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Review Article

Analytical And Bioanalytical Methods for The Determination of Naratriptan: A Critical Review with Regulatory Perspectives

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ABSTRACT

Naratriptan, a selective serotonin receptor agonist, is widely used for the treatment of acute migraine attacks. Accurate determination of naratriptan in pharmaceutical formulations and biological samples is crucial for quality control, pharmacokinetic evaluation, and bioequivalence assessment. Over the years, several analytical approaches have been developed for its quantification, including spectrophotometry, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Among these techniques, LC–MS/MS has demonstrated markedly superior sensitivity, with reported lower limits of quantification ranging from 0.05 to 0.2 ng/mL, significantly outperforming conventional HPLC and spectrophotometric methods. Although spectrophotometric and HPLC procedures remain useful for routine pharmaceutical quality control, LC–MS/MS methods offer enhanced selectivity, minimal matrix interference, and greater suitability for pharmacokinetic and bioequivalence investigations. This review provides a comprehensive and critical summary of the analytical and bioanalytical methods reported for the estimation of naratriptan. It integrates analytical performance with current regulatory expectations outlined by USFDA and ICH M10 guidelines and identifies existing methodological gaps in published assays.

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Particular attention is given to essential bioanalytical validation parameters such as selectivity, accuracy, precision recovery, matrix effects, stability, and calibration linearity in line with regulatory recommendations. Furthermore, the review emphasizes the growing importance of LC–MS/MS in pharmacokinetic and bioequivalence studies and underscores the need for robust, well-validated analytical methodologies for reliable naratriptan determination

INTRODUCTION

Naratriptan hydrochloride (NAR), chemically known as N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5ethanesulfonamide monohydrochloride, [1,2] appears as a white to pale yellow stable powder and is freely soluble in water. Naratriptan acts as a selective agonist of serotonin 5-HT1 receptors and is widely prescribed for the management of acute migraine episodes. However, it is not recommended for the prevention of migraines. The drug is administered orally in the form of its hydrochloride salt, while the dosage is expressed in terms of the base form. Approximately 1.11 mg of naratriptan hydrochloride is equivalent to 1 mg of naratriptan base. The usual recommended dose in the United Kingdom is 2.5 mg, whereas in the United States the recommended dose ranges from 1 mg to 2.5 mg. [3,4] The United States Pharmacopeia (USP) describes a high-performance liquid chromatography (HPLC) method coupled with ultraviolet (UV) detection for the determination of naratriptan (NAR). Previous studies have described several analytical techniques for naratriptan determination, including voltammetric, spectrophotometric, densitometric and chromatographic methods , densitometric analysis, highperformance liquid chromatography with UV detection, and spectrofluorimetric techniques.Among the advanced analytical techniques, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has gained considerable attention because of its superior sensitivity and selectivity. Mass spectrometry enables the detection of analytes at extremely low concentration levels, often several orders of magnitude lower than those achievable with conventional analytical methods. Due to these advantages, LC–MS/MS has become a widely accepted tool for the quantification of trace compounds in complex biological matrices and environmental samples. Furthermore, a comprehensive survey of the literature reveals that several LC–MS/MS methods have been developed and reported for the determination of naratriptan in different biological matrices. [2,5,6,9,10,11,12,13] Several liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods have been reported for the determination of naratriptan (NAR) in biological fluids. A review of these studies indicates that one method was developed for the analysis of NAR in rabbit plasma, while another was applied to human serum. Additional methods have been reported for the determination of NAR in human plasma; however, these approaches present certain limitations.In particular, the mobile phase used in some reported methods contained a high proportion of organic solvent. When these methods were applied to human plasma samples obtained from local sources, a significant ion suppression effect was observed for the NAR peak. This phenomenon negatively affected the analytical response and reduced the accuracy of the results. In addition, the internal standard used in those methods was not easily available, which limited their practical application.To address these issues, the proposed method investigated the use of structurally related compounds from the triptan class as potential internal standards. Almotriptan and zolmitriptan were selected because of their structural similarity to NAR. Both compounds showed satisfactory recovery and analytical response; however, almotriptan demonstrated better accuracy and precision compared with zolmitriptan.Therefore, the present study aimed to develop and validate a

reliable and practical analytical method for the determination of NAR in human plasma. [14,15,16,17]

