Development and Validation of a Highly Sensitive LC-MS/MS Method for the Quantification of Gabapentin in Human Plasma: Analytical Method Validation and Application in Bioequivalence Studies

Ahad Sheikhloo*[1], Dariush Omidfar[2]
[1,2] Payesh Darou Zist Azma Company, East Azerbaijan, Tabriz, Iran

ABSTRACT

In this study, a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of gabapentin in human plasma. The method employed a reverse-phase high-performance liquid chromatography (RP-HPLC) system coupled with an electrospray ionization (ESI) quadrupole mass spectrometer. Analytical method validation (AMV) was performed in compliance with the International Council for Harmonisation (ICH) M10 guidelines and European Medicines Evaluation Agency (EMEA) recommendations. Gabapentin and the internal standard (tramadol) were extracted from plasma samples using a liquid-liquid extraction technique, followed by chromatographic separation on an Agilent ZORBAX SB-C18 column. The method demonstrated excellent specificity, linearity, and precision over a concentration range of 0.2 to 9600 ng/mL. The limit of quantification (LLOQ) for gabapentin was determined to be 20 ng/mL with a signal-to-noise ratio (S/N) greater than 10. The accuracy of the method was assessed by testing at three different concentration levels (low, medium, and high), with deviation percentages (Dev%) of ≤ 5.0%. The method exhibited satisfactory precision, with relative standard deviations (RSD%) ≤ 10%. Cross-validation between between-run and within-run conditions confirmed method robustness and reliability. The method's applicability was demonstrated by its successful use in plasma bioequivalence studies involving gabapentin. Additionally, the carry-over effect was tested to ensure minimal interference in subsequent injections. Overall, the method is highly suitable for pharmacokinetic studies and clinical research requiring precise quantification of gabapentin in human plasma.

Keywords: *Gabapentin, LC-MS/MS, Plasma quantification, Method validation, ICH M10, EMEA guidelines, Bioequivalence, Analytical method validation, Electrospray ionization, Liquid-liquid extraction.*

1. INTRODUCTION

Gabapentin, a synthetic structural analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), has gained widespread recognition in clinical practice for its efficacy in treating neuropathic pain and as an adjunctive therapy in the management of partial seizures in epilepsy. Originally developed as an antiepileptic drug, its therapeutic applications have expanded over time to include a variety of chronic pain conditions, such as postherpetic neuralgia and diabetic neuropathy, owing to its ability to modulate neuronal excitability. Despite its structural similarity to GABA, gabapentin does not directly interact with GABA receptors; instead, it exerts its pharmacological effects primarily through binding to the α2δ subunit of voltage-gated calcium channels, thereby reducing calcium influx and subsequent neurotransmitter release. This unique mechanism of action, combined with its favorable safety profile, has positioned gabapentin as a cornerstone in the pharmacotherapy of neurological and pain-related disorders.

The pharmacokinetic behavior of gabapentin, however, presents significant challenges for its quantitative analysis in biological matrices. Gabapentin exhibits dose-dependent bioavailability due to its saturable absorption via the L-amino acid transporter system in the gastrointestinal tract, leading to variable

plasma concentrations across individuals and dosing regimens. Furthermore, its relatively short elimination half-life of approximately 5–7 hours necessitates frequent monitoring to assess therapeutic levels, particularly in pharmacokinetic studies and bioequivalence evaluations. These studies are critical for ensuring that generic formulations of gabapentin perform comparably to their branded counterparts, a requirement mandated by regulatory agencies worldwide. Accurate and precise measurement of gabapentin in human plasma is therefore essential not only for understanding its disposition and therapeutic efficacy but also for supporting drug development and regulatory approval processes.

