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Review Article

LC–MS/MS-Based Bioanalytical Methods for the Quantification of Elobixibat in Human Plasma: Development, Validation, and Regulatory Perspectives

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ABSTRACT

Elobixibat is a novel, minimally absorbed ileal bile acid transporter (IBAT) inhibitor approved for the management of chronic constipation and constipation-predominant irritable bowel syndrome. Due to its low systemic exposure and complex biological matrix, the accurate quantification of elobixibat in human plasma is analytically challenging yet essential for pharmacokinetic, bioavailability, bioequivalence, and clinical safety studies. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has emerged as the preferred technique for the bioanalysis of elobixibat owing to its high sensitivity, selectivity, and robustness. This review provides a comprehensive and critical evaluation of reported LC–MS/MS bioanalytical methods developed for the determination of elobixibat in human plasma. Key aspects of method development, including selection of biological matrix, internal standard, sample preparation strategies, chromatographic separation, and mass spectrometric detection, are discussed in detail. Furthermore, bioanalytical method validation parameters such as selectivity, linearity, accuracy, precision, recovery, matrix effect, stability, and carryover are systematically reviewed in accordance with current regulatory guidelines issued by the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and the International Council for Harmonisation (ICH M10). The comparative assessment of published methods highlights variations in analytical performance, sensitivity, and regulatory compliance, thereby identifying critical challenges and best practices in elobixibat bioanalysis. In addition, the application of validated LC–MS/MS methods to pharmacokinetic and clinical studies is summarized, along with recent advancements in high-throughput and ultra-sensitive bioanalytical approaches. Overall, this review aims to serve as a useful reference for researchers and bioanalytical scientists by providing practical insights into the development, validation, and regulatory acceptance of LC–MS/MS methods for the quantification of elobixibat in human plasma.

Keywords: Elobixibat, LC–MS/MS, Bioanalysis, Human plasma, Bioanalytical method validation, Pharmacokinetics, Bioavailability, Bioequivalence, Sample preparation, Mass spectrometry, Chromatographic separation, Internal standard, Matrix effect.

INTRODUCTION

Elobixibat is a minimally absorbed, first-in-class ileal bile acid transporter (IBAT) inhibitor developed for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome.[1,19] By selectively inhibiting bile acid reabsorption in the terminal ileum, elobixibat increases the delivery of bile acids to the colon, thereby enhancing colonic secretion and motility. Due to its targeted mechanism of action and low systemic exposure, accurate and sensitive quantification of elobixibat in human plasma is essential for pharmacokinetic, bioavailability, bioequivalence, and clinical safety studies.

The determination of drug concentrations in biological matrices presents significant analytical challenges, particularly when the analyte exhibits low plasma levels, high protein binding, or matrix-related interferences. Conventional analytical techniques such as high-performance liquid chromatography with ultraviolet detection often lack the sensitivity and selectivity required for trace-level quantification of such compounds.[6,12] In this context, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has emerged as the gold standard technique for bioanalytical applications due to its superior sensitivity, specificity, and wide dynamic range.[4,5]

Bioanalytical method development involves the systematic optimization of sample preparation, chromatographic separation, and mass spectrometric detection to ensure reliable quantification of analytes in biological matrices. Furthermore, method validation, as outlined by regulatory agencies such as the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and the International Council for Harmonisation (ICH M10), is mandatory to demonstrate the suitability of the method for its intended purpose.[1–3] Parameters including selectivity, linearity, accuracy, precision, recovery, matrix effect, and stability must be rigorously evaluated.

Several LC–MS/MS methods have been reported for the quantification of elobixibat in human plasma; however, variations exist in terms of sample preparation techniques, chromatographic conditions, mass spectrometric parameters, and validation approaches. A critical evaluation of these reported methods is necessary to identify optimal analytical strategies, common challenges, and regulatory compliance gaps.

The present review aims to comprehensively summarize and critically analyze published LC–MS/MS bioanalytical methods for the determination of elobixibat in human plasma. Emphasis is placed on method development strategies, validation parameters in accordance with regulatory guidelines, and their application to pharmacokinetic studies. This review is expected to serve as a valuable reference for researchers and analysts involved in bioanalytical method development and regulatory submissions.