The phenomenon of ion suppression or enhancement in LCMS/MS depends mainly on the sample matrix, sample preparation procedure, quality of chromatographic separation, mobile phase additives and ionization type. Electrospray ionization (ESI) is more prone to such effects than atmospheric pressure chemical ionisation (APCI). These effects may occur principally when other compounds co-elute with the analyte of interest. In bioanalysis, important sources of such co-eluting compounds are the (biological) sample matrix, exogenous compounds such as drugs and/or their metabolites, (stableisotopelabelled) internal standards (IS), or mobile phase additives such as trifluoroacetic acid (TFA). Ion suppression/enhancement effects from endogenous compounds have been reported for various biological matrices used in TDM or toxicology such as blood, plasma or serum, urine, and oral fluid. They are generally most pronounced for analytes with short retention times. Ion suppression/ enhancement is not uncommon at the void volume, hence the notable affect it has on analytes that elute early. However, caution has to be taken as matrix effects can also affect analytes that elute later in a chromatographic run. Ion suppression/enhancement from exogenous compounds and/or metabolites present in the sample may also occur. It is very difficult to assess the likelihood of such effects, since a variety of different drugs/drug classes other than the analyte may be present in authentic samples.(A,B)

Despite the availability of several analytical methods for naratriptan estimation, significant variability exists in sensitivity, matrix effect evaluation, validation depth, and regulatory compliance. Furthermore, no recent review has comprehensively integrated analytical performance with harmonized USFDA and ICH M10 bioanalytical validation requirements. Therefore, the present review critically evaluates existing analytical and bioanalytical methods and highlights current methodological and regulatory gaps.

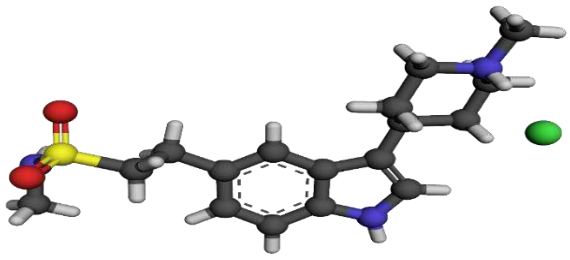

Figure 1. Three-dimensional molecular structure naratriptan

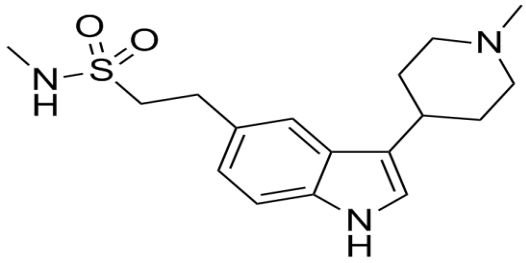

Figure 2: Naratriptan HCl structure

BIOANALYTICAL TECHNIQUES FOR NARATRIPTAN ESTIMATION: -

spectrophotometer: -

In this literature we 7 method were used to estimation of naratriptan using spectrometry with only naratriptan drug and 1 method were used to estimation of naratriptan using spectrometry with combination naratriptan.

Table 1: Spectrophotometric methods for analysis of Naratriptan

Sr no	Reference (author, year)	Method	Detection (nm)	solvent Used	Matrix	Linearity range	Reference
1	Ramu et al 2012	UV-spectrophotometric	530, 560	water	Bulk & tablets	8–70 µg/mL	19
2	Santosh Shelke et al 2015	UV-spectrophotometer	281	ethanol	Bulk & tablets	2–10 µg/mL	20
3	Ashutosh Gupta et al 2019	Stability-indicating UV Spectrophotometer	223	buffer	Bulk & oral film	1–40 µg/ml	21
4	Borse & Shirkhedkar, etal. 2012	First-order derivative UV spectrophotometer	294.20, 299.00	Methanol	Bulk & tablets	10 - 60 µg/ml.	22
5	Rizk et al. 2018	Native fluorescence	355.0, 230.0	water	Formulations	8.0-80.0 ng/ml	23
6	Ramesh et al 2011	Visible spectrophotometry	580, 620	water	Bulk & tablets	2–60 µg/mL	24
7	Sreelakshmi et al., 2013	Visible spectrophotometry (CT complexes)	400, 525	Chloroform	Tablets	2.5-12.5 µg/mL	25

Table 2: Spectrophotometric methods for analysis of Naratriptan in combination

Compound	Reference (author, year)	Method	Detection (nm)	solvent	Matrix	Linearity range	Reference
Naratriptan And Zolmitriptan	Kemisetti DP et al 2023	UV – visible spectrophotometer	238, 328	Methanol	Tablet	1-12µg/mL	26

HPLC methods: -

In the literature 3 methods were reported for the estimation of Naratriptan Alone using HPLC.