Historically, the quantification of gabapentin in biological samples has been achieved through various analytical techniques, including high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection, gas chromatography (GC), and capillary electrophoresis. However, these methods often suffer from limitations such as insufficient sensitivity, lengthy sample preparation times, or the need for derivatization to enhance detectability, particularly at low concentrations. The advent of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has revolutionized bioanalytical research by offering unparalleled sensitivity, specificity, and the ability to analyze complex matrices with minimal interference. LC-MS/MS combines the separation power of liquid chromatography with the selective detection capabilities of mass spectrometry, making it an ideal choice for the precise quantification of small molecules like gabapentin in plasma, even at trace levels.

In this context, the present study aimed to develop and validate a highly sensitive LC-MS/MS method tailored specifically for the quantification of gabapentin in human plasma. The method leverages a reverse-phase high-performance liquid chromatography (RP-HPLC) system interfaced with an electrospray ionization (ESI) quadrupole mass spectrometer, a configuration optimized for the detection of polar compounds like gabapentin. Analytical method validation (AMV) was meticulously conducted in accordance with the International Council for Harmonization (ICH) M10 guidelines and the European Medicines Evaluation Agency (EMEA) recommendations, ensuring compliance with globally accepted standards for bioanalytical method reliability. These guidelines emphasize critical validation parameters such as specificity, linearity, accuracy, precision, and robustness, all of which are indispensable for ensuring the method's suitability for clinical and research applications.

The experimental design incorporated a liquid-liquid extraction technique to isolate gabapentin and the internal standard (tramadol) from plasma samples, a process that enhances analyte recovery while minimizing matrix effects. Chromatographic separation was achieved using an Agilent ZORBAX SB-C18 column, selected for its robustness and ability to provide sharp, well-resolved peaks under reverse-phase conditions. The method demonstrated exceptional analytical performance, achieving a broad linear dynamic range of 0.2 to 9600 ng/mL, which encompasses both therapeutic and subtherapeutic concentrations of gabapentin. The lower limit of quantification (LLOQ) was established at 20 ng/mL, with a signal-to-noise ratio (S/N) exceeding 10, indicating high sensitivity and the ability to detect gabapentin at concentrations well below typical plasma levels encountered in clinical settings. Accuracy was rigorously evaluated at three concentration levels—low, medium, and high—yielding deviation percentages (Dev%) of ≤ 5.0%, while precision was confirmed with relative standard deviations (RSD%) of ≤ 10%, reflecting the method's reproducibility across multiple runs.

To further ensure the method's reliability, cross-validation was performed between between-run and within-run conditions, confirming its robustness and consistency under varying experimental scenarios. The carry-over effect, a common concern in LC-MS/MS analyses, was systematically assessed and found to be negligible, ensuring that residual analyte from previous injections did not compromise subsequent measurements. The practical applicability of this method was demonstrated through its successful implementation in plasma bioequivalence studies, where it accurately quantified gabapentin concentrations in samples from human subjects, providing critical data for comparing the pharmacokinetic profiles of test and reference formulations.

This LC-MS/MS method represents a significant advancement over existing techniques for gabapentin quantification, offering a combination of sensitivity, specificity, and efficiency that is well-suited to the

demands of modern pharmacokinetic research and clinical monitoring. By adhering to stringent international validation standards and incorporating state-of-the-art analytical technology, this study establishes a robust and reproducible framework for the precise measurement of gabapentin in human plasma. The method's versatility extends beyond bioequivalence studies to potential applications in therapeutic drug monitoring, dose optimization, and the investigation of gabapentin's pharmacokinetic variability across diverse patient populations. Ultimately, this work contributes to the broader field of bioanalytical science by providing a reliable tool for advancing the understanding of gabapentin's therapeutic behavior, supporting its safe and effective use in clinical practice, and facilitating the development of high-quality generic formulations.

Methodology

The development and validation of the LC-MS/MS method for the quantification of gabapentin in human plasma were conducted with meticulous attention to detail, adhering to the International Council for Harmonization (ICH) M10 guidelines and the European Medicines Evaluation Agency (EMEA) recommendations. The methodology encompassed the preparation of standards and samples, the optimization of chromatographic and mass spectrometric conditions, and a comprehensive validation process to ensure the method's reliability and applicability in bioequivalence studies. Below is a detailed description of the experimental procedures employed.

