RESEARCH PAPER

QUALITY BY DESIGN (QBD) APPROACH FOR DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR ESTIMATION OF ETIFOXINE HYDROCHLORIDE

Dr.Archana Gorle Ingle[1]*, Hemangi Jadhav[1], Dr. Rupesh Pingale[1]

*[1]Department of Pharmaceutical Quality Assurance, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai 400706, Maharashtra, India (0000-0001-5092-1666) archana.gorle@ncrdsip.com

Corresponding author:

Dr.Archana Gorle Ingle

Department of Pharmaceutical Quality Assurance, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai 400706, Maharashtra, India (0000-0001-5092-1666) Email: archana.gorle@ncrdsip.com

ABSTRACT

Anxiety disorders are among the most prevalent mental health conditions worldwide, significantly affecting quality of life and daily functioning. These disorders are commonly managed using anti-anxiety (anxiolytic) drugs such as benzodiazepines; however, their use is limited by sedation, cognitive impairment, tolerance, dependence, and withdrawal symptoms. Etifoxine hydrochloride is a non-benzodiazepine anxiolytic with a dual mechanism of action — direct modulation of the GABA-A receptor and indirect promotion of neurosteroid synthesis via activation of the mitochondrial translocator protein (TSPO) — offering comparable efficacy to standard benzodiazepines with better tolerability. Owing to its moderate lipophilicity and poor aqueous solubility, its formulation and analytical estimation present challenges. In the present work, a Quality by Design (QbD) approach was applied for the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for estimation of etifoxine hydrochloride. The Analytical Target Profile (ATP) and Critical Quality Attributes (CQAs) — retention time, peak area, theoretical plates, and tailing factor — were identified, and a 13-run Central Composite Design (CCD) was employed to study the effect of the Critical Process Parameters (CPPs), mobile phase composition and flow rate, on these CQAs. Chromatographic separation was achieved on an RP C-18 column using methanol: 0.1% orthophosphoric acid (76.9: 23.1 v/v) as the mobile phase in isocratic mode, at a flow rate of 0.98 mL/min and detection at 255 nm, giving a retention time of 2.577 min. The method was validated as per ICH Q2(R1) guidelines for linearity and range, precision, accuracy, LOD, LOQ, robustness, and ruggedness. The correlation coefficient (r) was within the specified limit of 0.999; % RSD values for precision, repeatability, robustness, and ruggedness were below 2.0%; and % recovery was within 98–102% at all levels. The developed RP-HPLC method was simple, precise, accurate, robust, and suitable for the routine analysis and quality control of etifoxine hydrochloride.

Keywords: Etifoxine hydrochloride; RP-HPLC; Quality by Design (QbD); Design of Experiments (DoE); Central Composite Design (CCD); Method validation

How to cite this article: Archana Gorle Ingle, Hemangi Jadhav, RupePingale. QUALITY BY DESIGN (QBD) APPROACH FOR DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR ESTIMATION OF ETIFOXINE HYDROCHLORIDE. Int J Drug Deliv Technol. 2026;16(79s):377-385. DOI: 10.25258/ijddt.16.79s.69

.1. INTRODUCTION

Anxiety disorders are among the most prevalent mental health conditions worldwide, significantly affecting quality of life and daily functioning. These disorders are commonly managed using anti-anxiety (anxiolytic) drugs such as benzodiazepines, which act by enhancing γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission. However, despite their effectiveness, benzodiazepines are associated with several limitations, including sedation, cognitive impairment, tolerance, dependence, and withdrawal symptoms. These drawbacks highlight the need for safer and more effective alternative therapies. Etifoxine hydrochloride is a non-benzodiazepine anxiolytic that has gained attention due to its improved safety and efficacy profile. It exhibits a unique dual mechanism of action by directly modulating the GABA-A receptor and indirectly promoting neurosteroid synthesis through activation of the translocator protein (TSPO) in mitochondria. This results in enhanced inhibitory neurotransmission without the significant sedative and dependency-related adverse effects commonly seen with benzodiazepines[1].

Etifoxine has been widely used in the treatment of adjustment disorders with anxiety and has demonstrated comparable efficacy to standard benzodiazepines with better tolerability. Additionally, its moderate lipophilicity and poor aqueous solubility present challenges in formulation and analytical estimation. The selection of etifoxine hydrochloride for the present study is based on its growing therapeutic importance, favorable safety profile, and the need for reliable analytical methods for its quantification

in pharmaceutical dosage forms. Furthermore, the application of Quality by Design (QbD) in analytical method development ensures a systematic, robust, and regulatory-compliant approach, making it highly suitable for the accurate estimation of etifoxine[2].