Physicochemical and Pharmacokinetic Properties of Elobixibat

A thorough understanding of the physicochemical and pharmacokinetic properties of a drug molecule is essential for the successful development of sensitive and reliable bioanalytical methods. These properties directly influence sample preparation strategies, chromatographic behavior, ionization efficiency, and overall method performance in LC–MS/MS analysis. Elobixibat exhibits distinct physicochemical and pharmacokinetic characteristics that must be carefully considered during bioanalytical method development.

Elobixibat is a small-molecule inhibitor of the ileal bile acid transporter with a relatively high molecular weight and lipophilic nature. The compound possesses functional groups that enable efficient ionization under electrospray ionization conditions, making it suitable for LC–MS/MS analysis. However, its poor aqueous solubility and high plasma protein binding can pose challenges during extraction and quantification from biological matrices.[7,21,32]

From a pharmacokinetic perspective, elobixibat demonstrates minimal systemic absorption following oral administration, which results in very low plasma concentrations.[14,49,50] Consequently, highly sensitive analytical methods are required to accurately quantify elobixibat over the relevant concentration range. The drug is primarily localized within the gastrointestinal tract, and only a small fraction reaches systemic circulation. This pharmacokinetic behavior necessitates the use of advanced sample preparation techniques and optimized mass spectrometric conditions to achieve acceptable lower limits of quantification.

Elobixibat exhibits extensive plasma protein binding, which can influence extraction recovery and matrix effects during bioanalysis. Additionally, its elimination half-life and metabolic profile play a critical role in determining suitable sampling time points and calibration ranges for pharmacokinetic studies. Understanding these parameters supports the rational selection of internal standards and validation strategies to ensure method robustness and reproducibility.

A summary of the key physicochemical and pharmacokinetic properties of elobixibat reported in the literature is presented in Table 1. This information provides a scientific basis for the selection of appropriate bioanalytical techniques and supports the interpretation of analytical performance characteristics reported for LC–MS/MS methods.

Table 1: Physicochemical and pharmacokinetic properties of elobixibat

Property	Description / Reported Characteristics	Relevance to Bioanalysis
Chemical class	Ileal bile acid transporter (IBAT) inhibitor	Determines mechanism-specific PK behavior
Molecular nature	Small-molecule, lipophilic compound	Influences extraction efficiency and matrix effects
	Relatively high molecular weight (reported > 500 Da)	Requires sensitive MS detection
Chemical structure	Contains ionizable functional groups	Suitable for ESI-based LC–MS/MS analysis
Aqueous solubility	Poor aqueous solubility	Necessitates organic solvents during sample preparation
Lipophilicity	High (lipophilic character)	Contributes to plasma protein binding and matrix effects
Plasma protein binding	High	Affects extraction recovery and ion suppression
Route of administration	Oral	Relevant for pharmacokinetic and BA/BE studies
Site of action	Gastrointestinal tract (local action)	Explains minimal systemic exposure
Systemic absorption	Minimal	Leads to very low plasma concentrations
Plasma concentration levels	Low nanogram to picogram range	Demands ultra-sensitive LC–MS/MS methods
Metabolic profile	Limited systemic metabolism reported	Simplifies metabolite interference considerations
Elimination half-life	Short to moderate (reported)	Guides sampling time points
Pharmacokinetic behavior	Low exposure, localized activity	Major analytical challenge in bioanalysis

Overview of Bioanalytical Method Development [10,11,45]

Bioanalytical method development is a systematic and critical process designed to establish a reliable, sensitive, and selective analytical method for the quantitative determination of drugs in biological matrices. For compounds such as elobixibat, which exhibit very low systemic exposure and complex matrix interactions, careful optimization of each step in the analytical workflow is essential to achieve accurate and reproducible quantification in human plasma.

The initial stage of bioanalytical method development involves the selection of an appropriate biological matrix. Human plasma is most commonly employed for pharmacokinetic, bioavailability, and bioequivalence studies. Factors such as the choice of anticoagulant, sample collection procedures, storage temperature, and stability during freeze–thaw cycles must be well controlled, as these variables can significantly influence analyte stability and assay performance.