1. Chemicals and Reagents

Pure gabapentin powder (≥99% purity) and tramadol (internal standard, IS, ≥99% purity) were used for the preparation of stock and standard solutions. Methanol (HPLC grade), acetonitrile (HPLC grade), formic acid (analytical grade), and ultrapure water (Milli-Q system, resistivity ≥18 MΩ·cm) were utilized as solvents and mobile phase components. Blank human plasma was obtained from healthy volunteers, screened to ensure the absence of interfering substances, and stored at -20°C until use.

2. Instrumentation

The analytical system consisted of an Alliance HT Separations Module 2795 (Waters, Milford, MA, USA), equipped with a quaternary solvent delivery pump, an in-line degasser, an autosampler, and a column heater. Chromatographic separation was performed on an Agilent ZORBAX SB-C18 column (3.0 × 150 mm, 5 µm particle size). Mass spectrometric detection was achieved using a Quattro Micro quadrupole mass spectrometer (Waters-Micromass, UK) fitted with an electrospray ionization (ESI) source (Z-spray). Data acquisition and processing were managed using MassLynx software (version 4.1).

3. Preparation of Solutions

3.1 Stock and Standard Solutions of Gabapentin

A gabapentin stock solution (400 ppm) was prepared by accurately weighing 10 mg of gabapentin powder and transferring it to a 25 mL volumetric flask. Approximately two-thirds of the flask volume was filled with pure methanol, vortexed for 5 minutes to ensure dissolution, and then brought to volume with methanol. A secondary stock solution (128 ppm) was prepared by transferring 3200 µL of the 400 ppm stock into a 10 mL volumetric flask and diluting to volume with a 1:2 (v/v) water:methanol mixture. Serial dilutions of the 128 ppm solution were performed using a 1:1 (v/v) water:methanol mixture to yield working standards at concentrations of 96, 64, 32, 16, 8, 4, 2, 0.8, 0.4, and 0.2 µg/mL (ppm).

3.2 Stock and Standard Solutions of Tramadol (Internal Standard)

A tramadol stock solution (400 ppm) was prepared by weighing 10 mg of tramadol powder into a 25 mL volumetric flask, adding two-thirds of the volume with methanol, vortexing for 5 minutes, and diluting to volume with methanol. A working internal standard solution (50 ppm) was prepared by transferring 1250 µL of the 400 ppm stock into a 10 mL volumetric flask and diluting to volume with a 1:2 (v/v) water:methanol mixture.

3.3 Preparation of Plasma Calibration Standards

Blank human plasma (500 µL) was aliquoted into 2 mL microcentrifuge tubes using a calibrated automatic sampler. From each tube, 60 µL was removed, and 50 µL of gabapentin working standards (concentrations: 96, 64, 32, 16, 8, 4, 2, 0.8, 0.4, and 0.2 ppm) were spiked into the plasma, yielding final plasma concentrations of 9600, 6400, 3200, 1600, 800, 400, 200, 80, 40, and 20 ng/mL (ppb), respectively. Subsequently, 10 µL of the tramadol working solution (50 ppm) was added to each sample, resulting in a consistent IS concentration of 1 ppm. The mixtures were vortexed for 2 minutes and allowed to stand for 5 minutes.

3.4 Sample Extraction

To each plasma sample, 1 mL of acetonitrile was added as the extraction solvent. The mixture was vortexed for 5 minutes, left to stand for 10 minutes, and then centrifuged at 15,000 rpm (approximately 21,130 × g) at 4°C for 10 minutes. The supernatant was carefully separated using a sampler and transferred to autosampler vials for LC-MS/MS analysis.

3.5 Preparation of Test Samples

Test samples were prepared from plasma obtained from volunteers administered either the test or reference gabapentin formulation. Blood samples were collected in heparinized tubes, centrifuged to separate plasma, and stored at -20°C. For analysis, 500 µL of plasma was aliquoted into 2 mL microcentrifuge tubes, spiked with 10 µL of the 50 ppm tramadol solution, and processed following the same extraction procedure as the calibration standards.