Pharmaceutical analysis is an extensive field that includes various methods for identifying, measuring, separating, purifying, and determining the structure of compounds found in pharmaceutical formulations. This analytical approach is mainly focused on active pharmaceutical ingredients (APIs), excipients, possible contaminants in pharmaceutical products, and drug metabolites, and is broadly divided into qualitative analysis (identification of chemical constituents) and quantitative analysis (determination of the concentration of specific components)[3].

Analytical techniques provide excellent accuracy, precision, and user-friendliness, ensuring dependable results. These methods include UV-Visible Spectroscopy, Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (IR), Titrimetric Analysis, Thin-Layer Chromatography (TLC), and High-Performance Thin-Layer Chromatography (HPTLC), which are essential for separating complex chemical mixtures containing multiple components. Analytical method development follows a defined set of steps: defining the purpose of method development, characterization of the analyte, review of literature and previous methods, choosing an analytical method, setting up instruments, optimization of the method, documentation of analytical figures of merit, evaluation of the developed method, and sample estimation, quantitative demonstration and analysis of the sample[4].

1.1 Quality by Design (QbD) Approach for Analytical Method Development

Analytical method development is a crucial part of the pharmaceutical product lifecycle, ensuring reliable analysis of drugs, impurities, and degradation products. Application of Quality by Design (QbD) in analytical method development ensures a systematic and scientific process, enhancing method robustness and reliability. It begins with defining the Analytical Target Profile (ATP) and identifying Critical Quality Attributes (CQAs) and uses risk-assessment tools such as Ishikawa diagrams and Design of Experiments (DoE) to optimize method parameters and minimize variability. Analytical QbD (AQbD) integrates risk management and continuous improvement, leading to efficient, regulatory-compliant, and robust analytical techniques for pharmaceutical quality control[5].

The Analytical Target Profile (ATP) defines the method's purpose, guiding method selection, design, and development by specifying the requirements for the test procedure's reportable result, including performance characteristics such as precision, accuracy, range, and sensitivity. ICH Q8 guidelines describe Critical Quality Attributes (CQAs) as the chemical, microbiological, physical, and biological properties of a drug that must be within defined constraints to guarantee the desired quality of the end product; for an HPLC technique, theoretical plate count, peak tailing, resolution between impurities and the main analyte, capacity factor, and peak purity are typically considered CQAs. Risk assessment is a science-based process used in quality risk management that can be carried out from the initial stage of development to monitor ongoing processes, with tools such as the Ishikawa fishbone diagram used for risk identification and assessment[6].

Design of Experiments (DoE) is a well-proven characterization approach and a key aspect of QbD, applied to method development, method validation, and assessment of the influence of analytical methods on product acceptance and out-of-specification (OOS) rates. Once Critical Analytical Method Variables (CMVs) are identified through risk assessment, DoE is implemented to systematically evaluate their interactions and effects on Critical Method Attributes; Factorial Design is one of the most commonly used screening techniques for determining individual and interactive effects on method performance, helping identify significant Critical Process Parameters (CPPs) that impact retention time, peak area, theoretical plates, and tailing factor[7,8].

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques used to model and analyze the relationships between multiple independent variables and one or more response variables, aiming to optimize a process or product[9]. Central Composite Design (CCD), a type of RSM, is a statistical technique used to optimize analytical methods by efficiently exploring the effects of independent variables on a response variable, requiring fewer experiments than a full factorial design. CCD combines factorial points, central points, and axial points to study quadratic relationships and optimize method conditions; statistical analysis including ANOVA, regression modeling, and response surface plots helps finalize the most robust method conditions, with Lack-of-Fit tests and R^2 values used to validate the model as per ICH Q8 guidelines[2].

Chromatography is a process that divides a mixture's components into two phases by continuously distributing the components between them, as one phase (mobile phase) passes continuously over the other (stationary phase)[10]. High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying

components in complex mixtures from sources such as food, chemicals, biological samples, and pharmaceuticals, using high pressure to pass a liquid mobile phase through a column packed with a stationary phase; separated components are detected using detectors such as UV detectors, generating a chromatogram of signal intensity over time[11]. Reverse-Phase HPLC (RP-HPLC) is characterized by an aqueous, somewhat polar mobile phase and a non-polar stationary phase; hydrophobic interactions between the polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase form the basis of RPC's operation[12].