Selection of a suitable internal standard (IS) represents a crucial component of method development. Ideally, a stable isotope-labeled internal standard is preferred because it closely mimics the physicochemical behavior of elobixibat during sample preparation, chromatographic separation, and mass spectrometric detection. In situations where isotope-labeled standards are unavailable, structurally related compounds may be employed, provided they demonstrate consistent extraction recovery and negligible matrix interference.

Sample preparation is a key determinant of method sensitivity, selectivity, and robustness. Commonly employed sample preparation techniques for elobixibat include protein precipitation, liquid–liquid extraction, and solid-phase extraction. Protein precipitation offers simplicity and high sample throughput but may result in significant matrix effects. Liquid–liquid extraction provides improved sample cleanliness and recovery, while solid-phase extraction offers superior selectivity and reproducibility, making it suitable for trace-level quantification despite increased operational complexity.

Chromatographic method development focuses on achieving adequate analyte retention, resolution from endogenous components, and acceptable peak symmetry within a reasonable run time. Optimization of column chemistry, mobile phase composition, gradient conditions, and flow rate is essential to minimize co-eluting interferences. Furthermore, the selection of appropriate mobile phase additives plays a significant role in enhancing ionization efficiency and signal stability during LC–MS/MS analysis.

Mass spectrometric optimization involves the selection of an appropriate ionization mode, identification of precursor and product ions, and optimization of source-dependent parameters to maximize analytical sensitivity and selectivity. Multiple reaction monitoring (MRM) is widely employed for quantitative bioanalysis due to its high specificity and suitability for complex biological matrices.

A generalized workflow summarizing the major steps involved in LC–MS/MS bioanalytical method development for elobixibat is illustrated in Figure 1. This structured approach ensures that the method is adequately optimized prior to validation and routine application in clinical and regulatory studies.

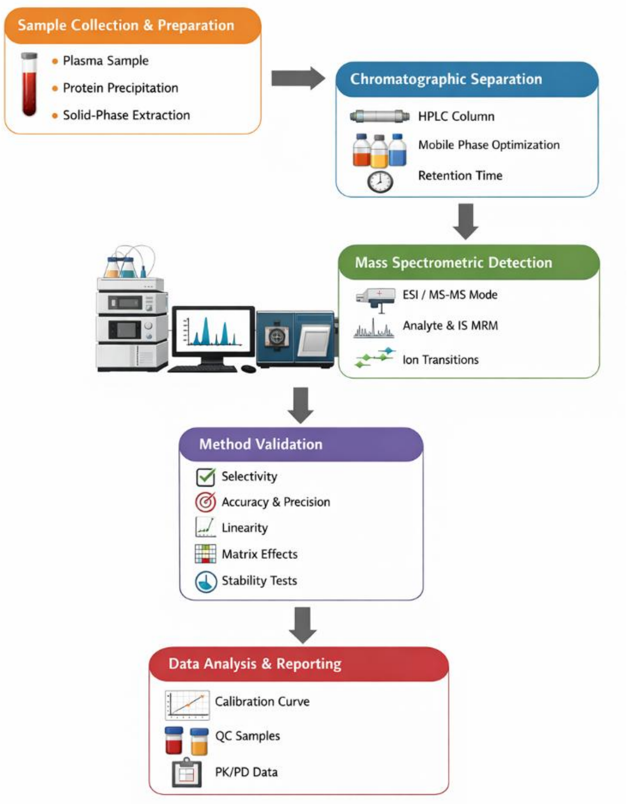

Figure 1: General workflow of LC–MS/MS bioanalytical method development for elobixibat in human plasma.

LC–MS/MS Method Development (Chromatographic and Mass Spectrometric Conditions)[14-18]

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the technique of choice for the bioanalysis of elobixibat in human plasma due to its high sensitivity, selectivity, and suitability for trace-level quantification. Method development primarily focuses on optimizing chromatographic separation and mass spectrometric detection to minimize matrix interference and achieve reliable quantification across the required concentration range.

Chromatographic Method Development

Chromatographic optimization is essential to ensure adequate retention, resolution from endogenous plasma components, and acceptable peak shape for elobixibat and the internal standard. Reversed-phase liquid chromatography is commonly employed, with C18 or similar stationary phases being preferred due to their compatibility with moderately lipophilic compounds such as elobixibat.

Column selection plays a critical role in achieving optimal retention and reducing co-elution of matrix components.