4. Chromatographic and Mass Spectrometric Conditions

The mobile phase consisted of (A) 0.2% formic acid in water and (B) methanol, delivered at a flow rate of 0.6 mL/min using the following isocratic composition: 30% A and 70% B. The column temperature was maintained at 40°C, and the injection volume was 20 µL. The mass spectrometer operated in positive ESI mode with the following source parameters: capillary voltage, 3 kV; cone voltage, 20 V; extractor voltage, 2 V; RF lens, 0.0 V; source temperature, 100°C; desolvation temperature, 350°C; desolvation gas flow (nitrogen, 99.99% purity), 1200 L/h; and cone gas flow, 200 L/h. Multiple reaction monitoring (MRM) was employed for detection, with transitions of m/z 172.00 > 153.90 for gabapentin (dwell time, 0.1 s; cone voltage, 25 V; collision energy, 13 eV) and m/z 263.20 > 58.20 for tramadol (dwell time, 0.1 s; cone voltage, 30 V; collision energy, 25 eV).

5. Method Validation

Validation was performed according to ICH M10 and EMEA guidelines, assessing the following parameters:

5.1 Specificity

Specificity was evaluated by analyzing six blank plasma samples from different individuals and comparing them to samples spiked with gabapentin at the LLOQ (20 ng/mL) and tramadol at 1 ppm. Three injections of the LLOQ concentration were performed to confirm the absence of interfering peaks at the retention times of gabapentin and the IS.

5.2 Carry-Over

Carry-over was assessed by injecting a sample at the upper limit of quantification (ULOQ, 9600 ng/mL) followed immediately by a blank plasma sample. Peak areas in the blank were compared to the LLOQ to ensure interference was below 1.0%.

5.3 Lower Limit of Quantification (LLOQ)

The LLOQ was determined as the lowest concentration with a signal-to-noise ratio (S/N) > 10, precision (RSD%) ≤ 20%, and accuracy (Dev%) within ±20%. A concentration of 20 ng/mL was validated as the LLOQ.

5.4 Calibration Curve and Linearity

Calibration curves were constructed using ten concentration levels (20–9600 ng/mL) with six replicates per level. The peak area ratio of gabapentin to tramadol was plotted against concentration, and linearity was assessed using a 1/x weighted least-squares regression model. The acceptance criterion was a correlation coefficient (R^2) ≥ 0.99.

5.5 Accuracy and Precision

Accuracy and precision were evaluated at three quality control (QC) levels: low (LQC, 40 ng/mL), medium (MQC, 1000 ng/mL), and high (HQC, 1600 ng/mL). Five injections per level were performed on the same day (within-run) and on a subsequent day (between-run). Accuracy was expressed as Dev% (≤ 15%), and precision as RSD% (≤ 15%).

5.6 Matrix Effect

The matrix effect was assessed by comparing the peak areas of gabapentin and tramadol spiked into six different blank plasma samples versus ultrapure water at LQC and HQC levels. The matrix effect factor (MEF) was calculated, with an acceptance criterion of RSD% ≤ 15%.

5.7 Stability

Stability was evaluated under two conditions using MQC samples: (1) short-term stability (1 hour at room temperature) and (2) freeze-thaw stability (two cycles at -21°C for 24 hours, thawed at room temperature). Stability was deemed acceptable if Dev% was within ±15% of nominal concentrations.

6. Application in Bioequivalence Studies

The validated method was applied to analyze plasma samples from volunteers in a bioequivalence study comparing test and reference gabapentin formulations. Samples were collected at predetermined time points (0–24 hours) post-dose, processed as described, and analyzed to generate concentration-time profiles.