2. MATERIALS AND METHODS

2.1 Drug Profile

The physicochemical characteristics of etifoxine hydrochloride relevant to the present study are summarized in Table 1.

Parameter	Details
Drug Name	Etifoxine Hydrochloride
Category	Non-benzodiazepine anxiolytic drug
Molecular Formula	C17H18Cl2N2O
IUPAC Name	6-chloro-2-ethyl-2-methyl-N-(4-chlorophenyl)-4-oxo-3,4-dihydroquinazoline-3-carboxamide hydrochloride
Molecular Weight	337.23 g/mol
Solubility	Soluble in methanol, ethanol, acetonitrile and DMSO; slightly soluble in water
Appearance	White crystalline powder
Melting Point	158–162 °C
Mechanism of Action	Etifoxine produces anxiolytic effects by direct modulation of GABA-A receptors and indirect stimulation of neurosteroid synthesis via mitochondrial TSPO activation, enhancing GABAergic neurotransmission.

Table 1. Drug profile of etifoxine hydrochloride

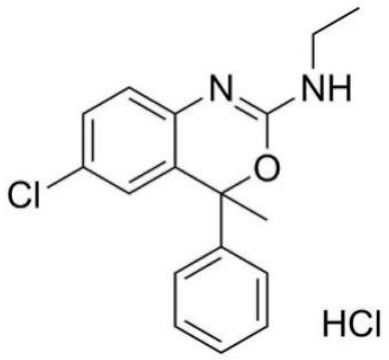

Figure 1. Chemical structure of etifoxine hydrochloride

2.2 Chemicals, Reagents and Instruments

Etifoxine Hydrochloride API was obtained as a gift sample. All solvents and reagents used, including acetonitrile, methanol, 0.1% orthophosphoric acid, and Milli-Q water, were of HPLC grade.

The analysis of the drug was carried out on an Agilent Technologies gradient HPLC system with an autoinjector and diode-array detector (DAD), equipped with a reverse-phase (Agilent) C18 column and a UV730D absorbance detector, running Chemstation 10.1 software. Other instruments used included a pH meter, UV spectrophotometer, analytical balance, and sonicator.

2.3 Solubility Determination

The solubility of the drug was determined using solvents including water, methanol, 0.1% orthophosphoric acid (OPA), DMSO, and DMFO.

2.4 Preparation of Standard Stock Solution

An accurately weighed quantity of 10 mg of Etifoxine HCl (ETF) was dissolved in methanol in a 50 mL volumetric flask and the volume made up to 50 mL to produce a solution of 200 µg/mL. From the freshly prepared standard stock solution (200 µg/mL), 0.1–0.5 mL of stock solution was pipetted into a 10 mL volumetric flask and the volume made up to 10 mL with mobile phase to obtain the final concentrations of the various dilutions.

2.5 Preparation of Tablet Powder Solution

To determine the content of Etifoxine HCl in marketed capsules (label claim 10 mg of Etifoxine HCl), 20 capsules were weighed (1.3 g) and the average weight of powder was calculated as 0.193 g. Capsule powder equivalent to 38.6 mg of drug was extracted from the powder with 10 mL methanol. To ensure complete extraction, the mixture was sonicated for 15 min. 0.2 mL of the supernatant was then diluted to 10 mL with mobile phase.

2.6 Optimised Chromatographic Conditions

The optimised chromatographic conditions finalized for the method are summarized in Table 2.

Sr. No.	Specification	Description
1	Equipment	Agilent (1100) Gradient System with DAD detector
2	Software	Chemstation
3	Column	4.6 × 250 mm i.d.
4	Detection wavelength	255 nm
5	Injection volume	20 µL
6	Flow rate	1 mL/min
7	Mobile phase	Methanol: 0.1% OPA (75:25 v/v)

Table 2. Optimised chromatographic conditions

2.7 QbD Approach and Experimental Design

A Design of Experiments (DoE) approach was employed using Design Expert® software (Version

10) to optimize an HPLC method for the estimation of etifoxine hydrochloride in accordance with Quality by Design (QbD) principles. Two independent variables (Factors), i.e., Mobile phase composition and Flow rate, were studied for their effect on key responses: retention time (RT), peak area , theoretical plates (TP), and tailing factor (TF). The design used was a Central Composite Design (CCD).

