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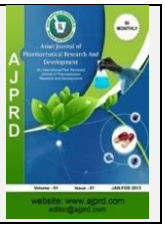

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Research Article

LC–MS/MS Bioanalysis of Gabapentin in Human Plasma: Method Development, Validation, and Regulatory Perspectives under USFDA and ICH M10 Guidelines

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ABSTRACT

Gabapentin, a structural analogue of gamma-aminobutyric acid, is widely used in the treatment of epilepsy, neuropathic pain, and related neurological disorders. Reliable quantification of gabapentin in human plasma is critical for pharmacokinetic, bioavailability, and bioequivalence studies. However, due to its high polarity, zwitterionic nature, and lack of strong chromophores, conventional analytical methods such as HPLC and UV spectroscopy often exhibit limited sensitivity and selectivity. In this context, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has emerged as the method of choice for gabapentin bioanalysis owing to its superior sensitivity, selectivity, and robustness. This review provides a comprehensive evaluation of LC–MS/MS methods developed for the quantification of gabapentin in human plasma, with particular emphasis on bioanalytical method development strategies and validation requirements as per United States Food and Drug Administration (USFDA) and International Council for Harmonisation (ICH M10) guidelines. Critical aspects including sample preparation techniques, chromatographic optimization, mass spectrometric parameters, and internal standard selection are systematically discussed. A comparative assessment of reported methods highlights variations in extraction efficiency, matrix effect evaluation, analytical sensitivity, and regulatory compliance. Furthermore, this review identifies key limitations in existing methods, such as inadequate assessment of matrix effects, incomplete stability studies, lack of appropriate internal standards, and insufficient evaluation of carryover, dilution integrity, and incurred sample reanalysis. These gaps may affect the regulatory acceptability and reproducibility of bioanalytical data. In conclusion, although LC–MS/MS offers a highly effective platform for gabapentin bioanalysis, strict adherence to harmonized regulatory guidelines and implementation of a lifecycle-based validation approach are essential to ensure method reliability, reproducibility, and global regulatory acceptance. This review aims to serve as a practical reference for the development of robust and compliant bioanalytical methods for gabapentin.

Keyword: Gabapentin, Gamma-aminobutyric, USFDA and ICH M10 Guidelines, Regulatory Perspectives.

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INTRODUCTION

Gabapentin is a widely used antiepileptic and analgesic drug, chemically related to gamma-aminobutyric acid (GABA). It is primarily prescribed for the treatment of epilepsy, neuropathic pain, post-herpetic neuralgia, and restless leg syndrome. Due to its favorable safety profile, minimal drug–

drug interactions, and effectiveness in chronic therapy, gabapentin is extensively used in both acute and long-term clinical practice. As a result, accurate measurement of gabapentin levels in biological matrices is essential for pharmacokinetic, bioavailability, bioequivalence, and therapeutic drug monitoring studies.

Pharmacokinetic evaluation of gabapentin requires a sensitive, selective, and reliable bioanalytical method because the drug is present at low concentrations in human plasma, especially during the elimination phase. Conventional analytical techniques such as UV spectrophotometry and HPLC have been reported for gabapentin estimation; however, these methods often lack sufficient sensitivity and selectivity when applied to complex biological matrices like plasma. Interference from endogenous substances and limited detection capability make these techniques unsuitable for regulatory pharmacokinetic studies.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has emerged as the most powerful and preferred technique for bioanalytical quantification of drugs in biological samples. LC-MS/MS offers high sensitivity, excellent selectivity, rapid analysis, and the ability to accurately quantify analytes in complex matrices. These advantages make LC-MS/MS the method of choice for gabapentin analysis in human plasma, particularly for studies conducted in accordance with regulatory requirements.

Human plasma collected using potassium ethylene diamine tetra acetic acid (K-EDTA) as an anticoagulant is commonly used in bioanalytical studies due to its ability to prevent blood coagulation and preserve sample integrity. The use of K-EDTA plasma ensures consistent sample quality and compatibility with LC-MS/MS analysis. Proper sample preparation techniques, such as protein precipitation, are essential to remove plasma proteins and minimize matrix effects, thereby improving method reliability and reproducibility.[1-4]

Development of a bioanalytical LC-MS/MS method involves optimization of chromatographic conditions, mass spectrometric parameters, sample preparation procedures, and selection of a suitable internal standard. Once developed, the method must be validated to demonstrate its suitability for the intended purpose. Bioanalytical method validation is performed in accordance with regulatory guidelines such as the United States Food and Drug Administration (USFDA) Bioanalytical Method Validation Guidance and the International Council for Harmonisation (ICH) M10 guideline. Validation parameters include selectivity, sensitivity, linearity, accuracy, precision, recovery, matrix effect, stability, carryover, and dilution integrity.

