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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND ETIZOLAM IN BULK DRUG AND DOSAGE FORM BY RP-HPLC

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Keywords:

RP-HPLC, Escitalopram, Etizolam, Method Validation, ICH Guidelines, Simultaneous Estimation

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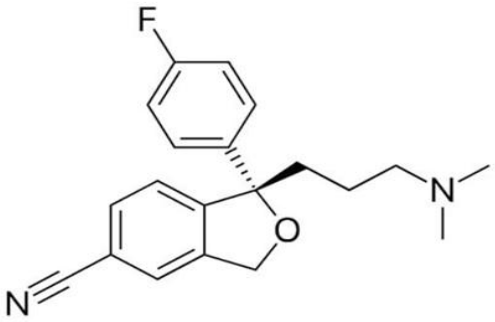
ABSTRACT

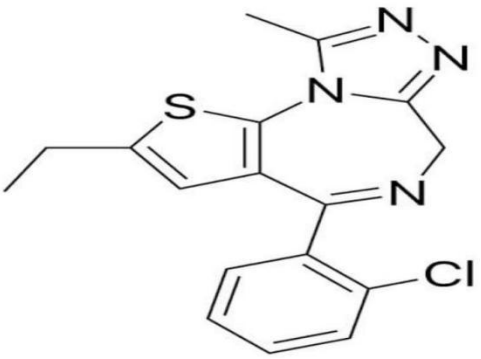
A simple, accurate, precise, and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous quantitative estimation of Escitalopram (ESC) and Etizolam (ETI) in bulk drug and combined tablet dosage form. Chromatographic separation was achieved using a Grace C18 column (4.6 mm × 250 mm, 5 µm) with a mobile phase consisting of acetonitrile and 0.2 mm ammonium acetate buffer (pH 5.5) in a ratio of 60:40 (v/v), at a flow rate of 1.0 mL/min, and UV detection at 250 nm. The retention times for ESC and ETI were found to be 3.57 and 6.45 minutes, respectively. The method was validated according to ICH Q2(R1) guidelines for parameters including linearity, precision, accuracy, specificity, robustness, limit of detection (LOD), and limit of quantitation (LOQ). ESC exhibited linearity over 5–50 µg/mL and ETI over 2–20 µg/mL, with correlation coefficients (R^2) of 0.9997 and 0.9999, respectively. Recovery studies yielded mean recoveries of 100.37% and 100.58% for ESC and ETI, respectively, indicating excellent accuracy. The proposed method can be successfully applied for routine quality control analysis of Escitalopram and Etizolam in pharmaceutical formulations.

1. INTRODUCTION

Pharmaceutical analytical method development plays a critical role in ensuring the quality, safety, and efficacy of drug products. Escitalopram (ESC) and Etizolam (ETI) are increasingly prescribed in combination for the management of neuropsychiatric conditions, particularly anxiety disorders comorbid with depression. The simultaneous determination of these two active molecular formula of $C_{20}H_{21}FN_2O$ and a molecular weight of 324.39 g/mol. It is used clinically for the treatment of major depressive disorder (MDD) and generalized anxiety disorder(GAD).



Etizolam is a thienodiazepine derivative that acts as a full agonist at GABA-A receptors, producing central nervous system depression. Its chemical name is 4-(2-Chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine with a molecular formula of $C_8H_{11}N_5O_5S$ and molecular weight of 342.85 g/mol. Etizolam is commonly prescribed for short-term management of insomnia and anxiety disorders.



A fixed-dose combination (FDC) tablet containing ESC (5 mg) and ETI (0.5 mg), commercially available as Etizola Plus 5, is widely used in clinical practice. Despite the increasing use of this combination, limited analytical methods are available for their simultaneous determination. The existing literature reports individual methods for these drugs, but there is a need for a validated, isocratic RP-HPLC method for their concurrent analysis.

The present work aimed to develop and validate a simple, rapid, and economical RP-HPLC method for the simultaneous estimation of ESC and ETI in bulk and tablet dosage form, in compliance with ICH Q2(R1) validation guidelines.

pharmaceutical ingredients (APIs) from a single dosage form requires the development of a reliable, rapid, and validated analytical method.