The study aimed to identify and control Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) to ensure robust and reliable chromatographic performance. A total of 13 experimental runs were conducted, and the effect of each factor on the response was modeled using a quadratic polynomial equation. ANOVA was performed to determine statistical significance, and response surface plots were generated to visualize factor–response relationships. The final method was chosen based on maximum desirability.

2.8 Method Validation

The developed method was validated as per ICH Q2(R2) guidelines for the following parameters:

3. RESULTS AND DISCUSSION

3.1HPLC Method Development

Solubility studies were first performed to select a suitable solvent for the drug, followed by determination of the working wavelength at 255 nm. Various solvents were then screened to achieve a well-resolved, sharp, and symmetrical peak. After multiple trials, methanol and 0.1% OPA in water were finalized as the optimal mobile phase for HPLC method development, yielding a retention time of 2.577 min for etifoxine hydrochloride (Figure 2).

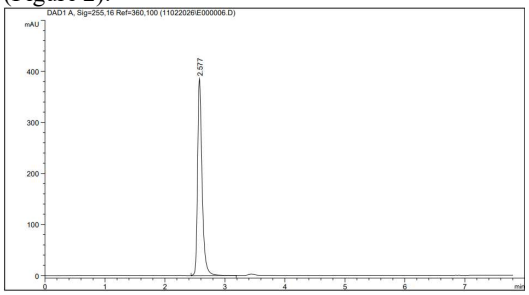

Figure 2. Standard chromatogram of etifoxine hydrochloride

Two independent variables — mobile phase composition (%) and flow rate (mL/min) — were studied for their effect on the key responses: retention time (R1), peak area (R2), theoretical plates (R3), and tailing factor (R4). The 13-run Central Composite Design experimental matrix, showing the effect of mobile phase composition (Factor A) and flow rate (Factor B) on the four measured responses, is presented in Table 3.

Std	Run	A: Mobile phase (%)	B: Flow rate (mL/min)	R1: RT (min)	R2: Area (AUC)	R3: TP	R4: TF
4	1	80	1.1	2.368	1747.51501	6233	0.72
3	2	70	1.1	2.352	1738.21045	5456	0.73
10	3	75	1	2.582	2021.48169	6463	0.67
13	4	75	1	2.584	2020.76489	6448	0.67
8	5	75	1.14142	2.269	1772.41809	5849	0.7
7	6	75	0.858579	3.031	2370.1499	7154	0.64
2	7	80	0.9	2.838	2268.93311	7759	0.64
12	8	75	1	2.582	2021.65872	6587	0.67
5	9	67.9289	1	2.627	1986.31396	5402	0.74
11	10	75	1	2.582	2019.77563	6426	0.67
9	11	75	1	2.58	2018.14456	6445	0.65
6	12	82.0711	1	2.55	2025.49878	7281	0.68
1	13	70	0.9	2.903	2222.71289	6060	0.68

Table 3. Central Composite Design (CCD) experimental matrix for method optimization

A representative chromatogram from the mid-level trial run is shown in Figure 3.

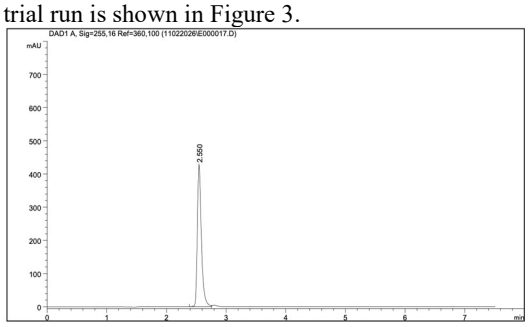

Figure 3. Representative chromatogram of the mid-level trial run of etifoxine hydrochloride

3.2 ANOVA and Response Surface Analysis

Analysis of variance (ANOVA) was performed for the response surface quadratic models fitted to each response. The results for retention time (R1), peak area (R2), and theoretical plates (R3) are summarized in Tables 4, 5, and 6 respectively.