In this context, the present review focuses on the development and validation of a rapid and reliable LC-MS/MS method for the quantification of gabapentin in human plasma using K-EDTA as an anticoagulant. The review emphasizes method development strategies, validation requirements as per USFDA and ICH M10 guidelines, and the regulatory importance of robust bioanalytical methods. This work aims to provide a comprehensive and practical reference for researchers and analysts involved in pharmacokinetic and bioequivalence studies of gabapentin.[5-7]

OVERVIEW OF BIOANALYTICAL METHOD VALIDATION GUIDELINES

Bioanalytical method validation is a critical process that ensures the reliability, accuracy, precision, and reproducibility of analytical data generated from biological samples. In pharmacokinetic, bioavailability, and bioequivalence studies, validated bioanalytical methods are essential for regulatory acceptance of study results. For the quantification of gabapentin in human plasma, method validation is particularly important due to the low plasma concentrations of the drug and the complex nature of biological matrices.[3-4]

Regulatory agencies such as the United States Food and Drug Administration (USFDA) and the International Council for Harmonisation (ICH) have established detailed guidelines to standardize bioanalytical method validation. These guidelines ensure that LC-MS/MS methods used for drug quantification are scientifically sound and suitable for their intended purpose.

1. USFDA Bioanalytical Method Validation Guidelines

The USFDA Bioanalytical Method Validation Guidance provides a comprehensive framework for the development and validation of bioanalytical methods used in regulated studies. According to the USFDA, a bioanalytical method must demonstrate adequate selectivity, sensitivity, linearity, accuracy, precision, recovery, matrix effect, stability, carryover, and dilution integrity.For gabapentin analysis in human plasma using LC-MS/MS, selectivity must be evaluated using blank K-EDTA plasma from multiple sources to ensure the absence of interference from endogenous components at the retention time of gabapentin and the internal standard. Sensitivity is assessed through the determination of the lower limit of quantification (LLOQ), which should be sufficiently low to accurately measure plasma concentrations during all pharmacokinetic phases.Linearity of the calibration curve should be established over an appropriate concentration range, with back-calculated concentrations falling within ±15% of nominal values, except at the LLOQ where ±20% is acceptable. Accuracy and precision must be evaluated at multiple quality control (QC) levels, including LLOQ, low, medium, and high QC samples.The USFDA also emphasizes the evaluation of matrix effects, especially for LC-MS/MS methods, as ion suppression or enhancement can significantly affect quantification accuracy. Stability studies under various conditions such as bench-top, freeze–thaw, long-term storage, and autosampler stability are mandatory to ensure the integrity of gabapentin in K-EDTA plasma samples throughout the analytical process.

2. ICH M10 Bioanalytical Method Validation Guideline

The ICH M10 guideline represents a harmonized international standard for bioanalytical method validation, integrating the requirements of major regulatory agencies including the USFDA, EMA, and PMDA. This guideline strengthens and expands upon earlier validation principles

by introducing a lifecycle approach to bioanalytical methods.

According to ICH M10, validation of LC-MS/MS methods for gabapentin must not only demonstrate acceptable performance during initial validation but also maintain reproducibility throughout the entire study period. The guideline places increased emphasis on matrix effect evaluation, stability studies, and incurred sample reanalysis (ISR) to confirm method reliability under real study conditions.

ICH M10 strongly recommends the use of stable isotope-labeled internal standards for LC-MS/MS analysis to effectively compensate for variability arising from sample preparation and ionization processes. For gabapentin, the use of a deuterated internal standard improves method robustness and regulatory acceptability.

3. Validation Parameters Applicable to Gabapentin LC-MS/MS Method

For the quantification of gabapentin in human plasma using K-EDTA, the following validation parameters are essential as per USFDA and ICH M10 guidelines:

Selectivity: Absence of interference in at least six different K-EDTA plasma lots

Sensitivity (LLOQ): Lowest concentration quantified with acceptable accuracy and precision

Linearity: Demonstrated over the calibration range suitable for pharmacokinetic studies

Accuracy and Precision: Within ±15% (±20% at LLOQ)

Recovery: Consistent and reproducible extraction efficiency

Matrix Effect: Evaluation of ion suppression or enhancement across plasma lots

Stability: Assessment under bench-top, freeze–thaw, long-term, and autosampler conditions

Carryover: Ensuring no significant analyte carryover between injections

Dilution Integrity: Accurate quantification of samples above the calibration range

4. ICH M10 GUIDELINE: HARMONIZATION OF BIOANALYTICAL METHOD VALIDATION

The ICH M10 Bioanalytical Method Validation Guideline was introduced by the International Council for Harmonisation (ICH) to establish a unified and globally accepted framework for the validation of bioanalytical methods. This guideline harmonizes the requirements of major regulatory authorities, including the US Food and Drug Administration (USFDA), European Medicines Agency (EMA), and Pharmaceuticals and Medical Devices Agency (PMDA), thereby reducing regional differences and ensuring consistency in bioanalytical data generated worldwide.

The primary objective of the ICH M10 guideline is to ensure that bioanalytical methods used in pharmacokinetic, bioavailability, and bioequivalence studies are reliable, reproducible, and scientifically robust throughout the entire lifecycle of a drug development program. For the quantification of gabapentin in human plasma using K-EDTA and LC-MS/MS, compliance with ICH M10 is essential to achieve global regulatory acceptance.