Table 3: Representative HPLC methods for analysis of Naratriptan

Sr no	Reference (author, year)	Method	Mobile phase	Detection	Linearity	FR	DT	Reference
1	Swati N. Lade et al. 2022	RP-HPLC	Potassium dihydrogen phosphate (KH2PO4): Acetonitrile (25: 75)	224	5-25 mg/ml	1.0 ml/ min	0.25 mg/ml.	27
2	Divya B. et al 2012	RP-HPLC	Ammonium acetate Buffer (pH 3) and Acetonitrile in the ratio (50:50).	225	2.5-15.5µg/mL	1.0 mL /mi n	(±0.1 ml/min).	28
3	Vaibhav Kulkarni et al. 2017	HPLC	acetonitrile: water (50: 50)	223	µg/mL	1ml /mi n	(±0.1 ml/min).	29

LC–MS/MS Method: -

In the literature 3 methods were reported for the estimation of Naratriptan Alone using LC–MS/MS.

Table 4: Representative LC-MS/ MS methods for analysis of Naratriptan

Sr no	Reference (author, year)	Method	Matrix	Mobile Phase	Column	Linearity	Ft	Mode	Reference
1	Ali M, Rizk M et al. 2020	LCMS /MS (MRM)	K_2E DT A hum an plas ma	Methanol: 0.02 M ammonium format (pH 3.5) (40:60 v/v)	reversed phase C8 with simple isocratic	0.05-20 ngmL-1	0.6 mLm in-1	+ve Ion Mode	30
2	B.R. Challa et al. 2011	LC–MS/M S	K_2E DT A	0.1% formic acid: acetonitrile	Zorbax SB-C18,	0.1-25.0 ng/mL	0.6 mL/ min.	+ve Mode	31

		(SRM)	human plasma	(50:50 v/v)	with isocratic				
3	Ali, M., et al. 2019	LCMS/MS (MRM)	K_2EDTA human plasma	methanol: 0.02 M ammonium formate (pH 3.5) (40:60 v/v)	reversed phase C8 with simple isocratic	0.05-20 ngmL-1.	0.6 mLmin-1.	+ve ion mode	32

UPLC Method: -

In the literature 2 methods were reported for the estimation of Naratriptan Alone using UPLC method And UPLC tandem mass spectrometry

Table 5: Representative UPLC methods for analysis of Naratriptan

Sr no	Reference (author, year)	Method	Matrix	Mobile Phase	Column	Accuracy	Ft	Mode	Reference
1	G. Shiva Kumar et al. 2013	UPLC–MS/MS	K_2EDTA human plasma	ammonium formate 5mM and acetonitrile	sub-2µm column with gradient	(94.0–105.7%)	0.4 ml/min	ESI+	33
2	Kuldeep Patel et al 2011	Isocratic UPLC method	Bulk drug	water: acetonitrile (pH3.4) (60:40)	UPLC BEH C18	(94.0–105.7%)	0.3 mL min-1	+ve Mode	34

OVERVIEW BIO-ANALITICAL METHOD VALIDATION: -

USFDA Bioanalytical Method Validation Guidance

The United States Food and Drug Administration (USFDA) has issued detailed guidance outlining expectations for the validation of bioanalytical methods used in the quantitative analysis of drugs and metabolites in biological samples. The primary objective of this guidance is to ensure accuracy, precision, selectivity, and reproducibility of analytical data throughout the

lifecycle of pharmacokinetic and bioequivalence studies.

According to the USFDA, a bioanalytical method intended for plasma analysis of naratriptan must be systematically validated for parameters such as selectivity, calibration model performance, accuracy, precision, recovery, matrix effects, stability, carryover, and dilution integrity. These parameters collectively demonstrate that the method can reliably quantify naratriptan without interference from endogenous plasma components or analytical artifacts.

Special emphasis is placed on the evaluation of matrix effects in LC–MS/MS methods, as ion suppression or enhancement can significantly influence quantification accuracy. The guidance also recognizes different levels of validation, including full validation, partial validation, and cross-validation, depending on the nature of method modifications or inter-laboratory transfers. This flexibility is particularly relevant for naratriptan assays that may be adapted for different study designs or analytical platforms. [35]

The transition from traditional validation approaches toward lifecycle-based bioanalytical method management under ICH M10 represents a major advancement in ensuring long-term reliability and global regulatory acceptability of naratriptan assays.