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	0.5629	5	0.1126	886.15	< 0.0001 (significant)
A – Mobile phase	0.0031	1	0.0031	24.53	0.0017
B – Flow rate	0.5505	1	0.5505	4333.44	< 0.0001
AB	0.0016	1	0.0016	12.91	0.0088
A^2	0.0000	1	0.0000	0.2772	0.6148

B^2	0.0076	1	0.0076	59.63	0.0001
Residual	0.0009	7	0.0001		
Lack of Fit	0.0009	3	0.0003	146.88	0.0002 (significant)
Pure Error	8.000E-06	4	2.000E-06		
Cor Total	0.5638	12			

Table 4. ANOVA for the quadratic model — R1(Retention Time)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	4.346E+05	5	86917.35	85.11	< 0.0001 (significant)
A – Mobile phase	1538.47	1	1538.47	1.51	0.2594
B – Flow rate	4.284E+05	1	4.284E+05	419.46	< 0.0001
AB	340.69	1	340.69	0.3336	0.5816
A^2	2327.75	1	2327.75	2.28	0.1749
B^2	1441.78	1	1441.78	1.41	0.2735
Residual	7148.94	7	1021.28		
Lack of Fit	7140.59	3	2380.20	1139.08	< 0.0001 (significant)
Pure Error	8.36	4	2.09		
Cor Total	4.417E+05	12			

Table 5. ANOVA for the quadratic model — R2 (Area)

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	5.525E+06	5	1.105E+06	221.66	< 0.0001 (significant)
A – Mobile phase	3.294E+06	1	3.294E+06	660.76	< 0.0001
B – Flow rate	1.976E+06	1	1.976E+06	396.32	< 0.0001
AB	2.125E+05	1	2.125E+05	42.63	0.0003
A^2	41540.35	1	41540.35	8.33	0.0234
B^2	51.66	1	51.66	0.0104	0.9218
Residual	34894.41	7	4984.92		
Lack of Fit	18183.61	3	6061.20	1.45	0.3536 (not significant)
Pure Error	16710.80	4	4177.70		
Cor Total	5.560E+06	12			

Table 6. ANOVA for the quadratic model — R3(Theoretical Plates)

The 3D response-surface plots depicting the effect of mobile phase composition and flow rate on theoretical plates and peak area are shown in Figures 4 and 5 respectively.

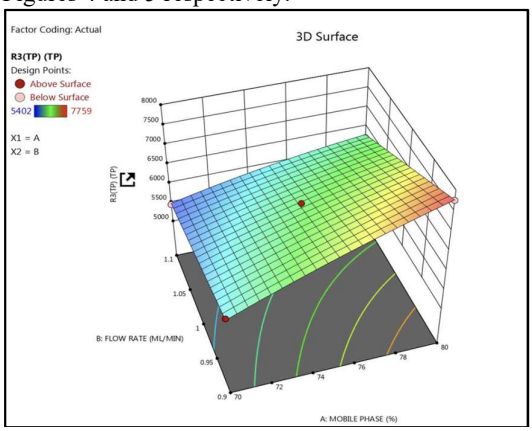

Figure 4. 3D response surface plot (Predicted vs. Actual) for theoretical plates (TP) of etifoxine hydrochloride against mobile phase and flow rate

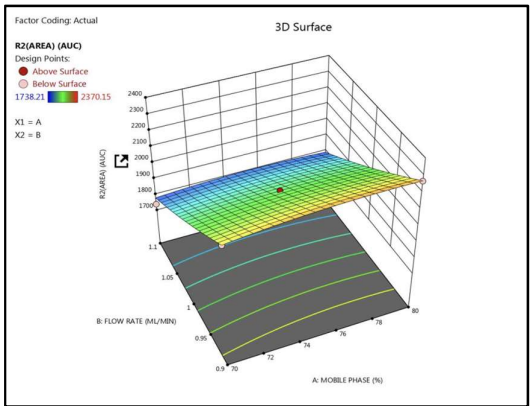

Figure 5. 3D response surface plot (Predicted vs. Actual) for peak area of etifoxine hydrochloride against mobile phase and flow rate

3.3 Design Space and Optimized Conditions

Overlay plots were generated to identify the optimum design space, as shown in Figure 6, with a desirability value of 1.000 achieved at a mobile phase composition of 76.93% methanol and a flow rate of 0.98 mL/min. The corresponding overlay plot of the optimized region is shown in Figure 7, and the factor levels used in the design are summarized in Table 7.