LC–MS/MS CONSIDERATIONS RELEVANT TO GABAPENTIN BIOANALYSIS

Gabapentin possesses unique physicochemical properties that influence its behavior during LC–MS/MS analysis. It is a highly polar, zwitterionic compound with low protein binding and lacks strong chromophores, making conventional UV-based analytical techniques less suitable for sensitive plasma quantification. These characteristics necessitate careful optimization of chromatographic and mass spectrometric conditions to achieve accurate and reliable bioanalysis in human plasma.

1. Mass Spectrometric Considerations

Gabapentin is typically analyzed using positive electrospray ionization (ESI+) due to its ability to undergo protonation. In LC–MS/MS analysis, gabapentin forms a stable protonated precursor ion, which can be efficiently monitored using multiple reaction monitoring (MRM). Select appropriateprecursor-to-product ion transitions is critical to achieve high sensitivity and selectivity while minimizing interference from endogenous plasma components.

2. Optimization of mass spectrometric parameters

Collision energy, declustering potential, dwell time, and source conditions plays a vital role in improving signal intensity and reproducibility. Since gabapentin is highly polar, careful control of ionization conditions is required to reduce variability caused by ion suppression.

The use of a stable Isotope-labeled internal standard (e.g., gabapentin-d_{10}) is strongly recommended, as it closely mimics the ionization and fragmentation behavior of the analyte. This helps compensate for variations arising from sample preparation, ionization efficiency, and matrix effects.

3. Chromatographic Considerations

Chromatographic separation of gabapentin presents challenges due to its high polarity and weak retention on conventional reversed-phase columns. To overcome this, LC–MS/MS methods commonly employ C18 columns with optimized mobile phase composition, or alternative approaches such as ion-pairing or hydrophilic interaction chromatography (HILIC).

Mobile phases typically consist of organic solvents such as acetonitrile or methanol combined with volatile additives like formic acid or ammonium acetate to enhance ionization efficiency and improve peak shape. Gradient or isocratic elution modes may be selected depending on the desired balance between resolution and run time.

Short run times are preferred in bioequivalence and pharmacokinetic studies to increase sample throughput. However, rapid analysis must not compromise chromatographic resolution or selectivity from endogenous plasma components.

4. Sample Preparation Considerations

Efficient sample preparation is essential to remove plasma proteins and potential interfering substances. Protein precipitation using organic solvents such as acetonitrile or methanol is the most commonly employed technique for gabapentin analysis due to its simplicity, speed, and suitability for high-throughput analysis.

However, protein precipitation may result in higher matrix effects compared to more selective extraction techniques. Therefore, careful evaluation of matrix effects is mandatory, especially when using electrospray ionization. In some cases, solid-phase extraction (SPE) may be employed to achieve cleaner extracts and improved method robustness, particularly for regulated studies.

Human plasma collected using K-EDTA as an anticoagulant is widely preferred because it prevents clotting and maintains sample stability without interfering with LC–MS/MS detection.

5. Matrix Effect and Ion Suppression

Matrix effects are a critical consideration in LC–MS/MS bioanalysis, particularly for polar compounds like gabapentin. Endogenous plasma components may co-elute with the analyte and cause ion suppression or enhancement, leading to inaccurate quantification.

Evaluation of matrix effects across multiple K-EDTA plasma lots is required as per USFDA and ICH M10 guidelines. The use of a stable isotope-labeled internal standard significantly reduces the impact of matrix-related variability and improves method reliability.

6. Internal Standard Selection

Selection of an appropriate internal standard is a key factor influencing the accuracy and precision of LC–MS/MS methods. For gabapentin bioanalysis, isotope-labeled internal standards are preferred because they exhibit similar extraction recovery, chromatographic behavior, and ionization efficiency as the analyte.

Use of non-isotopic or structurally unrelated internal standards may inadequately compensate for matrix effects and should be avoided in regulatory bioanalytical studies whenever possible.[8-11].

COMPARATIVE REVIEW OF REPORTED LC–MS/MS METHODS

The development of LC–MS/MS methods for the quantification of gabapentin in human plasma has advanced significantly over the past two decades, driven by the growing requirement for highly sensitive, selective, and rapid bioanalytical methods suitable for pharmacokinetic, bioavailability, and bioequivalence studies. Gabapentin presents specific analytical challenges due to its high polarity, lack of chromophoric groups, and poor retention on conventional reversed-phase columns, making LC–MS/MS the technique of choice for its reliable quantification.

Several research groups have reported bioanalytical LC–MS/MS methods for gabapentin determination in human plasma; however, considerable variability exists among these methods with respect to sample preparation strategies, chromatographic conditions, internal standard selection, and extent of validation. These methodological differences significantly influence sensitivity, robustness, and regulatory acceptability of the reported methods.

Most published methods employ reversed-phase liquid chromatography, predominantly using C18 stationary phases, despite gabapentin's highly polar nature. In such cases, chromatographic retention is often achieved through optimization of mobile phase composition, including the use of volatile acidic or buffered modifiers such as formic acid, ammonium acetate, or ammonium formate. More recently, hydrophilic interaction liquid chromatography (HILIC) has been increasingly explored to improve retention and peak shape for gabapentin. Electrospray ionization in positive ion mode is consistently reported across studies due to the presence of basic amine functional groups in the gabapentin molecule.