Importance of Bioanalytical Method Validation in Naratriptan Studies

Naratriptan hydrochloride is administered at relatively low therapeutic doses, resulting in plasma concentrations typically in the nanogram per millilitre range. Accurate quantification of such low drug levels in complex biological matrices is essential for pharmacokinetic characterization, bioavailability assessment, and bioequivalence evaluation. Consequently, the reliability of analytical data generated during these studies depends strongly on the use of well-validated bioanalytical methods. [38,39]

Bioanalytical method validation provides documented evidence that an analytical procedure is suitable for its intended purpose and consistently produces reliable results. For naratriptan, which is commonly analysed using LC–MS/MS due to its sensitivity requirements, method validation ensures that variability arising from biological matrices, sample preparation, and instrumental conditions is adequately controlled. Regulatory agencies have therefore established comprehensive guidelines to standardize validation practices and ensure data integrity in regulated studies. [35]

The validation of the proposed analytical method was performed in accordance with the bioanalytical method validation guidelines issued by the U.S. Food and Drug Administration (May 2018) and the European Medicines Agency. The validation process included the evaluation of key analytical performance parameters such as selectivity, linearity and calibration range, accuracy, precision, recovery, matrix effect, dilution integrity, and stability. Furthermore, the validated method was applied to the analysis of incurred samples, and incurred sample reanalysis (ISR) was conducted to confirm the reliability and reproducibility of the analytical results. (Ali, M., et al.

Development and Validation of LC-MS/MS Method for Determination of Naratriptan in Human Plasma. An Application to a Pharmacokinetic Study. (2019) J Anal Bioanal

Separation Tech 4(1): 14- 20.)

This validation involves two different drugs, a parent drug with its metabolites or the enantiomers or isomers of a drug. In these cases, the principles

of validation and analysis apply to all analytes of interest. (m 10)

Ich Harmonised M10 Guideline - Bioanalytical Method Validation and Study Sample Analysis:

This guideline provides recommendations for the validation of bioanalytical methods used for the quantification of chemical and biological drugs and their application in the analysis of study samples. Following the principles outlined in this guideline helps ensure the reliability, quality, and consistency of bioanalytical data generated during drug development and regulatory submission. Such validated data are essential for supporting both the development process and the market authorization of pharmaceutical products.The primary objective of bioanalytical method validation is to confirm that the analytical procedure is reliable and appropriate for its intended application. Although the guideline outlines standard validation practices, deviations from these recommendations may be acceptable when supported by adequate scientific justification. In cases where alternative validation strategies are considered, applicants are advised to consult the relevant regulatory authorities before implementing significant modifications to the recommended validation procedures.[36]

Validation Of the Proposed Method: -

The proposed analytical method was validated by evaluating key performance characteristics, including linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness, and stability. These parameters were assessed to confirm the reliability and suitability of the method for quantitative analysis. The objective of bioanalytical method development is to establish appropriate experimental design, optimize operating conditions, and identify the limitations and applicability of the analytical procedure. This process ensures that the method is adequately optimized and suitable for subsequent validation in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use M10 guideline for bioanalytical method validation. [37]

Plasma Sample preparation:

Plasma samples were stored at a temperature of ≤ −70 °C until analysis, while all sample preparation and analytical procedures were performed at room temperature. For the determination of naratriptan (NAR), 0.50 mL aliquots of plasma were transferred into appropriate tubes and spiked with 50 µL of an internal standard solution (200 ng mL^{-1}) along with 50 µL of 0.3% ammonia solution. The mixture was vortex-mixed for 10 s to ensure proper homogenization.

Liquid–liquid extraction was then performed by adding 3.50 mL of diethyl ether to the sample, followed by vortex mixing at 2000 rpm for 3 min. The samples were subsequently centrifuged at 4000 rpm (1789 × g) for 10 min at 5 °C to achieve phase separation. Approximately 3 mL of the clear upper organic layer was carefully collected and evaporated under vacuum at 45 °C for 30 min. The resulting residue was reconstituted with 300 µL of a methanol–water mixture (7:3, v/v). After vortex mixing, a 3 µL aliquot of the final solution was injected into the LC–MS/MS system for analysis.

Method Development: -

The analysis of the proposed drug formulations was carried out using different analytical media. The selection of the appropriate medium was based on several important factors, including the solubility characteristics of the drug, the cost and availability of solvents, the sensitivity of the analytical method, its applicability to routine

analysis, and the overall robustness of the method. (Verma et al., 2014)

Development of Sample preparation techniques: -

Initially, different sample preparation techniques were evaluated to identify the most suitable extraction procedure. Liquid–liquid extraction was tested using several organic solvents, including diethyl ether, tert-butyl methyl ether, ethyl acetate, n-hexane, and dichloromethane. In addition, protein precipitation methods were investigated using methanol and acetonitrile under neutral, acidic, and alkaline conditions.