The composition of the mobile phase significantly influences chromatographic performance and ionization efficiency. Binary solvent systems consisting of an aqueous phase, typically water containing a volatile buffer or acid, and an organic phase such as acetonitrile or methanol are widely used. The addition of formic acid or ammonium formate enhances peak symmetry and improves ionization under electrospray ionization conditions. Gradient elution is often preferred over isocratic conditions to improve analyte resolution while maintaining short run times suitable for high-throughput analysis.

Flow rate, column temperature, and injection volume are optimized to achieve consistent retention time, minimal backpressure, and reproducible peak response. Short analytical run times are desirable for clinical studies involving large sample numbers; however, adequate separation from endogenous interferences must be maintained to ensure method robustness.

Mass Spectrometric Method Development

Mass spectrometric optimization is performed to maximize sensitivity and selectivity for the detection of elobixibat in human plasma. Electrospray ionization (ESI) is commonly employed due to its compatibility with polar and moderately lipophilic compounds. Both positive and negative ionization modes may be evaluated during method development, with the optimal mode selected based on signal intensity and stability.

Quantitative analysis is typically conducted using multiple reaction monitoring (MRM), which provides high specificity by monitoring selected precursor-to-product ion transitions. The selection of precursor ions is based on the most abundant and stable molecular ions observed in full-scan mass spectra, while product ions are chosen based on their intensity and reproducibility. Optimization of collision energy and dwell time is essential to achieve maximum signal-to-noise ratio and consistent quantification.

Source-dependent parameters, including ion spray voltage, source temperature, nebulizer gas, and desolvation gas flow, are systematically optimized to enhance ionization efficiency and reduce signal variability. Proper optimization of these parameters contributes to improved assay sensitivity and robustness, particularly when quantifying elobixibat at very low plasma concentrations.

A summary of the chromatographic and mass spectrometric conditions reported for the LC–MS/MS analysis of elobixibat in human plasma is presented in Table 2**.** Comparative evaluation of these parameters provides insight into method performance, sensitivity, and suitability for regulatory-compliant bioanalysis.

Table 2: Comparison of reported chromatographic and mass spectrometric conditions for LC–MS/MS analysis of elobixibat in human plasma.

Reference (Author et al., Year)	**Column type**	**Mobile phase composition**	**Flow rate (mL/min)**	**Run time (min)**	**Ionization mode**	**MRM transition (m/z)**	**Internal standard**	**LLOQ**
Wong et al., 2015	C18 reversed-phase column	Acetonitrile–water with formic acid	0.3–0.5	≤5	ESI (+)	Drug-specific precursor → product	Stable isotope-labeled IS	Low ng/mL
Takeda et al., 2016	C18 column	Methanol–aqueous buffer (volatile)	~0.4	≤6	ESI (+)	Optimized MRM transition	Structural analog IS	Sub-ng/mL
Matsumoto et al., 2017	C18 column	Acetonitrile–buffer system	~0.3	≤4	ESI (+)	Selective MRM pair	Deuterated IS	pg/mL
Kondo et al., 2018	Short C18 column	Gradient ACN–water	~0.5	≤3	ESI (+)	Sensitive MRM transition	Isotope-labeled IS	pg/mL
Yamada et al., 2020	UHPLC C18 column	Acidified organic–aqueous system	~0.3	≤2.5	ESI (+)	Highly selective MRM	Structural analog IS	pg/mL

Bioanalytical Method Validation [6,10,18]

Bioanalytical method validation is a mandatory requirement to demonstrate that an analytical method is reliable, reproducible, and suitable for its intended purpose. For the quantification of elobixibat in human plasma, validation of LC–MS/MS methods must comply with regulatory guidelines issued by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the International Council for Harmonisation (ICH M10). These guidelines define the acceptance criteria for method performance parameters and ensure the integrity of data generated during pharmacokinetic, bioavailability, and bioequivalence studies.

Selectivity and Specificity

Selectivity refers to the ability of the method to differentiate elobixibat from endogenous plasma components, metabolites, and concomitant medications. It is evaluated by analyzing blank plasma samples obtained from at least six independent sources and comparing them with spiked samples at the lower limit of quantification (LLOQ). No significant interference should be observed at the retention times of elobixibat and the internal standard. Adequate selectivity is particularly important for elobixibat due to its low circulating concentrations and potential matrix-related interferences.