Escitalopram oxalate is a selective serotonin reuptake inhibitor (SSRI) classified under antidepressants. Chemically designated as (S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, it has a

2. MATERIALS AND METHODS

2.1 CHEMICALS AND REAGENTS

Escitalopram (purity 99.7%) and Etizolam (purity 99.8%) were obtained as gift samples from MG Lab India.The marketed formulation Etizola Plus 5 (Escitalopram 5 mg + Etizolam 0.5 mg per tablet) manufactured by Macleods Pharmaceuticals Pvt. Ltd. was procured from a local pharmacy. HPLC-grade acetonitrile and methanol were obtained from Merck Ltd. (India). Ammonium acetate (AR grade) was used for buffer preparation. HPLC-grade water was produced using a Milli-Q water purification system.

2.2 INSTRUMENTATION

The HPLC system used was a Waters HPLC instrument equipped with a UV detector. A Grace ODS C18 analytical column (4.6 mm × 150 mm, 5 µm particle size) was employed for separation. A Shimadzu Double Beam UV-1800 spectrophotometer was used for UV spectral studies. An Equip-tronics pH meter (Model Eq-614A) was used for buffer pH adjustment. A Citizen analytical balance (Model CY 104) and a Meta-lab ultrasonicator (1.5 L, 50 Hz) were also used throughout the study.

2.3 PREPARATION OF MOBILE PHASE

Ammonium acetate buffer (0.2 mM) was prepared by dissolving an appropriate quantity of ammonium acetate in HPLC-grade water and adjusting the pH to 5.5 using glacial acetic acid. The buffer was filtered through a 0.45 µm Millipore membrane filter. The mobile phase was prepared by mixing acetonitrile and ammonium acetate buffer in a ratio of 60:40 (v/v), sonicated for 15 minutes to degas, and filtered before use.

2.4 PREPARATION OF STANDARD SOLUTIONS

Standard stock solutions of ESC (100 µg/mL) and ETI (100 µg/mL) were prepared separately by dissolving accurately weighed quantities of each drug in methanol and making up the volume in 100 mL volumetric flasks. Working standard solutions were prepared by appropriate dilution of stock solutions with the mobile phase to obtain concentrations in the range of 5–50 µg/mL for ESC and 2–20 µg/mL for ETI.

2.5 CHROMATOGRAPHIC CONDITIONS

Parameter	Condition
Column	Grace ODS C18 (4.6 × 250 mm, 5 µm)
Mobile Phase	Acetonitrile : 0.2 mm Ammonium Acetate Buffer (60:40, v/v), pH 5.5
Detection Wavelength	250 nm
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Column Temperature	Ambient (~25°C)
Run Time	8 minutes

Table 1: Optimized Chromatographic Conditions

2.6 METHOD VALIDATION

The developed RP-HPLC method was validated as per ICH Q2(R1) guidelines for the following parameters: system suitability, specificity, linearity and range, precision (repeatability and intermediate precision), accuracy (recovery), limit of detection (LOD), limit of quantitation (LOQ), robustness, and stability of analytical solutions.

Linearity and Range: Calibration curves were constructed by plotting peak area versus concentration for both drugs over the ranges 5–50 µg/mL (ESC) and 2–20 µg/mL (ETI). Ten concentration levels were used for each compound.

Precision: Intraday precision was evaluated by analyzing three concentrations in triplicate on the same day. Interday (intermediate) precision was assessed over three consecutive days. Results were expressed as % RSD.

Accuracy: Recovery studies were performed by the standard addition method at three concentration levels (80%, 100%, 120% of label claim). Known amounts of standard drug were added to pre-analyzed formulation sample.

LOD and LOQ: Both were calculated based on the standard deviation of the response (σ) and slope (S) of the calibration curve using the ICH formulae: LOD = 3.3σ/S and LOQ = 10σ/S.

Robustness: The method was evaluated for minor deliberate variations in mobile phase composition (±5%), flow rate (±0.1 mL/min), pH (±0.2 units), and column oven temperature (±5°C).