The results indicated that liquid–liquid extraction with diethyl ether after alkalinization of the plasma sample using 0.3% ammonia solution provided the most effective approach. This procedure involved centrifugation, evaporation of the organic phase under vacuum, and subsequent reconstitution of the residue with a methanol– water mixture (7:3, v/v). The selected method demonstrated advantages in terms of simplicity, cost-effectiveness, and satisfactory recovery of the analyte.

The improved extraction efficiency of naratriptan (NAR) under alkaline conditions can be attributed to its chemical behavior. In an alkaline medium, NAR predominantly exists in its non-ionized form, which exhibits higher solubility in the organic solvent (diethyl ether). In contrast, under acidic conditions the drug mainly exists in a protonated ionic form, resulting in reduced solubility in the organic extraction solvent and consequently lower extraction efficiency. (35)

COMPARATIVE EVALUATION OF REPORTED LC–MS/MS METHODS FOR NARATRIPTAN

Over the past several years, a number of LC–MS/MS methods have been developed for the quantitative determination of naratriptan in human plasma, largely driven by the growing demand for sensitive bioanalytical techniques suitable for pharmacokinetic and bioequivalence investigations. Although these methods share the common objective of trace-level quantification, notable differences exist in terms of sample preparation strategies, chromatographic conditions, internal standard selection, and overall validation rigor.

Most reported methods employ reversed-phase liquid chromatography using C18 stationary phases, reflecting the moderate lipophilicity and favourable chromatographic behaviour of naratriptan under such conditions. Mobile phase compositions generally consist of organic solvents such as acetonitrile or methanol combined with volatile buffers or acidic modifiers to facilitate efficient ionization in positive electrospray mode. Advances in column technology and mass spectrometric sensitivity have contributed to reduced analysis times and improved throughput in more recent studies.

Sample preparation approaches reported in the literature range from simple protein precipitation to more selective liquid–liquid extraction and solid-phase extraction techniques. Protein precipitation remains attractive due to its operational simplicity and rapid processing; however, this approach may be more susceptible to matrix-related effects if not carefully optimized. In contrast, extraction-based methods offer improved cleanliness at the expense of longer preparation time and higher cost.

The choice of internal standard also varies among reported methods. While some studies rely on structurally related analogues, others utilize stable isotope-labelled internal standards to compensate

more effectively for matrix effects and extraction variability. Regulatory guidance increasingly favours the use of isotopically labelled internal standards, particularly for LC–MS/MS assays intended for pivotal bioequivalence studies.

Among the available methods, the robust LC–MS/MS method developed for estimating naratriptan levels in K_2EDTA anticoagulated human plasma demonstrates a well-balanced combination of sensitivity, selectivity, and regulatory compliance. The method achieves low nanogram-per-millilitre quantification limits, employs appropriate control of matrix effects, and includes comprehensive stability and validation assessments in line with current regulatory expectations. These attributes collectively support its suitability for routine application in pharmacokinetic and bioequivalence studies. [40-43]

Table: Comparison of Reported LC–MS/MS Bioanalytical Methods for Naratriptan in Human Plasma

Parameter	**Method I**	**Method II**	**Method III**	**Preferred Method**
Biological matrix	Human plasma	Human plasma	Human plasma	K_2EDTA human plasma
Sample preparation	Protein precipitation	Liquid–liquid extraction	Solid-phase extraction	Protein precipitation
Internal standard	Structural analogue	Deuterated IS	Non-labelled analogue	Deuterated IS
Chromatographic column	C18	C18	C18	C18
Mobile phase composition	ACN + acid modifier	MeOH + buffer	ACN + buffer	ACN + formic acid
Ionization mode	ESI positive	ESI positive	ESI positive	ESI positive
Run time (min)	~6.0	~4.5	~5.0	~3.0
LLOQ	~1.0 ng/mL	~0.5 ng/mL	~0.8 ng/mL	≤0.2 ng/mL
Matrix effect evaluation	Limited	Reported	Not specified	Thoroughly evaluated
Stability studies	Partial	Complete	Partial	Complete

Regulatory compliance	USFDA	USFDA	Not specified	USFDA / ICH M10

CRITICAL ASSESSMENT OF COMPARATIVE BIOANALYTICAL DATA FOR NARATRIPTAN

A detailed evaluation of published bioanalytical methods for naratriptan indicates that, although most reported LC–MS/MS assays achieve the sensitivity required for plasma quantification, the extent of regulatory rigor and methodological robustness varies considerably across studies. Differences are particularly evident in internal standard selection, assessment of matrix effects, and completeness of stability investigations.