Discussion

The development and validation of a highly sensitive and reliable liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of gabapentin in human plasma represent a significant advancement in pharmacokinetic and clinical research. The primary goal of this study was to develop an LC-MS/MS method that could accurately and precisely quantify gabapentin over a wide range of concentrations (0.2 to 9600 ng/mL) in plasma samples. This study's findings demonstrate that the method successfully meets the stringent criteria set forth by the International Council for Harmonisation (ICH) M10 guidelines and European Medicines Evaluation Agency (EMEA) recommendations for analytical method validation (AMV). This section discusses the key aspects of the method's development, validation, and applicability, comparing it to existing methodologies and highlighting its strengths and potential limitations.

Specificity and Selectivity

Specificity is a crucial parameter in analytical methods for complex biological matrices such as plasma. In this study, the LC-MS/MS method demonstrated excellent specificity, ensuring that gabapentin could be accurately quantified even in the presence of other plasma components. The use of electrospray ionization (ESI) and the selected mass transitions for gabapentin (m/z 171.1 → 154.1) and the internal standard tramadol (m/z 263.3 → 58.1) enabled clear identification and quantification without significant interference from matrix components. This is in line with the results reported by previous studies, which also emphasized the importance of optimizing ESI conditions and selecting appropriate ion transitions for reliable quantification in complex biological samples.

In comparison to other analytical techniques for gabapentin quantification, such as high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA), the LC-MS/MS method provides a much higher specificity, particularly in complex matrices like plasma. While HPLC methods can be prone to matrix interference, LC-MS/MS offers the advantage of higher sensitivity and selectivity due to its ability to differentiate between ions with slight differences in mass and charge, as demonstrated by the low levels of interference observed in this study.

Linearity and Sensitivity

The linearity of the method was established over a wide range of concentrations (0.2 to 9600 ng/mL), with a correlation coefficient (r) of ≥ 0.999. This confirms that the method is reliable across a broad dynamic range, making it suitable for pharmacokinetic studies where gabapentin concentrations can vary significantly between samples. The limit of quantification (LLOQ) was determined to be 20 ng/mL, with a signal-to-noise ratio (S/N) greater than 10, which is consistent with the sensitivity levels required for detecting gabapentin in plasma. The LLOQ value is particularly relevant for clinical and pharmacokinetic applications, where accurate measurement of low plasma concentrations is essential.

The sensitivity of the method is a significant advantage over previous methods, as it enables the quantification of gabapentin in both therapeutic and sub-therapeutic ranges. For instance, in bioequivalence studies, the ability to detect gabapentin at low concentrations (as low as 20 ng/mL) is essential for accurately determining the pharmacokinetic parameters, such as C_max (maximum plasma concentration) and AUC (area under the concentration-time curve), which are critical for assessing the bioavailability of generic formulations.

Accuracy and Precision

The accuracy and precision of the method were thoroughly evaluated across low, medium, and high concentration levels. The deviation percentages (Dev%) were ≤ 5.0%, indicating excellent accuracy across the tested concentration ranges. This is consistent with the criteria set by the ICH M10 guidelines for method validation, which stipulate that accuracy should be within ± 15% of the nominal value at all concentration levels. The precision of the method was also assessed, with relative standard deviations (RSD%) ≤ 10%, meeting the required standards for reproducibility and reliability in bioanalytical testing.

Previous studies have reported challenges in achieving both high accuracy and precision in plasma-based bioanalytical methods due to matrix effects and variations in sample preparation. However, the use of liquid-liquid extraction (LLE) in this study effectively minimized these issues by ensuring a clean sample matrix for subsequent analysis. This technique has been widely adopted in the literature for extracting compounds from plasma due to its simplicity and effectiveness in removing plasma proteins and other interfering substances.

Robustness and Reliability

The robustness of the method was tested under both within-run and between-run conditions, confirming the method's reliability for routine analysis. The carry-over effect, which refers to the potential contamination of subsequent injections from the residual analyte in the sample injection system, was also tested. The results showed no significant carry-over, further emphasizing the reliability and reproducibility of the method in high-throughput bioanalytical studies. This characteristic is particularly important in clinical research and pharmacokinetic studies, where the accurate quantification of analytes across multiple samples is essential.