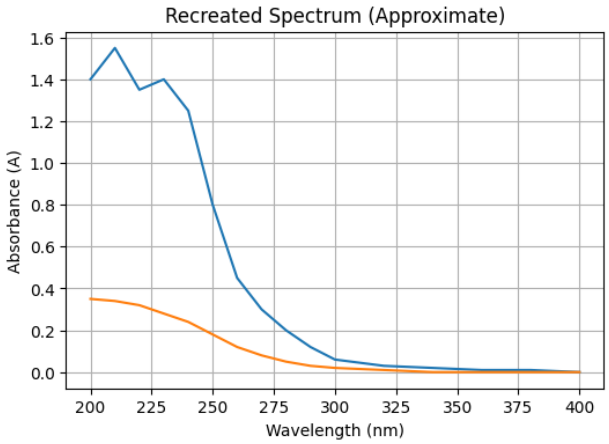
2.7 ANALYSIS OF MARKETED FORMULATION

Twenty tablets of Etizola Plus 5 were accurately weighed and finely powdered. A quantity equivalent to 5 mg of ESC and 0.5 mg of ETI was transferred to a 100 mL volumetric flask, dissolved in 50 mL of mobile phase with ultrasonication for 10 minutes, and diluted to volume. The solution was filtered through Whatman filter paper No. 41 prior to injection.

3. RESULTS

3.1 UV SPECTROSCOPIC STUDIES

The UV absorption spectra of ESC and ETI were recorded over the wavelength range 200–400 nm. The wavelength maxima (λmax) were found to be 262 nm for ESC and 239 nm for ETI. An isoabsorptive wavelength of 250 nm was selected for HPLC detection as it provided adequate sensitivity for both analytes simultaneously.



3.2 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

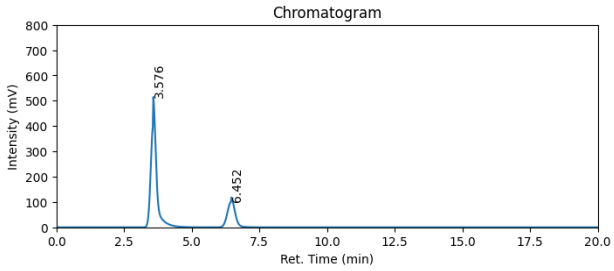
Several mobile phase compositions were screened including methanol:water (90:10 and 80:20, v/v), acetonitrile:water (90:10, v/v), and various ratios of acetonitrile:ammonium acetate buffer (90:10, 80:20, 70:30, 60:40, v/v). Mobile phases containing only methanol or acetonitrile with water provided poor resolution or excessively long retention times. The addition of ammonium acetate buffer significantly improved peak shape and resolution.

The mobile phase consisting of acetonitrile and 0.2 mM ammonium acetate buffer (pH 5.5) in the ratio 60:40 (v/v) provided optimal separation with well-

resolved, symmetrical peaks for both drugs within a reasonable run time of 8 minutes. The retention times were 3.57 ± 0.03 min for ESC and 6.45 ± 0.04 min for ETI, with a resolution of 4.8 between the two peaks, well exceeding the minimum acceptable value of 1.5.

3.3 SYSTEM SUITABILITY

System suitability was evaluated from five replicate injections of the standard mixture containing 50 µg/mL ESC and 20 µg/mL ETI. The results are summarized in Table 2.All system suitability parameters met the acceptance criteria, confirming adequate column efficiency and method performance.