Methods employing stable isotope-labelled internal standards generally demonstrate improved control over analytical variability arising from sample preparation and ionization processes. Such approaches provide more reliable compensation for matrix-related effects and extraction losses, thereby enhancing reproducibility and alignment with current regulatory expectations outlined by the USFDA and ICH M10 guidelines. In contrast, assays relying on structurally related but non-isotopic internal standards may insufficiently correct for ion suppression or enhancement, especially when simplified sample preparation techniques such as protein precipitation are employed.

Evaluation of matrix effects, which has gained increasing regulatory importance in recent years, is inconsistently reported among naratriptan LC–MS/MS methods. While some studies include systematic assessment across multiple plasma sources, others provide only limited or no information regarding ionization interference. Given the known susceptibility of electrospray-based mass spectrometric methods to matrix-induced variability, inadequate documentation of matrix effects represents a potential limitation in the regulatory acceptability of such assays.

Stability testing is another area where variability in methodological thoroughness is observed. Although shortterm and freeze–thaw stability is commonly assessed, long-term frozen stability and autosampler stability are not always comprehensively evaluated. These parameters are critical for ensuring the integrity of samples during extended storage and batch analysis, particularly in large-scale pharmacokinetic and bioequivalence studies.

Furthermore, explicit alignment with harmonized regulatory frameworks such as ICH M10 is rarely discussed in earlier publications. While many authors report compliance with USFDA guidance, fewer studies address incurred sample reanalysis or method lifecycle considerations, which are now regarded as essential components of contemporary bioanalytical validation practice.

Overall, the comparative analysis suggests that while several LC–MS/MS methods for naratriptan are analytically sound, their suitability for regulated applications depends strongly on the depth of validation and transparency of reporting. These observations underscore the need for adopting harmonized validation strategies and comprehensive documentation to ensure global regulatory acceptance of bioanalytical data [37,40-43]

CRITICAL REVIEW OF BIOANALYTICAL METHOD VALIDATION PARAMETERS FOR NARATRIPTAN

Validation of bioanalytical methods is essential to confirm that analytical procedures provide accurate, precise, and reproducible quantification

of drugs in biological samples . For naratriptan, which is typically present in plasma at low nanogram concentrations, method validation is particularly critical to ensure consistent performance across pharmacokinetic and bioequivalence studies. Regulatory agencies such as the US Food and Drug Administration (USFDA) and the International Council for Harmonisation (ICH) have issued detailed guidance to standardize validation practices and promote data acceptability across regions.

An assessment of published LC–MS/MS methods for naratriptan reveals that while most studies report compliance with regulatory requirements, the extent and depth of validation vary across individual parameters. The following sections critically examine key validation attributes with reference to reported naratriptan assays.

5.1 Selectivity and Specificity

Selectivity refers to the ability of a bioanalytical method to distinguish the analyte and internal standard from endogenous matrix components and potential co-eluting substances. According to USFDA and ICH M10 guidelines, selectivity should be demonstrated using multiple independent sources of blank biological matrix, including haemolyzed and lipemic samples where appropriate.

Most reported LC–MS/MS methods for naratriptan demonstrate acceptable selectivity based on the absence of interfering peaks at the retention times of the analyte and internal standard. However, several studies rely primarily on visual inspection of chromatograms without providing quantitative evaluation relative to the lower limit of quantification (LLOQ). Limited assessment of potential interference from metabolites or co-administered drugs may restrict confidence in the method's broader applicability, particularly in clinical study settings.

5.2 Linearity and Calibration Model Performance

Linearity assessment ensures that the analytical response is directly proportional to analyte concentration over the intended calibration range. Regulatory guidelines require the use of appropriately distributed calibration standards with acceptable back-calculated accuracy across the range, including the LLOQ.

Published naratriptan LC–MS/MS methods generally report linear calibration curves over nanogram-per-millilitre concentration ranges suitable for pharmacokinetic analysis. Nevertheless, variability exists in calibration range selection and weighting strategies. Some studies extend calibration ranges without sufficient justification based on expected plasma concentrations, while others provide limited discussion of regression models and weighting factors. Inadequate documentation of calibration model selection may impact method reproducibility and regulatory transparency.

5.3 Accuracy and Precision

Accuracy and precision are fundamental indicators of method reliability and are typically evaluated using quality control samples at multiple concentration levels. Regulatory guidelines specify acceptance criteria for both intraday and inter-day performance, with tighter limits at higher concentrations and slightly relaxed criteria at the LLOQ.

Most naratriptan bioanalytical methods report acceptable accuracy and precision within regulatory limits. However, certain publications present summarized data without clear differentiation between within-run and between-

run variability. Such reporting practices may obscure assessment of intermediate precision and limit the interpretability of method robustness under routine analytical conditions.