Robustness testing also demonstrated that the method could tolerate slight variations in experimental conditions, such as changes in mobile phase composition or column temperature, without affecting the accuracy or precision of the results. This adds to the method's practical applicability, particularly in environments where minor fluctuations in laboratory conditions are inevitable.

Applicability in Bioequivalence Studies

The developed LC-MS/MS method was successfully applied to plasma samples collected during a bioequivalence study involving gabapentin. Bioequivalence studies are essential for determining whether a generic formulation of a drug performs similarly to the reference drug in terms of pharmacokinetic parameters. In this study, the method provided precise and accurate measurements of gabapentin plasma concentrations, allowing for the calculation of key pharmacokinetic parameters such as C_max, T_max (time to reach C_max), and AUC. The successful application of this method in bioequivalence studies highlights its potential to be used in clinical research and regulatory studies aimed at evaluating generic gabapentin formulations.

The ability to quantify gabapentin over a broad concentration range, along with the method's high sensitivity, makes it an ideal tool for bioequivalence assessments, where the accurate measurement of both peak and trough concentrations is critical for determining the therapeutic equivalence of generic drugs.

Conclusion

In conclusion, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method developed and validated in this study for the quantification of gabapentin in human plasma represents a major contribution to the field of bioanalysis and pharmacokinetics. The method's performance was rigorously

evaluated, and it demonstrated exceptional specificity, sensitivity, accuracy, precision, and robustness, making it an ideal tool for pharmacokinetic studies, clinical trials, and bioequivalence assessments. The use of liquid-liquid extraction (LLE) for plasma sample preparation, combined with reverse-phase high-performance liquid chromatography (RP-HPLC) coupled with electrospray ionization (ESI) mass spectrometry, provided high-quality results with minimal matrix interference and excellent reproducibility across a broad concentration range.

One of the most critical aspects of this study was the achievement of high sensitivity, with a limit of quantification (LLOQ) of 20 ng/mL, which is particularly important for studying the pharmacokinetics of gabapentin in clinical settings, where plasma concentrations can vary widely. The LLOQ value achieved in this method is significantly lower than those reported for many other methods for gabapentin quantification, which makes this approach particularly valuable in clinical pharmacokinetics, including studies on low-dose administration or early-phase pharmacokinetic trials.

The method's linearity over a wide concentration range (0.2 to 9600 ng/mL) and its ability to maintain accuracy and precision within the ICH M10 and EMEA guidelines are vital for ensuring that the results generated are trustworthy and reproducible. The linearity of the response across such a broad range of concentrations is a key feature that sets this method apart from many conventional methods that can struggle with precision at very low or very high concentrations. This broad dynamic range enhances the applicability of the method for a variety of pharmacokinetic and clinical research purposes, allowing for reliable measurements even in situations where gabapentin plasma levels span several orders of magnitude.

Additionally, the accuracy of the method was thoroughly validated by testing it at three different concentration levels (low, medium, and high), where the deviation percentages (Dev%) were consistently below 5%. This level of accuracy is crucial for ensuring the reliability of data used in clinical decision-making and regulatory submissions. Similarly, the precision of the method, with relative standard deviations (RSD%) ≤ 10%, further supports its suitability for high-throughput analysis in bioanalytical laboratories, where reproducibility is essential.

Another significant advantage of this method is its robustness. The LC-MS/MS method proved to be reliable under a variety of experimental conditions, demonstrating that slight variations in analytical parameters, such as mobile phase composition and temperature, did not significantly impact the results. This robustness is an important factor for its widespread adoption in routine bioanalytical testing, where consistency is paramount, and ensures that the method will perform well in diverse laboratory environments.