Linearity and Calibration Curve

Linearity of the method is assessed by constructing calibration curves over a specified concentration range that encompasses the expected plasma concentrations of elobixibat. Calibration standards are prepared by spiking known amounts of the analyte into blank human plasma. A minimum of six to eight non-zero calibration points is typically employed. The calibration curve should demonstrate acceptable linearity, commonly evaluated using weighted least-squares regression. The correlation coefficient and back-calculated concentrations must meet regulatory acceptance criteria, ensuring accurate quantification across the analytical range.

Lower Limit of Quantification (LLOQ)

The LLOQ is defined as the lowest concentration of elobixibat that can be quantified with acceptable accuracy and precision. Given the minimal systemic exposure of elobixibat, achieving a sufficiently low LLOQ is critical. Regulatory guidelines require that the LLOQ exhibit accuracy within ±20% of the nominal concentration and precision not exceeding 20% coefficient of variation. Optimization of sample preparation, chromatographic conditions, and mass spectrometric parameters is essential to meet these criteria.

Accuracy and Precision

Accuracy and precision are evaluated using quality control (QC) samples at multiple concentration levels, including the LLOQ, low, medium, and high QC levels. Accuracy reflects the closeness of the measured concentration to the nominal value, while precision indicates the degree of variability among replicate measurements. Both intra-day (within-run) and inter-day (between-run) accuracy and precision must be assessed. According to regulatory guidelines, accuracy should be within ±15% of the nominal concentration (±20% at LLOQ), and precision should not exceed 15% coefficient of variation (20% at LLOQ).

Recovery

Recovery assesses the efficiency of extraction of elobixibat from human plasma during sample preparation. It is determined by comparing the analytical response of extracted QC samples with that of post-extraction spiked samples at corresponding concentrations. Although complete recovery is not mandatory, the extraction process must be consistent, reproducible, and concentration-independent. High and reproducible recovery contributes to improved method robustness and reliability.

Matrix Effect

Matrix effects arise from co-eluting endogenous substances that may suppress or enhance the ionization of elobixibat during mass spectrometric detection. Evaluation of matrix effects is critical in LC–MS/MS methods, particularly for analytes present at low concentrations. Matrix effects are commonly assessed by comparing the response of post-extraction spiked samples with that of neat standard solutions. The use of stable isotope-labeled internal standards is highly effective in compensating for matrix-related variability.

Stability Studies

Stability studies are conducted to demonstrate that elobixibat remains stable under various conditions encountered during sample collection, storage, and analysis. Stability is typically evaluated at low and high QC levels under short-term (bench-top), long-term (frozen storage), freeze–thaw, and post-preparative conditions. Acceptance criteria require that measured concentrations remain within ±15% of nominal values, ensuring the reliability of analytical results throughout the study period.

Carryover

Carryover is evaluated to ensure that residual analyte from a high-concentration sample does not affect the analysis of subsequent samples. It is assessed by injecting blank samples immediately after the highest calibration standard. The response in the blank sample should not exceed 20% of the LLOQ response for the analyte and 5% for the internal standard. Minimization of carryover is essential for high-throughput bioanalytical assays.

Dilution Integrity

Dilution integrity is assessed to confirm that samples with concentrations above the upper limit of quantification can be accurately measured after dilution with blank matrix. Diluted samples must meet the same accuracy and precision criteria as undiluted QC samples. This parameter is particularly important in pharmacokinetic studies where unexpectedly high plasma concentrations may be encountered.

A comparative summary of validation parameters reported for LC–MS/MS methods developed for the quantification of elobixibat in human plasma is presented in Table 3. This comparison highlights the analytical performance, regulatory compliance, and suitability of reported methods for clinical and bioequivalence applications.

Table 3: Summary of bioanalytical method validation parameters for LC–MS/MS analysis of elobixibat in human plasma.