5.4 Recovery and Matrix Effect

Extraction recovery reflects the efficiency and consistency of sample preparation, whereas matrix effect evaluation assesses the influence of co-eluting matrix components on analyte ionization. Given the susceptibility of LC– MS/MS methods to ion suppression or enhancement, regulatory authorities emphasize systematic assessment of matrix effects across multiple plasma sources.

While recovery studies are commonly reported for naratriptan assays, matrix effect evaluation is inconsistently addressed. Some methods provide qualitative observations, whereas others lack detailed quantitative assessment. Insufficient characterization of matrix effects may compromise method reliability, particularly when simple extraction techniques such as protein precipitation are employed. [39]

5.5 Stability Studies

Stability testing confirms that the analyte remains unchanged during sample collection, storage, processing, and analysis. Regulatory guidance recommends evaluation of short-term, long-term, freeze–thaw, and autosampler stability.

Reported stability studies for naratriptan generally include short-term and freeze–thaw conditions; however, longterm frozen stability and processed sample stability are not always comprehensively evaluated. Given the extended timelines often associated with pharmacokinetic and bioequivalence studies, incomplete stability assessment may limit confidence in reported concentration data. [37, 40-43]

5.6 Carryover, and Dilution Integrity in Naratriptan LC–MS/MS Methods

Efficient and reproducible sample extraction is a critical requirement for LC–MS/MS bioanalysis, particularly for analytes such as naratriptan that are quantified at low nanogram-per-millilitre concentrations. Recovery studies provide insight into the consistency of analyte extraction, whereas matrix effect assessments evaluate the influence of co-eluting endogenous substances on ionization efficiency.

Most reported LC–MS/MS methods for naratriptan demonstrate acceptable and reproducible recovery using protein precipitation, liquid–liquid extraction, or solid-phase extraction techniques. However, while recovery is frequently reported, the depth of matrix effect evaluation varies considerably across studies. In several cases, matrix effects are assessed qualitatively or using a limited number of plasma sources, which may not adequately capture inter-individual variability. Given the susceptibility of electrospray ionization to matrix-induced signal suppression or enhancement, insufficient matrix effect characterization may impact the robustness of such methods in large-scale clinical studies.

Carryover assessment is another validation parameter that has gained increasing regulatory attention. Although high-sensitivity LC–MS/MS methods for naratriptan typically report negligible carryover, detailed experimental conditions and acceptance criteria are not consistently described. Inadequate documentation of carryover control may raise concerns regarding the reliability of results at the lower limit of quantification, particularly following high-concentration sample injections.

Dilution integrity testing, which confirms accurate quantification of samples exceeding the upper

limit of quantification after dilution, is also inconsistently reported. While some studies include dilution integrity experiments, others omit this parameter entirely, despite its relevance for pharmacokinetic studies involving variable exposure levels.

Overall, these observations suggest that while many naratriptan LC–MS/MS methods meet basic analytical performance requirements, comprehensive evaluation and transparent reporting of recovery, matrix effects, carryover, and dilution integrity remain areas for improvement in order to fully align with current regulatory expectations. (37 40-43)

Table: Alignment of Validation Parameters in Reported LC–MS/MS Methods for Naratriptan with Regulatory Guidelines

Validation Parameter	USFDA Requirement	ICH Emphasis	Status in Reported Naratriptan Methods
Selectivity	Mandatory	Mandatory	Generally acceptable, limited quantitative reporting
Linearity	±15% (±20% at LLOQ)	Harmonized	Mostly compliant
Accuracy & Precision	±15% CV (±20% at LLOQ)	Lifecycle oriented	Acceptable, often batch-limited
Matrix effect	Recommended	Strongly emphasized	Frequently underreported
Recovery	Consistent and reproducible	Required	Generally acceptable
Stability (all conditions)	Mandatory	Extended conditions	Partial compliance common
Carryover	Mandatory	Mandatory	Limited documentation
Dilution integrity	Required	Required	Often omitted
Incurred sample reanalysis	Recommended	Mandatory	Rarely reported

Identification of Methodological Gaps in Reported Naratriptan Lc– Ms/Ms Assays

Despite the availability of multiple LC–MS/MS methods for naratriptan quantification in human plasma, a number of methodological gaps can be identified upon critical review. Earlier studies primarily focused on achieving analytical sensitivity and basic validation compliance, with less emphasis on comprehensive matrix effect assessment, carryover evaluation, and method lifecycle considerations.

Additionally, while many methods claim compliance with USFDA guidance, explicit alignment with harmonized standards such as ICH M10 is seldom demonstrated. Key elements such as incurred sample reanalysis, extended stability evaluation, and detailed justification of calibration model selection are frequently absent or insufficiently discussed.