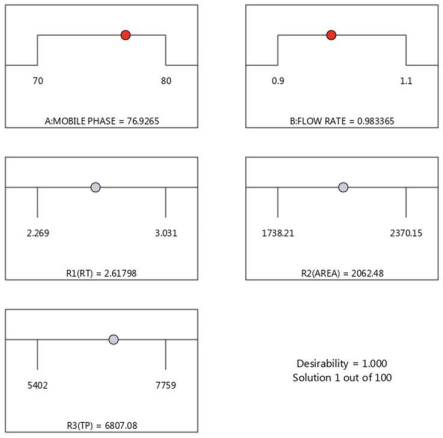

Figure 6. Overlay plots for all responses showing the optimum desirability solution

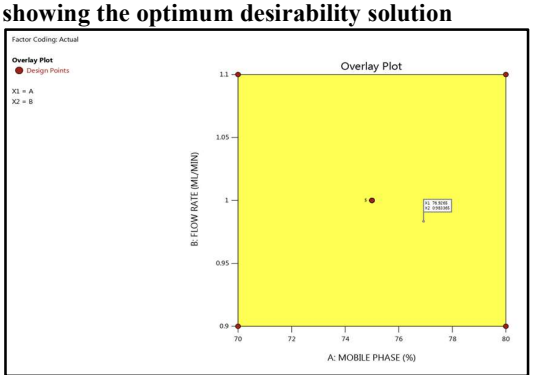

Figure 7. Overlay plot of the optimized design space for etifoxine hydrochloride

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Mobile Phase	76.93	70.00	80.00	0.0000	Actual
B	Flow Rate	0.98	0.9000	1.10	0.0000	Actual

Table 7. Factors and levels used in the Central Composite Design

3.4 Method Validation

The results of the HPLC validation study are summarized below. The method was found to be specific, accurate, and precise. The relationship between the concentration of etifoxine hydrochloride and response was linear across the range examined, since all points lay on a straight line and the correlation coefficient was well within the specified limit (NLT 0.999). LOD and LOQ were determined successfully for the drug. Accuracy by the % recovery method was found to be within the acceptable limit of 98–102%, and % RSD as per the precision study was within the limit of NMT 2%. For the robustness study, the system suitability parameters remained within the limit under all variable conditions.

3.4.1 System Suitability

By injecting the standard solution five times at the working concentration (n = 5), system suitability was determined. The tailing factor was less than 2.0, the theoretical plate count was more than 2000, and the % RSD of peak areas was within 2.0%. As a result, the HPLC system was deemed appropriate for analysis, as shown in Figure 8 and summarized in Table 8.

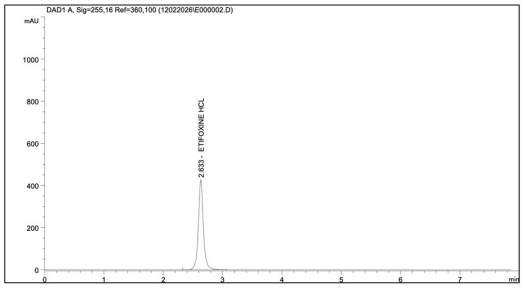

Figure 8. Chromatogram for system suitability parameters (4 µg/mL)

Sr. No.	Parameters	Results
1	Tailing factor	0.90
2	Number of theoretical plates	5595

Table 8. System suitability results

3.4.2 Linearity

The linearity of the analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample. The data obtained in the calibration experiments, when subjected to linear regression analysis, showed a linear relationship between peak area and concentration in the range 2–10 µg/mL for etifoxine HCl. The correlation coefficient was 0.999, as summarized in Table 9 and depicted in the calibration curve (Figure 9).

Concentration (µg/mL)	Average Area (Etifoxine HCl)
2	1312.5
4	2254.47
6	3081.63
8	3970.24
10	4861.44

Table 9. Linearity data of etifoxine hydrochloride

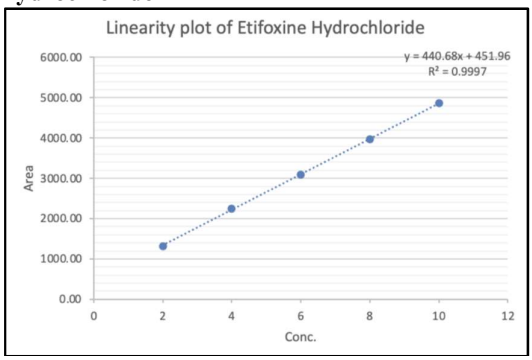

Figure 9. Calibration curve of Etifoxine Hydrochloride

3.4.3 Accuracy

Recovery studies were performed to validate the accuracy of the developed method. To the pre-analyzed tablet/capsule solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and the recovery was analyzed, as summarized in Table 10.