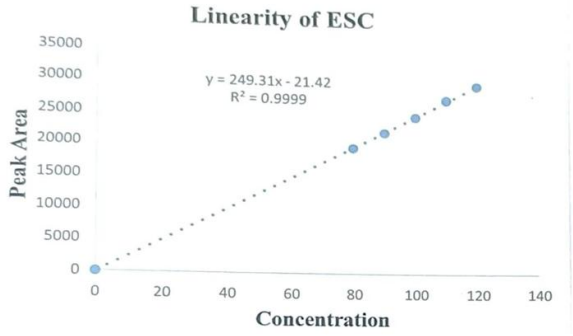


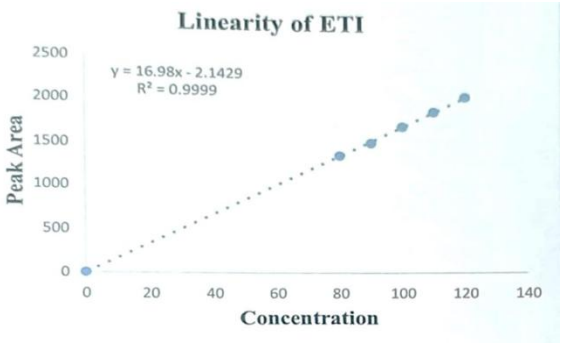
Parameter	ESC	ETI	Acceptance Criteria
Mean Peak Area	24840.1 ± 94.7	10920.9 ± 97.8	—
Retention Time (min)	3.572 ± 0.029	6.458 ± 0.033	—
Asymmetry Factor	2.407 ± 0.341	1.569 ± 0.050	≤ 2.0
Theoretical Plates (N)	344046 ± 474	31848 ± 64	> 2000
%RSD (Peak Area)	0.38	0.90	≤ 2.0%

Table 2: System Suitability Parameters (n = 5)

3.4 LINEARITY AND RANGE

Calibration curves were constructed by plotting peak area versus concentration for ESC (5–50µg/mL) and ETI (2–20 µg/mL). The calibration data are presented in Table 3. Both drugs exhibited excellent linearity within the tested ranges.





Parameter	ESC	ETI	Acceptance Criteria
Linearity Range (µg/mL)	5 – 50	2 – 20	–
Regression Equation	y = 500.89x − 136.73	y = 553.46x − 67.91	–
Correlation Coefficient (R^2)	0.9997	0.9999	≥ 0.999
Slope	500.89	553.46	–
Intercept	−136.73	−67.91	–

Table 3: Linearity Parameters for ESC and ET

3.5 PRECISION

Intraday precision was assessed at three concentrations (80%, 100%, 120% of test concentration) on the same day, while interday precision was evaluated over three different days.

The %RSD values for both intraday and interday studies were well within the ICH acceptable limit of ≤ 2.0% (Table 4), confirming the method's repeatability and reproducibility.

Study Type	Mean % Label Claim ESC	%RSD ESC	Mean % Label Claim ETI	%RSD ETI	Pass?
Intraday (n=3)	99.97	0.32	100.93	0.66	Yes
Interday (n=3)	99.70	0.10	100.03	0.55	Yes
Different Analyst (n=5)	99.86	0.55	100.78	0.71	Yes

Table 4: Precision Study Results

3.6 ACCURACY (RECOVERY STUDIES)

The accuracy of the method was determined by the standard addition method at three spiking levels (1,2, and 3 µg/mL added to pre-analyzed formulation). The percent recovery data are presented in Table 5.

All recovery values were within the ICH-specified range of 98–102% for assay methods, confirming the accuracy of the proposed method.

Spike Level (µg/mL)	% Recovery ESC	% Recovery ETI	Mean % Recovery ESC	Mean % Recovery ETI	Acceptance Limit
1 µg/mL (n=3)	100.1, 100.2, 99.7	99.9, 100.1, 100.8	100.0	100.3	98–102%
2 µg/mL (n=3)	100.4, 100.5, 101.0	100.9, 101.1, 101.5	100.6	101.2	98–102%
3 µg/mL (n=3)	101.1, 100.1, 100.2	100.2, 100.3, 100.4	100.5	100.3	98–102%
Overall Mean ± SD	100.37 ± 0.447	100.58 ± 0.526	–	–	–

Table 5: Accuracy (Recovery) Data for ESC and ETI

3.7 LIMITS OF DETECTION AND QUANTITATION

The LOD and LOQ were determined using the formula based on standard deviation of the responseand slope of the calibration curve. The LOD was 0.087 µg/mL for ESC and 0.041 µg/mL for ETI, while the LOQ was 0.430 µg/mL for ESC and0.198 µg/mL for ETI.. These low values indicate the high.