These gaps highlight the need for updated bioanalytical approaches that integrate both high analytical performance and rigorous regulatory compliance. Methods that incorporate isotope-labelled internal standards, systematic matrix effect evaluation, comprehensive stability testing, and transparent reporting practices are better positioned to support global regulatory submissions.

FUTURE REGULATORY EXPECTATIONS AND EMERGING ANALYTICAL TRENDS FOR NARATRIPTAN BIOANALYSIS

The regulatory framework governing bioanalytical method validation continues to advance in response to the growing demand for harmonized standards, enhanced reproducibility, and strengthened data integrity across global drug development programs. The introduction of the ICH M10 guideline marks a significant milestone in this evolution, promoting international consistency in validation practices and directly influencing the development of LC–MS/MS methods for drugs such as naratriptan.

A key regulatory trend is the increasing emphasis on a lifecycle-based approach to bioanalytical method management. Validation is no longer regarded as a one-time activity performed during method development; instead, regulatory agencies now expect continuous verification of method performance throughout clinical development and post-marketing phases. For naratriptan, which is frequently evaluated in pharmacokinetic, bioequivalence, and formulation comparison studies, this approach necessitates periodic reassessment of method robustness when applied across different studies, laboratories, or analytical platforms.

Future expectations also include more rigorous documentation of matrix effects, carryover control, and dilution integrity, particularly for highly sensitive LC–MS/MS assays operating at low concentration ranges. As mass spectrometric instrumentation becomes increasingly sensitive, regulatory authorities are placing greater scrutiny onion suppression or enhancement phenomena and their potential impact on quantitative reliability. Consequently, comprehensive matrix effect evaluation using multiple plasma sources and clear reporting of acceptance criteria are anticipated to become standard practice.

Another emerging trend is the broader adoption of stable isotope-labelled internal standards to improve compensation for extraction variability and matrix-related ionization effects. For naratriptan assays intended for regulatory submissions, the use of isotopically labelled analogues is expected to enhance method reproducibility and align more closely with ICH M10 expectations.

In addition, future regulatory assessments are likely to place greater importance on data integrity elements, including traceability of analytical runs, calibration model justification, and transparent reporting of incurred sample reanalysis. These requirements aim to ensure that bioanalytical data supporting clinical and regulatory decisions are both scientifically sound and auditable.

Overall, the future regulatory landscape underscores the need for naratriptan LC–MS/MS methods that combine high analytical sensitivity

with comprehensive validation strategies and transparent reporting. Adoption of harmonized guidelines, lifecycle-based validation principles, and advanced analytical practices will be critical to ensuring the long-term regulatory acceptability of bioanalytical data generated for naratriptan. [40-44]

CONCLUSION: -

This review provides a comprehensive and critical overview of analytical and bioanalytical methods reported for the estimation of naratriptan across pharmaceutical and biological matrices. Spectrophotometric, spectrofluorimetric, and chromatographic techniques have been widely employed for routine quality control and formulation analysis, offering simplicity, cost-effectiveness, and acceptable sensitivity for bulk drug and dosage forms. However, their applicability in complex biological matrices remains limited due to insufficient selectivity and sensitivity at low concentration levels.

Liquid chromatographic methods, particularly those coupled with tandem mass spectrometric detection, have emerged as the most reliable analytical tools for naratriptan quantification in human plasma. LC–MS/MS techniques demonstrate superior sensitivity, specificity, and robustness, making them well suited for pharmacokinetic, bioequivalence, and clinical studies. Comparative evaluation of reported methods indicates that advances in sample preparation strategies, chromatographic optimization, and mass spectrometric detection have significantly improved analytical performance and reduced run times.

Despite these advancements, the critical assessment presented in this review highlights notable variability in validation rigor and reporting practices across published LC–MS/MS methods. Key regulatory parameters such as matrix effect evaluation, carryover assessment, dilution integrity, and incurred sample reanalysis are inconsistently addressed, which may limit the regulatory acceptability of some methods. The growing emphasis on harmonized validation standards, particularly following the implementation of ICH M10 guidelines, underscores the need for more comprehensive and transparent validation approaches.

Among currently available analytical techniques, LC–MS/MS remains the most suitable platform for naratriptan bioanalysis because of its superior sensitivity, selectivity, and compatibility with regulatory requirements for pharmacokinetic and bioequivalence studies. However, methodological gaps persist regarding matrix effect evaluation, carryover assessment, incurred sample reanalysis, and lifecycle-based validation practices. Future research should focus on harmonized regulatory compliance, incorporation of isotope-labelled internal standards, and development of high-throughput, environmentally sustainable analytical workflows.

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