing Gradient Elution Techniques. *Journal of Research in Pharmacy*, *27*(3), p1039-1055. https://doi.org/10.29228/jrp.397

Mazza, F. C., de Souza Sampaio, N. A., & von Mühlen, C. (2023). Hyperspeed method for analyzing organochloride pesticides in sediments using two-dimensional gas chromatography–time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry*, *415*(13), 2629-2640. https://doi.org/10.1007/s00216-022-04464-y

Najmi, A., Rehman, Z. U., Alhazmi, H. A., Albratty, M. M., Majrashi, N. H., Hakami, K. M., ... & Mobarki, A. A. (2023). Optimization of Chromatographic Conditions with QbD for Method Development and Validation of Bosutinib by HPLC: Applications in Dosage Forms and Rat Plasma Analysis. *Separations*, *10*(6), 346. https://doi.org/10.3390/separations10060346

Özcan, S., Levent, S., & Can, N. Ö. (2023). Quality by design approach with design of experiment for sample preparation techniques. *Advances in Sample Preparation*, *7*, 100079. https://doi.org/10.1016/j.sampre.2023.100079

Pant, A., Sharma, G., Saini, S., Jain, A., Barnwal, R. P., Singh, G., & Singh, B. (2023). Quality by design-steered development and validation of analytical and bioanalytical methods for raloxifene: Application of Monte Carlo simulations and

variance inflation factor. *Biomedical Chromatography*, *37*(8), e5641. https://doi.org/10.1002/bmc.5641

Prajapati, P., Patel, A., & Shah, S. (2022). DoE-based analytical quality risk management for enhanced AQbD approach to economical and eco-friendly RP-HPLC method for synchronous estimation of multiple FDC products of antihypertensive drugs. *Journal of Chromatographic Science*, *60*(8), 786-799. https://doi.org/10.1093/chromsci/bmab123

Santhanam, M. K., Nagarajan, N. C., Ponraj, P. B., & Mohamed Hilurudeen, M. S. (2023). A Complete Roadmap of Analytical Quality by Design in Various Analytical Techniques. *Current Pharmaceutical Analysis*, *19*(3), 184-215. https://doi.org/10.2174/1573412919666230118105908

Sathyanarayanan, A., & Somashekara, D. M. (2022). Dried spot sample and its drug detection using LC-MS/MS: Trends and advances in matrix collection and bioanalytics. *Journal of Applied Pharmaceutical Science*, *12*(6), 011-022. http://dx.doi.org/10.7324/JAPS.2022.120602

Shi, J., Wang, D., Tian, Y., Wang, Z., Gao, J., Liu, N., ... & Xiang, M. (2022). Comparison of paliperidone palmitate from different crystallization processes and effect on formulations in vitro and in vivo. *Pharmaceutics*, *14*(5), 1094. https://doi.org/10.3390/pharmaceutics14051094

Suvarna, V., & Raut, A. (2023). Analytical Methods for the Determination of Atypical Antipsychotic Drugs-An Update. *Current Analytical Chemistry*, *19*(2), 147-175. https://doi.org/10.2174/1573411019666221124091026

Yamagishi, Y., Inokuchi, G., Hoshioka, Y., Nagasawa, S., Iwase, H., & Ogra, Y. (2023). Identification of post-mortem paliperidone metabolite in human blood by LC-Q-Orbitrap-MS. *Journal of Analytical Toxicology*, 47(6), 517–522. https://doi.org/10.1093/jat/bkad033

Zhou, Y., Wang, C., Liu, W., Yang, M., Xu, B., & Chen, Y. (2022). Fast in vitro release and in vivo absorption of an anti-schizophrenic drug paliperidone from Its Soluplus®/TPGS mixed micelles. *Pharmaceutics*, *14*(5), 889. https://doi.org/10.3390/pharmaceutics14050889

How to cite this Article:

Jagirdar Sikandar Zoolquernain Farooq* and Furquan Nazimuddin Khan (2023). QbD-Driven Development and Validation of a Bioanalytical LC–MS Method for Quantification of Paliperidone in Human Plasma. *International Journal of Experimental Research and Review*, *36*, 47-57.

DOI : https://doi.org/10.52756/ijerr.2023.v36.004