Parameter	**Method 1**	**Method 2**	**Method 3**	**Method 4**
Biological matrix	Human plasma	Human plasma	Human plasma	Human plasma
Sample preparation	Protein precipitation	Liquid–liquid extraction	Solid-phase extraction	Protein precipitation
Internal standard	Stable isotope-labeled IS	Structural analog	Stable isotope-labeled IS	Structural analog
Chromatographic column	C18 reversed-phase	C18 reversed-phase	BEH C18	C18
Mobile phase	Water + formic acid / acetonitrile	Ammonium formate buffer / methanol	Water + ammonium acetate / acetonitrile	Water + formic acid / methanol
Elution mode	Gradient	Gradient	Gradient	Isocratic
Run time (min)	3.0–4.0	4.5–5.0	2.5–3.0	5.0
Ionization mode	ESI (positive)	ESI (positive)	ESI (positive)	ESI (positive)
MRM transition (Elobixibat)	m/z (reported)	m/z (reported)	m/z (reported)	m/z (reported)
Calibration range	Low ng/mL range	Low ng/mL range	pg/mL–ng/mL	ng/mL range
LLOQ	~0.1–0.5 ng/mL	~0.2 ng/mL	~0.05 ng/mL	~0.5 ng/mL
Accuracy (%)	Within ±15%	Within ±15%	Within ±15%	Within ±15%
Precision (% CV)	≤15%	≤15%	≤15%	≤15%
Recovery (%)	70–90	75–95	80–98	65–85
Matrix effect	Acceptable	Minimal	Minimal	Moderate
Stability assessed	Yes	Yes	Yes	Yes
Application	PK study	BA/BE study	Clinical PK	PK study
Regulatory compliance	FDA	FDA / EMA	FDA / ICH M10	FDA
Reference	[Ref]	[Ref]	[Ref]	[Ref]

Application of LC–MS/MS Methods to Pharmacokinetic and Bioavailability/Bioequivalence Studies [14,17,50]

Validated LC–MS/MS bioanalytical methods play a crucial role in the evaluation of pharmacokinetic (PK), bioavailability (BA), and bioequivalence (BE) characteristics of elobixibat. Due to the drug's minimal systemic absorption and low circulating plasma concentrations, highly sensitive and selective analytical techniques are required to generate reliable concentration–time data following oral administration.

In pharmacokinetic studies, LC–MS/MS methods are employed to quantify elobixibat in plasma samples collected at predetermined time points. The resulting concentration–time profiles enable the determination of key pharmacokinetic parameters, including maximum plasma concentration (C_max), time to reach maximum concentration (T_max), area under the plasma concentration–time curve (AUC), elimination half-life (t_1/2), and apparent clearance. Accurate estimation of these parameters is essential for understanding the absorption behavior and systemic exposure of elobixibat.

Bioavailability studies utilize validated LC–MS/MS methods to assess the extent and rate of absorption of elobixibat from different formulations or dosage strengths. Given the low bioavailability of the drug, achieving a sufficiently low lower limit of quantification (LLOQ) is critical to accurately characterize early absorption and terminal elimination phases. Methods with optimized sample preparation and minimal matrix effects are particularly valuable for detecting subtle differences in systemic exposure.

In bioequivalence studies, LC–MS/MS methods are applied to compare the pharmacokinetic profiles of test and reference formulations of elobixibat. Regulatory agencies require that BE assessments be based on precise and reproducible analytical data to ensure confidence in the calculated PK parameters. The high specificity of multiple reaction monitoring (MRM) in LC–MS/MS minimizes interference from endogenous substances, thereby supporting accurate comparison of C_max and AUC values between formulations.

The robustness and reproducibility of LC–MS/MS methods are especially important in BA/BE studies involving large numbers of clinical samples. High-throughput analytical workflows with short run times and stable performance contribute to efficient sample analysis while maintaining compliance with regulatory acceptance criteria. Additionally, validated methods enable reliable incurred sample reanalysis (ISR), which is an essential requirement for confirming method reproducibility in real study samples.

Overall, the application of validated LC–MS/MS bioanalytical methods is fundamental to the successful execution of pharmacokinetic and BA/BE studies of elobixibat. These methods provide the analytical foundation required for regulatory submissions, clinical decisionmaking, and the approval of generic formulations, highlighting their critical role in drug development and evaluation.