Earlier LC–MS/MS methods were characterized by longer chromatographic run times, often exceeding five minutes, which limited sample throughput. In contrast, recent methods emphasize shorter run times (≤5 minutes) without compromising chromatographic resolution, thereby enhancing suitability for high-throughput bioanalysis.

Sample preparation approaches reported for gabapentin bioanalysis vary widely and include protein precipitation (PPT), liquid–liquid extraction (LLE), and solid-phase extraction (SPE). Protein precipitation using acetonitrile or methanol is the most commonly employed technique due to its simplicity, speed, and low cost. However, PPT-based methods are often associated with higher matrix effects and ion suppression, which may adversely affect quantitative accuracy if not thoroughly evaluated. In contrast, SPE-based methods provide cleaner extracts, improved recovery, and reduced matrix effects, albeit at the expense of additional sample preparation steps.

Internal standard (IS) selection also varies among reported methods. While stable isotope-labeled gabapentin (e.g., gabapentin-d_6) is considered the most appropriate internal standard due to its ability to effectively compensate for extraction variability and matrix effects, several studies continue to employ structurally unrelated or non-isotopically labeledanalogs. Although such choices may be driven by availability or cost constraints, they may limit method robustness, particularly under the more stringent validation expectations outlined in ICH M10 bioanalytical guidelines.

Table 1: Comparison of Reported LC–MS/MS Bioanalytical Methods for TZD in Human Plasma[12-18]

Parameter	Reported Method 1	Reported Method 2	Reported Method 3	Remarks / Compariso
Biological Matrix	Human plasma (K-EDTA)	Human plasma (K-EDTA)	Human plasma (K-EDTA)	KEDTA plasma is most commonly -used in PK and BA/BE studie
Sample Preparation	Protein precipitation (ACN / MeOH)	Solid-phase extraction (SPE)	Liquid–liquid extraction (LLE)	PPT is rapid but show higher matrix effect; SPE gives cleaner extract
Extraction Recovery (%)	60–80%	85–95%	70–85	SPE provides highest and consistent recovery
Chromatographic Column	C18 column	HILIC column	C18 column	HILIC offers better retention for polar gabapentin
Mobile Phase	ACN : Water + Formic acid	ACN: Ammonium formate	buffer Me OH : Buffer	Volatile buffers improve MS response
Ionization Mode	ESI (+)	ESI (+)	ESI (+)	Positive mode preferred due to amine groups
MRM Transition (Gabapentin)	m/z 172 → 154	m/z 172 → 154	m/z 172 → 137	Similar precursor ions used across studies
Internal Standard	Structural analog	Gabapentin-d_6 (isotope-labelled)	Structural analog	Isotope-labelled IS minimizes matrix effect
Run Time	5–7 min	2–4 min	6–8 min	Shorter run time improves throughput
Calibration Range (ng/mL)	50–10,000	10–10,000	25–8,000	Wider linearity enhances clinical applicability
LLOQ (ng/mL)	50	10	25	SPE + HILIC achieves lowest LLOQ
Precision (%CV)	≤10%	≤15%	≤15%	All comply with regulatory limits
Accuracy (%)	90–110%	95–105%	90–110	Acceptable as per FDA/ICH guidelines
Matrix Effect (%)	75–90%	95–105%	80–95%	Clean extraction reduces ion suppression
Stability Studies	Short-term, freeze-thaw	Full stability (bench-top, autosampler	Partial stability	Comprehensive stability strengthens validation
Regulatory Compliance	FDA	FDA / ICH M10	FDA	Recent methods align with ICH M10 guidance

Critical Interpretation of Comparative Data for Gabapentin

A critical examination of the reported LC–MS/MS methods for gabapentin quantification in human plasma reveals that, while most studies achieve acceptable levels of sensitivity and selectivity, consistency in compliance with regulatory validation requirements remains variable. Many published methods successfully demonstrate quantification of gabapentin within therapeutically relevant concentration ranges; however, the extent and rigor of method validation differ substantially across studies.

Methods employing stable isotope-labeled internal standards, such as gabapentin-d_6, consistently demonstrate superior control over matrix effects, extraction variability, and ionization efficiency, resulting in improved reproducibility and accuracy. These approaches align more closely with the expectations outlined in both USFDA bioanalytical guidelines and the harmonized ICH M10 guideline. In contrast, methods relying on non-isotopic or structurally unrelated internal standards may inadequately compensate for variability introduced during sample preparation and electrospray ionization, particularly in methods utilizing protein precipitation as the sole extraction technique.

Matrix effect evaluation, now recognized as a critical regulatory parameter, is insufficiently described or entirely absent in several reported gabapentin assays. Given the highly polar nature of gabapentin and its early elution in reversed-phase chromatography, the risk of ion suppression or enhancement from co-eluting endogenous plasma components is significant. Failure to comprehensively evaluate and report matrix effects limits the regulatory applicability and robustness of such methods.

Similarly, stability studies in many publications are restricted to short-term or bench-top stability, with limited assessment of long-term frozen stability, freeze–thaw stability, or autosampler stability. Comprehensive stability evaluation is essential to ensure data integrity during routine bioanalytical workflows, particularly for pharmacokinetic and bioequivalence studies involving extended sample storage and repeated analysis.