Parameter	ESC	ETI
LOD (µg/mL)	0.087	0.041
LOQ (µg/mL)	0.430	0.198

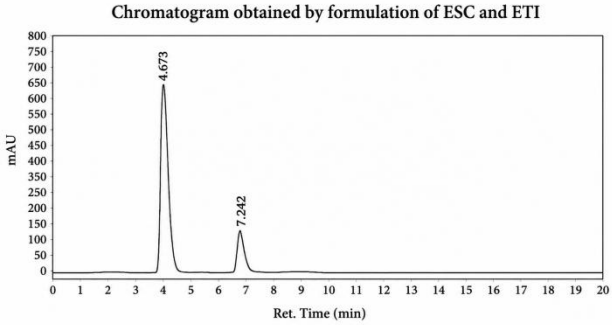
Table 6: LOD and LOQ Values

3.8 SPECIFICITY

Specificity was demonstrated by analyzing blank (diluent), individual drug solutions, placebo, and the combined formulation sample. The retention times of ESC (3.576 min) and ETI (6.452 min) were well resolved from each other and from formulation excipients. No interfering peaks were observed in the blank or placebo chromatograms at the retention times of either analyte. The peak purity for both drugs was confirmed using a photodiode array detector, with purity angles well within the threshold angles.

3.9 ANALYSIS OF MARKETED FORMULATION

The validated method was applied to the simultaneous estimation of ESC and ETI in the commercially available combined tablet formulation Etizola Plus 5. The results, presented in Table 7, show that the drugcontent was within 98–102% of the stated label claim, confirming the reliability of the developed method for quality control analysis.



Brand Name	Label Claim	% Label Claim ESC (Mean ± SD)	% Label Claim ETI (Mean ± SD)
Etizola Plus 5	ESC 5 mg + ETI 0.5 mg	100.47 ± 1.012	100.43 ± 0.723

Table 7: Analysis of Marketed Formulation (n = 3)

4. DISCUSSION

The developed RP-HPLC method utilizes a reversed-phase C18 column with an isocratic mobile phase system, making it simple, cost-effective, and amenable to routine laboratory use. The selection of acetonitrile as the organic modifier over methanol was justified by the sharper and more symmetrical peaks observed with acetonitrile. The addition of ammonium acetate buffer at pH 5.5 improved the ionization

state of the basic nitrogen atoms in both ESC and ETI, leading to improved peak shape and reproducible retention times.

The relatively short run time of approximately 8 minutes for complete elution of both peaks, combined with the isocratic mode of operation, makes the method suitable for high-throughput quality control laboratories. The system suitability parameters—including theoretical plate count (>2000 for both peaks), tailing factor (<2.0), and %RSD of peak areas (<1%)—all met pharmacopoeial requirements.

Comparison with existing literature reveals that while individual HPLC methods exist for ESC and ETI separately, few methods report their simultaneous determination. The present method compares favorably in terms of run time, sensitivity, and validation compliance. The method described by Vyas et al. (2019) for amitriptyline and pregabalin and that by Kasawar and Farooqui (2010) for pregabalin capsules share similar mobile phase principles, lending further credibility to the current approach.

The validated analytical parameters demonstrate that the method meets all ICH Q2(R1) requirements. The high sensitivity (LOD 0.087 µg/mL for ESC and 0.041 µg/mL for ETI) indicates that the method could also be applied for related substance and degradation product profiling. Ruggedness studies across different analysts and days confirmed robustness under routine laboratory conditions.

5. CONCLUSION

A simple, rapid, sensitive, precise, and accurate RP-HPLC method has been successfully developed and validated for the simultaneous estimation of Escitalopram and Etizolam in bulk drug and tablet dosage form. The method employs an isocratic mobile phase (acetonitrile:0.2 mM ammonium acetate buffer, 60:40, pH 5.5) on a C18 column with UV detection at 250 nm, providing well-resolved peaks within 8 minutes.

The method was validated in accordance with ICH Q2(R1) guidelines for all relevant parameters: linearity, accuracy, precision, specificity, LOD, LOQ, and robustness. All validation results were within acceptable limits. The method demonstrated excellent linearity (R^2 > 0.999), high sensitivity (LOD < 0.1 µg/mL for both drugs), and acceptable accuracy (recovery 98–102%).

The successful application of the method to the analysis of a marketed combined tablet formulation confirms its suitability for routine quality control and stability testing. Future work should explore the applicability of this method to biological matrix analysis and pharmacokinetic studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this research.

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