Method	Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
RP-HPLC Method	80%	100.03	0.08	0.08
RP-HPLC Method	100%	99.28	0.96	0.97
RP-HPLC Method	120%	101.5	0.12	0.11

Table 10. Statistical validation of recovery studies of etifoxine hydrochloride (*mean of three determinations)

3.4.4 Precision

The method precision was established by analyzing various replicate standards of Etifoxine HCl. All solutions were analyzed in triplicate to record intraday and interday variation. Intraday and interday precision studies on the RP-HPLC method for Etifoxine HCl showed % amount found between 98% and 102%, confirming the precision of the analytical method (Table 11). Representative chromatograms of the interday and intraday precision studies are shown in Figures 10 and 11 respectively.

Method	Concentration (µg/mL)	Intraday: Avg. peak area ± SD	Intraday: % amount found	Intraday: % RSD	Interday: Avg. peak area ± SD	Interday: % amount found	Interday: % RSD
RP-HPLC Method	4	2208.4 ±0.65	99.8	0.03	2211.5 ±8.25	99.98	0.37
RP-HPLC Method	6	3086.2 ±1.53	99.71	0.05	3085.43±2.49	99.68	0.08
RP-HPLC Method	8	3972.72±1.6	99.91	0.04	3984.05±4.2	100.23	0.11

Table 11. Results of intraday and interday precision studies on the RP-HPLC method (mean of three readings)

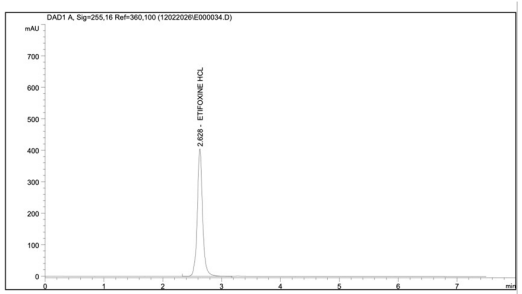

Figure 10. Chromatogram of interday precision study (4 µg/mL)

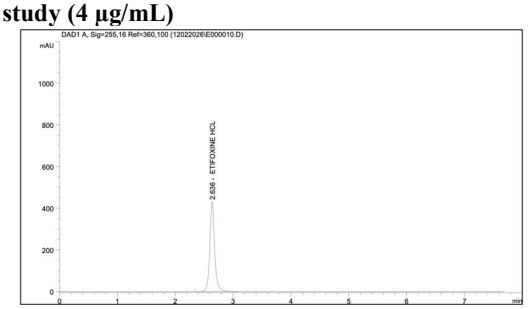

Figure 11. Chromatogram of intraday precision study (4 µg/mL)

3.4.5 Robustness

The robustness of a method is its ability to remain unaffected by small, deliberate changes in parameters. The mobile phase composition and flow rate were each varied by ±1 mL/min, and the detection wavelength was varied by ±1 nm from the optimized chromatographic conditions. The results of the robustness study are shown in Table 12; all robustness parameters were found to be satisfactory.

Parameter	Conc. (µg/mL)	Amount detected (mean ± SD)	% RSD
Mobile phase 75.9% Methanol + 24.1% Water	4	2271.916±1.7	0.07
Mobile phase 77.9% Methanol + 22.1% Water	4	2285.16±23.6	1.03
Wavelength change 254 nm	4	2268.91±1.13	0.05
Wavelength change 256 nm	4	2204.48±1.83	0.08

Table 12. Results of the robustness study of etifoxine hydrochloride (mean of three readings)

3.4.6 Ruggedness

Ruggedness, assessed as inter-analyst reproducibility, gave a mean amount found of 99.70% (Analyst 1) and 99.89% (Analyst 2), with a % RSD of 0.133% for both analysts, confirming that the method is rugged and reproducible between analysts.

3.5 Analysis of Marketed Formulation

The validated method was applied to the marketed capsule formulation (Tranxiety®, label claim 50 mg etifoxine HCl). The amount of drug present was

calculated from the regression equation of the calibration curve, as shown in Table 13.

Conc. Taken (µg/mL)	Amount Found (µg/mL)	% Label Claim	SD	% RSD
4	4.0005	100.01	0.022	0.554
4	4.0321	100.80	0.586	0.554
4	4.041	101.03	0.55	0.55

Table 13. Assay of etifoxine hydrochloride in the marketed capsule formulation

The label claim recovered was in the range of 100–101%, in good agreement with the labelled amount, confirming that the developed and validated RP-HPLC method is suitable for the routine quality control estimation of etifoxine hydrochloride in bulk drug and in the marketed capsule formulation.