Another notable limitation across several reports is the lack of explicit alignment with harmonized regulatory guidelines, particularly ICH M10. Although many authors claim compliance with USFDA guidance, fewer studies address newer regulatory expectations such as incurred sample reanalysis (ISR), cross-validation, and lifecycle management

of bioanalytical methods. This omission reflects a gap between methodological development and evolving global regulatory requirements.

Overall, the comparative analysis indicates that while technically sound LC–MS/MS methods for gabapentin quantification are widely available, their regulatory robustness and transparency vary considerably. These findings emphasize the necessity of adopting harmonized validation practices, comprehensive matrix effect and stability evaluations, and transparent reporting in accordance with ICH M10 and USFDA guidelines. Such practices are essential to ensure that bioanalytical data generated for gabapentin are reliable, reproducible, and acceptable for global regulatory submissions.

CRITICAL EVALUATION OF BIOANALYTICAL METHOD VALIDATION PARAMETERS FOR GABAPENTIN

Bioanalytical method validation ensures that an analytical procedure is reliable, reproducible, and fit for its intended purpose. Regulatory authorities such as the United States Food and Drug Administration (USFDA) and, more recently, the International Council for Harmonisation (ICH) through the M10 guideline, have established comprehensive validation criteria to harmonize bioanalytical data generation across laboratories and regulatory regions.

In the context of LC–MS/MS assays for gabapentin quantification in human plasma, a critical evaluation of validation parameters reveals both methodological strengths and recurring deficiencies in the published literature. Although numerous methods have been reported, variability in validation rigor and regulatory alignment remains evident.

1. Selectivity and Specificity

Selectivity refers to the ability of a bioanalytical method to unequivocally distinguish gabapentin and the internal standard from endogenous plasma components, metabolites, and potential co-administered drugs. According to USFDA and ICH M10 guidelines, selectivity must be demonstrated using at least six independent sources of blank human plasma, including lipemic and hemolyzed matrices where appropriate.

Most reported LC–MS/MS methods for gabapentin claim acceptable selectivity based on the absence of interfering peaks at the retention times of the analyte and internal standard. However, several studies provide limited chromatographic evidence, often presenting only representative chromatograms without quantitative evaluation of interference relative to the lower limit of quantification (LLOQ). Such qualitative demonstrations may be insufficient under ICH M10, which emphasizes transparent and quantitative assessment.

Additionally, only a limited number of studies evaluate potential interference from gabapentin metabolites or co-administered antiepileptic and analgesic drugs, despite the frequent clinical use of gabapentin in polypharmacy settings. The omission of such evaluations may restrict the applicability of these methods in real-world pharmacokinetic and bioequivalence studies.

2. Linearity and Calibration Curve Performance

Linearity assessment ensures that the analytical response is directly proportional to gabapentin concentration over the defined calibration range. Regulatory guidelines require calibration curves to include a minimum of six to eight non-zero standards, with back-calculated concentrations within ±15% of nominal values (±20% at LLOQ).

Most published LC–MS/MS methods for gabapentin demonstrate acceptable linearity across low nanogram to microgram per milliliter ranges, suitable for therapeutic pharmacokinetic profiling. However, differences in calibration range selection and justification are apparent. Some studies report narrow calibration ranges without adequate justification for their clinical relevance, while others extend calibration to higher concentrations without demonstrating robustness at the lower end.

ICH M10 further emphasizes consistent calibration model selection, weighting factors, and documentation of regression parameters, which are often underreported in published gabapentin methods. Such omissions reduce method reproducibility and regulatory transparency.

3. Accuracy and Precision

Accuracy and precision are fundamental indicators of method reliability and are evaluated using quality control (QC) samples at LLOQ, low, medium, and high concentration levels. Both USFDA and ICH M10 specify acceptance criteria of ±15% for accuracy and ≤15% CV for precision (±20% at LLOQ).

Most reported gabapentin LC–MS/MS methods demonstrate acceptable intra-day and inter-day accuracy and precision. However, several publications present aggregated performance data, without clear distinction between within-run and between-run variability. While common in earlier literature, this practice does not fully align with current regulatory expectations.

Furthermore, some studies rely on a limited number of validation batches, which may inadequately capture analytical variability, particularly when protein precipitation is employed as the primary sample preparation technique.

4. Recovery and Matrix Effect

Recovery and matrix effect assessments are particularly critical for gabapentin due to its high polarity and early elution, which increase susceptibility to ion suppression or enhancement during electrospray ionization.

While most published methods report acceptable recovery values, the extent of matrix effect evaluation varies considerably. Several studies either omit matrix effect experiments entirely or provide only qualitative discussion without quantitative data. This represents a significant limitation, as both USFDA and ICH M10 explicitly require systematic matrix effect assessment across multiple plasma lots.

Methods employing stable isotope-labeled internal standards (e.g., gabapentin-d_6) consistently demonstrate

improved control of matrix effects, supporting regulatory recommendations favoring isotopically labeled standards. In contrast, methods using non-deuterated analogs may inadequately compensate for ionization variability, especially when simple protein precipitation is used.