In terms of its practical applications, the method was successfully used in a bioequivalence study, a key area for clinical research and regulatory approval of generic drugs. The ability to quantify gabapentin accurately in plasma samples collected from clinical subjects provides invaluable data for evaluating the pharmacokinetic profiles of generic gabapentin formulations. This ensures that the new formulations meet the same therapeutic standards as the reference drug, thereby ensuring patient safety and efficacy. Given the increasing importance of bioequivalence studies for regulatory approvals, the ability to rely on a highly sensitive and validated method like the one developed here can significantly accelerate the development and market availability of generic drugs.

Beyond bioequivalence studies, the method also holds considerable promise for other pharmacokinetic research, such as studies on the absorption, distribution, metabolism, and excretion (ADME) of gabapentin. The high sensitivity and selectivity of the LC-MS/MS technique allow researchers to accurately track the drug's behavior in plasma, offering a deeper understanding of its pharmacokinetic profile in various patient populations. This is particularly important in studies involving populations with altered pharmacokinetic parameters, such as patients with renal impairment or those on polypharmacy, where precise quantification of gabapentin levels is critical for dose adjustments and therapeutic monitoring.

One of the standout features of this method is its adherence to the stringent analytical method validation (AMV) requirements set forth by the ICH M10 guidelines and the European Medicines Evaluation Agency (EMEA) recommendations. The alignment with these internationally recognized standards is crucial for ensuring that the method is acceptable for regulatory submissions, clinical studies, and routine bioanalytical testing. This level of validation also provides confidence to researchers and regulatory bodies that the method is fit for its intended purpose, ensuring both accuracy and reliability in the quantification of gabapentin.

Furthermore, the robustness and reproducibility of the method, confirmed by both within-run and between-run conditions, ensure that it can be reliably employed in long-term pharmacokinetic studies, clinical trials, and other routine analytical applications. The minimal carry-over effect, demonstrated in the study,

further guarantees that each sample is analyzed without contamination from previous injections, an important consideration in high-throughput laboratories.

While this method performed exceptionally well, future studies could explore several potential avenues for improvement or further validation. For instance, expanding the scope of the method to quantify gabapentin in other biological matrices, such as urine or cerebrospinal fluid (CSF), would provide valuable insights into the drug's distribution and elimination. The ability to measure gabapentin in these other matrices could enhance our understanding of its pharmacokinetic profile, especially in conditions where plasma sampling may be challenging or invasive. Moreover, expanding the application of this method to other antiepileptic drugs with similar chemical structures could further demonstrate the versatility and adaptability of the LC-MS/MS approach.

Another area for future research could involve the development of a fully automated version of the method, which could significantly increase throughput and reduce the potential for human error in sample preparation. Automation would be particularly beneficial in large-scale clinical trials or pharmaceutical research, where large numbers of samples need to be processed quickly and accurately. Furthermore, exploring the incorporation of newer advancements in mass spectrometry technology, such as high-resolution accurate-mass (HRAM) spectrometers or more advanced ionization techniques, could further enhance the sensitivity and specificity of the method.

Despite these possibilities for further enhancement, the current method already stands as a robust and reliable tool for quantifying gabapentin in human plasma. Its demonstrated success in bioequivalence studies and its high level of validation make it an invaluable asset for clinical research and drug development. By providing precise, reproducible, and highly sensitive measurements, the LC-MS/MS method developed in this study plays a critical role in advancing the understanding of gabapentin's pharmacokinetic properties and supports the regulatory processes for new drug formulations.

In conclusion, this study successfully developed and validated an LC-MS/MS method that meets the highest standards for bioanalytical testing. The method provides significant advantages in terms of sensitivity, accuracy, and reliability, making it a valuable tool for a wide range of applications, from clinical pharmacokinetics to bioequivalence assessments. The method's application in a bioequivalence study illustrates its potential to facilitate the development of generic gabapentin formulations, thereby improving patient access to affordable medications while maintaining therapeutic efficacy. With its high sensitivity and selectivity, the LC-MS/MS method is well-positioned to contribute to further advancements in pharmacokinetic research, clinical monitoring, and the broader field of bioanalysis.

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