4. CONCLUSION

A simple, precise, and selective analytical method has been developed by the RP-HPLC technique as per ICH Q2(R1) guidelines for the estimation of etifoxine hydrochloride. The method has several advantages, including a simple mobile phase, rapid analysis, a simple sample preparation method, and improved selectivity and sensitivity. The regression coefficient (R^2) is near 0.999, which shows good linearity. The percent recovery was within the acceptable range in the tablet dosage form. The %RSD values are less than 2%, indicating the proposed method's high degree of precision. The method was robust, as there was no significant change in peak area and retention time. The ruggedness study confirms that the method is reproducible under different conditions, such as different analysts. The developed technique may be used for routine analysis and quality control of etifoxine hydrochloride, alone or in combination.

ACKNOWLEDGEMENTS

The authors are grateful to the Management and Principal, NCRD's Sterling Institute of Pharmacy, Nerul of Department of Pharmaceutical Quality Assurance for their support and guidance throughout the study.

REFERENCES

1. P, Ferreri F, Bourin M. An update on the anxiolytic and neuroprotective properties of etifoxine: from brain GABA modulation to a whole-body mode of action. Neuropsychiatric Disease and Treatment. 2019;15:1781-1795. DOI:10.2147/ndt.s200568
2. Trivedi B. Quality by design (QbD) in pharmaceuticals. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(1):17-29.
3. Akash MSH, Rehman K. Introduction to pharmaceutical analysis. In: Essentials of Pharmaceutical Analysis. Singapore: Springer; 2020.
4. Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR Journal of Pharmacy. 2015;5(10):7-19.
5. Chauhan A, Mittu B, Chahan P. Analytical method development and validation: a concise review. Analytical & Bioanalytical Techniques. 2015;6:1000233.
6. Beg S, Haneef J, Rahman M, Peraman R, Taleuzzaman M, Almalki WH. Introduction to analytical quality by design. In: Beg S, Hasnain MS, Rahman M, Almalki WH, editors. Handbook of Analytical Quality by Design. San Diego: Academic Press; 2021. p. 1-14.
7. Alhakeem MA, Ghica MV, Pîrvu CD, Anuța V, Popa L. Analytical quality by design with the lifecycle approach: a modern epitome for analytical method development. Acta Medica Marisiensis. 2019;65(2):37-44. DOI:10.2478/amma-2019-0010 *(corrected — original manuscript truncated this title and omitted the journal)*
8. Dewi MK, Pratama R, Arifka M, Chaerunisa AY. Quality by design: approach to analytical method validation. Sciences of Pharmacy. 2022;1(1):27-33. DOI:10.58920/sciphar01010033 *(corrected — the journal is "Sciences of Pharmacy," not "Scientia Pharmaceutica"; page range and DOI also corrected)*
9. Little T. Design of experiments for analytical method development and validation. In: Beg S, Hasnain MS, Rahman M, Almalki WH, editors. Handbook of Analytical Quality by Design. San Diego: Academic Press; 2021. p. 87-97.
10. Response surface methodology. Wikipedia, The Free Encyclopedia [Internet]. Available from: https://en.wikipedia.org/wiki/Response_surface_methodology (accessed March 20, 2025).
11. High-performance liquid chromatography. Wikipedia, The Free Encyclopedia [Internet]. Available from: https://en.wikipedia.org/wiki/High-performance_liquid_chromatography (accessed March 20, 2025).
12. Sabir AM, Moloy M, Bhasin PS. HPLC method development and validation: a review. International Research Journal of Pharmacy. 2013;4(4):39-46.
13. Choudary ML, Srinivas M, Kumari BU.Nuss Method development and validation for estimation of etifoxine in capsule dosage form by using RP-HPLC/UV. Journal of Drug Delivery and

Therapeutics. 2024;14(7):64-70. DOI:10.22270/jddt.v14i7.6703

14. Ahuja S, Scypinski S. Handbook of Modern Pharmaceutical Analysis, Separation Science and Technology. 3rd ed. USA: Academic Press; 2001.
15. Das V, Bhairav B, Saudagar RB. Quality by design approaches to analytical method development. Research Journal of Pharmacy and Technology. 2017;10(9):3188-3194.
16. Malviya R, Bansal V, Pal OP, Sharma PK. High-performance liquid chromatography: a review. Journal of Global Pharma Technology. 2010;2(5):22-26.
17. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2018;10(6):8-15.
18. Jatto E, Okhamafe AO. An overview of pharmaceutical validation and process controls in drug development. Tropical Journal of Pharmaceutical Research. 2002;1(2):115-122.