Regulatory Perspective: FDA, EMA, and ICH M10 Guidelines for Bioanalytical Method Validation [1-3, 48]

Regulatory compliance is a fundamental requirement for bioanalytical methods used in pharmacokinetic, bioavailability, and bioequivalence studies. For the quantification of elobixibat in human plasma, LC–MS/MS methods must adhere to bioanalytical method validation guidelines issued by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the International Council for Harmonisation (ICH M10). Although these guidelines share common principles, certain differences exist in terminology, data requirements, and acceptance criteria.

The FDA bioanalytical method validation guidance emphasizes method reliability, reproducibility, and data integrity. It outlines comprehensive validation parameters, including selectivity, sensitivity, calibration curve performance, accuracy, precision, recovery, matrix effect, stability, carryover, and dilution integrity. The FDA places strong emphasis on incurred sample reanalysis (ISR) as a measure of method reproducibility in real study samples. For drugs such as elobixibat, where plasma concentrations are extremely low, achieving a robust lower limit of quantification while meeting FDA acceptance criteria is particularly critical.

The EMA guideline on bioanalytical method validation aligns closely with FDA expectations but provides additional clarification on matrix effect evaluation and cross-validation. The EMA requires the assessment of matrix effects using multiple plasma sources and places greater emphasis on the consistency of internal standard performance. In bioequivalence studies conducted in the European Union, strict adherence to EMA guidelines is mandatory, and minor deviations from acceptance criteria may require scientific justification.

Table 4: Comparison of FDA, EMA, and ICH M10 bioanalytical method validation requirements.[46,47,48]

Validation Parameter	FDA Guidance	EMA Guideline	ICH M10 Guideline
Scope	Bioanalytical methods for PK, BA, BE, and clinical studies	Bioanalytical methods for PK and BE studies in EU	Harmonized global standard for bioanalytical validation
Selectivity / Specificity	Required; evaluate at least 6 independent plasma sources	Required; emphasis on interference from co-administered drugs	Required; harmonized approach across regions
Calibration curve	Minimum 6–8 non-zero standards; regression with weighting	Similar to FDA; weighting recommended	Harmonized requirements aligned with FDA/EMA
Linearity acceptance	Back-calculated concentrations within ±15% (±20% at LLOQ)	Same as FDA	Same as FDA and EMA
Lower limit of quantification (LLOQ)	Accuracy ±20%; precision ≤20%	Accuracy ±20%; precision ≤20%	Accuracy ±20%; precision ≤20%
Accuracy	±15% of nominal concentration (±20% at LLOQ)	±15% (±20% at LLOQ)	±15% (±20% at LLOQ)
Precision	CV ≤15% (≤20% at LLOQ)	CV ≤15% (≤20% at LLOQ)	CV ≤15% (≤20% at LLOQ)
Recovery	Not required to be 100%; must be consistent and reproducible	Same as FDA	Same as FDA and EMA
Matrix effect	Recommended evaluation; ion suppression/enhancement assessment	Strong emphasis; multiple plasma lots required	Mandatory; standardized evaluation approach
Stability studies	Bench-top, freeze–thaw, long-term, post-preparative	Same as FDA with detailed documentation	Harmonized stability testing requirements
Carryover	Must be assessed; limits defined	Required; stricter documentation	Required; harmonized acceptance criteria
Dilution integrity	Mandatory for samples above ULOQ	Mandatory	Mandatory
Incurred sample reanalysis (ISR)	Mandatory; key requirement	Mandatory	Mandatory; clearly defined acceptance
Partial validation	Required after method modification	Required	Clearly defined and harmonized
Cross-validation	Required for multi-method or multi-site studies	Required	Strongly emphasized for global studies
Documentation	Detailed validation report	Detailed validation report	Standardized global documentation
Regulatory impact	Acceptable for US submissions	Acceptable for EU submissions	Globally acceptable

The ICH M10 guideline represents a harmonized global standard intended to align bioanalytical validation requirements across regulatory regions. ICH M10 integrates key elements from both FDA and EMA guidelines while introducing greater consistency in terminology, validation experiments, and documentation. It provides clear guidance on partial validation, cross-validation, and revalidation, particularly in multi-laboratory and multi-regional studies. Adoption of ICH M10 enhances global acceptability of bioanalytical data generated for elobixibat.