5. Stability Studies

Stability assessment ensures that gabapentin remains stable under conditions encountered during sample collection, storage, processing, and analysis. Regulatory guidelines require evaluation of bench-top, freeze–thaw, long-term frozen, and autosampler stability.

Many reported gabapentin methods demonstrate acceptable short-term and freeze–thaw stability; however, long-term stability data are frequently limited or insufficiently justified. In some cases, stability is evaluated at only a single concentration level, contrary to guideline recommendations requiring assessment at both low and high QC levels.

ICH M10 places additional emphasis on stability under conditions representative of actual study workflows, including extended autosampler residence times. Failure to address these aspects may limit the suitability of reported methods for large-scale or multicenter pharmacokinetic studies.

6. Carryover and Dilution Integrity

Carryover assessment ensures that high-concentration gabapentin samples do not contaminate subsequent injections. Although most methods claim negligible carryover, explicit experimental design and acceptance criteria are often lacking.

Dilution integrity, which evaluates the ability of the method to accurately quantify samples exceeding the upper limit of quantification after dilution, is inconsistently reported. Given the variability in gabapentin plasma concentrations across dosing regimens, omission of dilution integrity studies represents a notable validation gap.

Table 2: Evaluation of Validation Parameters in Reported Gabapentin LC–MS/MS Methods

Validation Parameter	USFDA Requirement	ICH M10 Emphasis	Compliance in Literature
Selectivity	Mandatory	Mandatory	Generally acceptable, limited reporting
Linearity	±15% (±20% LLOQ)	Harmonized	Mostly compliant
Accuracy & Precision.	≤15% CV	Lifecycle-based	Compliant, limited batch data
Matrix Effect	Recommended	Strongly emphasized	Frequently underreported
Recovery	Consistent & reproducible	Required	Generally acceptable
Stability	Multiple conditions	Extended conditions	Partial compliance
Carryover	Mandatory	Mandatory	Limited documentation
Dilution Integrity	Required	Required	Often omitted

Identification of Non-Compliance Gaps in Reported Lc–Ms/Ms Methods for Gabapentin

Despite the availability of numerous LC–MS/MS methods for the quantification of gabapentin in human plasma, a detailed regulatory-oriented evaluation reveals several recurring non-compliance gaps that may limit their suitability for use in regulated pharmacokinetic and bioequivalence studies. Although most reported methods demonstrate acceptable analytical performance, deficiencies in validation design, documentation, and alignment with current regulatory expectations significantly affect their regulatory robustness and global acceptability.

One of the most prominent gaps observed in reported gabapentin LC–MS/MS methods is the incomplete evaluation of matrix effects. Gabapentin is a highly polar and zwitterionic compound, making it particularly susceptible to ion suppression or enhancement during electrospray ionization, especially when simple protein precipitation is used as the primary sample preparation technique. Several published methods either omit matrix effect studies entirely or provide limited qualitative assessments using a single plasma source. According to USFDA and ICH M10 guidelines, matrix effects must be quantitatively evaluated using multiple independent plasma lots, including hemolyzed and lipemic matrices where applicable. Failure to adequately assess matrix effects raises concerns regarding method robustness and reproducibility when applied to diverse clinical samples.

Another significant non-compliance issue relates to internal standard selection. While stable isotope-labeled gabapentin is strongly recommended due to its ability to closely mimic the analyte's extraction efficiency and ionization behavior, many reported methods rely on non-isotopic or structurally unrelated internal standards. Such internal standards may inadequately compensate for variability introduced during sample preparation and ionization, particularly given gabapentin's poor chromatographic retention and high polarity. The continued use of non-deuterated internal standards represents a regulatory limitation, as both USFDA and ICH M10 increasingly favor isotopically labeled compounds to ensure analytical reliability.

Stability testing represents another area of partial compliance in published gabapentin methods. Although most studies report acceptable bench-top and freeze–thaw stability, long-term frozen stability and autosampler stability are often insufficiently evaluated or inadequately justified. In several cases, stability assessments are performed at only a single concentration level, contrary to guideline recommendations requiring evaluation at both low and high quality control levels. ICH M10 emphasizes stability assessment under

conditions reflective of actual bioanalytical workflows, and insufficient stability data may compromise confidence in pharmacokinetic results, particularly in large or multicenter studies.

Carryover assessment is also inconsistently addressed in reported gabapentin LC–MS/MS methods. While many authors state that carryover was negligible, detailed experimental designs, acceptance criteria, and quantitative results are frequently omitted. Given that gabapentin is administered at relatively high therapeutic doses, plasma concentrations can vary widely, making rigorous carryover evaluation essential to avoid contamination of subsequent injections. The lack of comprehensive carryover documentation represents a notable regulatory gap.

Dilution integrity studies are another commonly omitted validation parameter. Dilution integrity ensures that samples exceeding the upper limit of quantification can be accurately measured after dilution, which is particularly relevant for gabapentin due to dose-dependent variability, interindividual pharmacokinetic differences, and potential accumulation in patients with renal impairment. The absence of dilution integrity evaluation limits the applicability of several reported methods in real-world clinical scenarios and does not align with current regulatory expectations.