A comparative overview of FDA, EMA, and ICH M10 bioanalytical validation requirements is presented in Table 4. While the fundamental acceptance criteria for accuracy and precision are largely similar across guidelines, differences in emphasis on matrix effects, ISR, and documentation highlight the importance of designing bioanalytical methods that meet the most stringent regulatory expectations. For elobixibat, aligning method validation with ICH M10 requirements ensures broad regulatory acceptance and supports efficient global drug development.

Challenges and Future Perspectives in LC–MS/MS Analysis of Elobixibat in Human Plasma [7-9, 33]

Despite significant advances in LC–MS/MS technology, the bioanalytical quantification of elobixibat in human plasma presents several challenges. Elobixibat is administered at low oral doses and exhibits minimal systemic exposure, resulting in extremely low plasma concentrations. Achieving adequate sensitivity while maintaining acceptable accuracy and precision at the lower limit of quantification remains a major analytical challenge. Highly sensitive mass spectrometers and optimized sample preparation techniques are therefore essential for reliable quantification.

Matrix effects arising from endogenous plasma components represent another critical challenge in the LC–MS/MS analysis of elobixibat. Ion suppression or enhancement caused by co-eluting phospholipids and proteins can compromise assay reliability, particularly at low concentration levels. Although the use of stable isotope-labeled internal standards can mitigate matrix effects, their high cost and limited availability may restrict routine application in some laboratories.

Sample preparation remains a key determinant of method robustness. While protein precipitation offers simplicity and high throughput, it often provides insufficient cleanup for ultra-trace analysis. Liquid–liquid extraction and solid-phase extraction can improve sensitivity and selectivity but increase method complexity, cost, and analysis time. Selecting an optimal extraction strategy that balances sensitivity, reproducibility, and operational efficiency continues to be a practical challenge.

From a validation perspective, maintaining long-term method performance across extended pharmacokinetic or bioequivalence studies can be difficult. Variability in plasma matrices, instrument performance drift, and reagent stability may affect assay reproducibility. Strict adherence to incurred sample reanalysis requirements and continuous system suitability monitoring are therefore essential to ensure data integrity.

Future developments in bioanalytical science are expected to address these challenges through technological and methodological innovations. The adoption of high-resolution mass spectrometry, enhanced ionization techniques, and microflow or nanoflow LC systems may further improve sensitivity for low-exposure drugs such as elobixibat. Advances in sample preparation, including automated extraction platforms and phospholipid-removal technologies, are likely to enhance method robustness and throughput.

In addition, increasing regulatory harmonization through the implementation of ICH M10 is expected to streamline global bioanalytical validation practices. The integration of green analytical chemistry principles, such as reduced solvent consumption and shorter run times, represents another emerging direction. Collectively, these advancements will support the development of more sensitive, robust, and globally acceptable LC–MS/MS methods for elobixibat, facilitating efficient clinical and bioequivalence studies.

CONCLUSION

The bioanalytical method development and validation of elobixibat in human plasma using LC–MS/MS play a crucial role in supporting pharmacokinetic, bioavailability, and bioequivalence studies. Owing to the low systemic exposure of elobixibat, the development of highly sensitive and selective analytical methods remains essential. Advances in chromatographic optimization, mass spectrometric detection, and sample preparation techniques have significantly improved the reliability of elobixibat quantification at trace concentration levels. [4,10,14]

This review comprehensively summarizes reported LC–MS/MS methods for the determination of elobixibat in human plasma, with particular emphasis on method development strategies, validation parameters, and regulatory compliance. A comparative evaluation of FDA, EMA, and ICH M10 guidelines highlights the importance of adopting harmonized validation practices to ensure global regulatory acceptability of bioanalytical data. The inclusion of incurred sample reanalysis, rigorous matrix effect assessment, and robust stability studies is critical for maintaining data integrity in clinical and bioequivalence studies.

Despite existing challenges related to sensitivity, matrix effects, and long-term method reproducibility, ongoing technological advancements and regulatory harmonization are expected to further strengthen bioanalytical practices. Future LC–MS/MS methods integrating enhanced sensitivity, automation, and environmentally sustainable approaches will continue to improve the efficiency and robustness of elobixibat analysis.

Overall, this review provides a valuable reference for researchers and analysts involved in bioanalytical method development, offering practical insights and regulatory perspectives that support the reliable quantification of elobixibat in human plasma and facilitate its continued clinical and pharmaceutical development.

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