Incurred sample reanalysis is rarely addressed in published gabapentin LC–MS/MS methods. Although ISR was not consistently required in earlier regulatory guidance, ICH M10 strongly encourages its inclusion to confirm method reproducibility using real study samples. The absence of ISR in most published methods reflects misalignment with contemporary regulatory standards and may affect the acceptability of such methods in current and future regulatory submissions.

Finally, insufficient documentation and transparency in reporting validation experiments remain a recurring concern. Several studies provide summarized validation results without adequate details regarding the number of validation runs, replicates, acceptance criteria, or statistical treatment of data. Such practices limit reproducibility, complicate regulatory assessment, and reduce the scientific value of the published methods.

Overall, these non-compliance gaps indicate that while many LC–MS/MS methods for gabapentin are analytically sound, their regulatory rigor varies considerably. Addressing these deficiencies through comprehensive matrix effect evaluation, appropriate internal standard selection, complete stability testing, inclusion of dilution integrity and incurred sample reanalysis, and transparent reporting is essential to ensure that bioanalytical data generated using gabapentin LC–MS/MS methods are acceptable for global regulatory submissions under USFDA and ICH M10 guidelines.[24-29]

PRACTICAL RECOMMENDATIONS FOR BIOANALYTICAL SCIENTISTS (GABAPENTIN)

Based on the critical evaluation of reported LC–MS/MS methods for gabapentin quantification in human plasma and the identified regulatory gaps, several practical recommendations can be proposed to support the development of robust and regulatory-compliant bioanalytical assays. These recommendations aim to ensure that gabapentin bioanalysis achieves not only acceptable analytical performance but also compliance with current USFDA and ICH M10 requirements for regulated pharmacokinetic and bioequivalence studies.

A key consideration in gabapentin bioanalysis is the selection of an appropriate internal standard. Gabapentin is a highly polar, zwitterionic compound with limited chromatographic retention under conventional reversed-phase conditions, making it particularly sensitive to ionization variability. Whenever feasible, a stable isotope-labeled internal standard such as gabapentin-d_6 should be employed, as it closely matches the analyte's extraction behavior and ionization efficiency. This approach provides superior compensation for matrix effects and sample preparation variability. When isotopically labeled standards are unavailable, structurally similar analogs may be used; however, their limitations should be clearly acknowledged, and additional validation experiments should be conducted to demonstrate adequate control of matrix-related effects.

The choice of sample preparation technique should be guided by a balance between simplicity, throughput, and analytical robustness. Protein precipitation is commonly used for gabapentin due to its operational simplicity and high throughput; however, this approach often results in greater exposure to endogenous plasma components, increasing the risk of ion suppression. For studies requiring higher regulatory confidence, such as pivotal bioequivalence trials, liquid–liquid extraction or solid-phase extraction may offer improved selectivity and reduced matrix interference. Regardless of the extraction strategy employed, recovery and matrix effect assessments should be rigorously performed across multiple independent plasma sources to ensure reproducibility.

During chromatographic and mass spectrometric optimization, priority should be given not only to achieving rapid run times but also to ensuring sufficient separation of gabapentin from early-eluting endogenous compounds. Optimization of mobile phase composition, buffer strength, and ionization conditions is particularly critical for gabapentin due to its poor retention and high polarity. Transparent reporting of MRM transitions, collision energies, dwell times, and source parameters is essential to support method reproducibility and regulatory review.

Validation studies must be designed in full accordance with USFDA and ICH M10 guidelines. Accuracy and precision should be evaluated using multiple independent validation runs, with clear distinction between intra-day and inter-day performance. Calibration curve models, weighting factors, and acceptance criteria should be explicitly stated and scientifically justified. Stability assessments should include bench-top stability, freeze–thaw stability, long-term frozen stability, and autosampler stability, evaluated at both low and high quality control levels to reflect realistic bioanalytical workflows.

Special attention should be given to carryover and dilution integrity evaluations, which are frequently underreported in gabapentin LC–MS/MS methods. Carryover should be systematically assessed following injections at the upper limit of quantification to ensure that high-concentration samples do not contaminate subsequent analyses. Dilution

integrity studies are particularly important for gabapentin, as plasma concentrations may vary widely depending on dose, renal function, and patient population. Accurate quantification of diluted samples must therefore be demonstrated.

With increasing regulatory emphasis on bioanalytical method lifecycle management, incurred sample reanalysis should be incorporated wherever applicable. ISR provides confirmation of method reproducibility using real study samples and is strongly encouraged under ICH M10. Inclusion of ISR enhances confidence in the reliability of gabapentin pharmacokinetic and bioequivalence data.

Finally, comprehensive documentation and transparent reporting of validation experiments are essential. Detailed description of experimental design, number of validation batches, acceptance criteria, and statistical treatment of data facilitates regulatory assessment and enhances the scientific value of published gabapentin LC–MS/MS methods. By adopting these practices, bioanalytical scientists can develop gabapentin assays that are analytically sound, reproducible, and fully aligned with current and future global regulatory expectations.[30-34]

FUTURE REGULATORY EXPECTATIONS AND ANALYTICAL TRENDS FOR GABAPENTIN

The regulatory landscape governing bioanalytical method validation continues to evolve in response to the growing demand for harmonization, reproducibility, and data integrity in global drug development programs. The implementation of the ICH M10 guideline represents a major advancement toward unified international standards, and its impact on LC–MS/MS bioanalysis of highly polar compounds such as gabapentin is expected to be significant.

One of the most important future regulatory expectations is the broader adoption of lifecycle-based bioanalytical method management. Regulatory authorities increasingly view method validation not as a one-time activity but as an ongoing process requiring continuous performance monitoring throughout clinical development and post-approval phases. For gabapentin, which is widely used across multiple indications and formulations, this approach necessitates repeated evaluation of method robustness when applied across different studies, laboratories, analytical platforms, and patient populations, particularly those with altered renal function.

Incurred sample reanalysis is expected to become an integral component of regulatory submissions involving gabapentin. Although ISR was selectively applied under earlier guidance, ICH M10 emphasizes its importance in confirming method reproducibility using real study samples. Future gabapentin bioanalytical methods are therefore expected to routinely incorporate ISR, especially in pivotal pharmacokinetic and bioequivalence studies, to ensure confidence in reported concentration data.[35-39]

Advancements in LC–MS/MS instrumentation are also shaping analytical trends in gabapentin bioanalysis. Modern mass spectrometers provide enhanced sensitivity, improved robustness, and faster acquisition rates, enabling lower limits of quantification and shorter chromatographic run times. These improvements are particularly beneficial for gabapentin, which exhibits poor retention under conventional reversed-phase conditions. However, despite these technological advances, regulatory authorities continue to prioritize data quality, selectivity, and reproducibility over analytical speed. Method developers must therefore ensure that reduced run times do not compromise chromatographic separation or validation rigor.

An emerging regulatory and analytical trend is the increased emphasis on matrix effect characterization and mitigation. Gabapentin's high polarity and early elution make it especially vulnerable to ion suppression from endogenous plasma components. As bioanalytical methods are increasingly applied to diverse patient populations, including those with varying physiological and pathological conditions, regulatory expectations are likely to further emphasize comprehensive matrix effect evaluation using multiple plasma sources, including hemolyzed and lipemic samples.

Automation and high-throughput bioanalysis are also expected to play an expanding role in future gabapentin studies, particularly in large-scale clinical trials and post-marketing investigations. Automated sample preparation systems and integrated data processing platforms can improve reproducibility, reduce analyst-dependent variability, and enhance laboratory efficiency. However, the adoption of such technologies must be accompanied by appropriate validation, cross-validation, and system suitability assessments to meet regulatory requirements.

Finally, there is a growing regulatory emphasis on data integrity, transparency, and traceability, aligned with broader initiatives addressing good laboratory practices and electronic data management. For gabapentin bioanalysis, clear documentation of method development, validation, and ongoing performance, along with adherence to standardized reporting formats, will be essential to maintain regulatory confidence. Transparent reporting of analytical parameters, validation outcomes, and any method modifications throughout the method lifecycle will be critical under the ICH M10 framework.

Overall, future LC–MS/MS methods for gabapentin are expected to reflect a convergence of advanced analytical technologies and increasingly stringent regulatory oversight. Bioanalytical scientists who proactively align their method development, validation, and lifecycle management strategies with these evolving regulatory expectations will be better positioned to generate gabapentin bioanalytical data that are scientifically robust, reproducible, and globally acceptable.[40-51]

CONCLUSION

The accurate and reliable quantification of gabapentin in human plasma is essential for pharmacokinetic, bioavailability, and bioequivalence studies that support both clinical development and regulatory submissions. Owing to its high sensitivity, selectivity, and suitability for complex biological matrices, LC–MS/MS has become the analytical technique of choice for gabapentin bioanalysis. However, as highlighted in this critical review, substantial variability exists among reported LC–MS/MS methods in terms of methodological design, validation rigor, and alignment with current regulatory expectations.

Although most published methods achieve adequate sensitivity and acceptable chromatographic performance for gabapentin quantification, inconsistencies in adherence to bioanalytical method validation guidelines are evident. Common limitations include incomplete evaluation of matrix effects, suboptimal selection of internal standards, restricted stability assessments, and insufficient documentation of carryover, dilution integrity, and incurred sample reanalysis. These deficiencies do not necessarily compromise the analytical capability of the methods but may significantly limit their suitability for regulated pharmacokinetic and bioequivalence studies, particularly under the harmonized framework established by the ICH M10 guideline.

This review emphasizes the importance of adopting a guideline-driven and lifecycle-based approach to the development and validation of gabapentin bioanalytical methods. Alignment with both USFDA and ICH M10 requirements, combined with comprehensive validation design and transparent reporting, is essential to ensure data integrity, reproducibility, and global regulatory acceptability. The practical recommendations outlined in this review are intended to assist bioanalytical scientists in designing robust and reliable LC–MS/MS assays for gabapentin that meet contemporary regulatory standards.

In conclusion, future method development and research efforts in gabapentin bioanalysis should prioritize regulatory compliance alongside analytical performance. By integrating advances in LC–MS/MS technology with rigorous validation practices and proactive consideration of evolving regulatory expectations, bioanalytical laboratories can generate high-quality data that effectively support the clinical evaluation and regulatory approval of gabapentin and gabapentin-containing pharmaceutical products.

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