

Bioanalytical Method Development and Validation For The Estimation of Fexuprazan Hcl in Marketed Formulations

Tarun Acharya, Govind Nayak*, Surendra Kumar Jain, Priyanka Namdeo

Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal, Madhya Pradesh, India -462022
Corresponding Author*: Dr. Govind Nayak. Email ID: govindn@lnct.ac.in

ABSTRACT

The present study was undertaken for the development and validation of an RP-HPLC method for the quantitative estimation of Fexuprazan HCl and its application to the analysis of marketed tablet formulation. The work included preformulation characterization of the drug, optimization of chromatographic conditions, preparation of standard and sample solutions, and evaluation of important analytical method validation parameters. Fexuprazan Hydrochloride, a novel potassium-competitive acid blocker, requires a reliable analytical method for quality control. In the present study, a simple, accurate, precise, and stability-indicating RP-HPLC method was successfully developed and validated as per ICH guidelines. The method demonstrated excellent linearity ($R^2 = 0.999$), satisfactory precision, high accuracy (100.02–100.06% recovery), and good sensitivity. The assay of the marketed formulation was found to be 99.60%.

KEYWORDS: Method Development, Validation, AQBD, Robust, LOD, LOQ, Methanol, Acetonitrile, RP-HPLC.

1. INTRODUCTION

Fexuprazan Hydrochloride is a medication primarily used to treat conditions related to excess stomach acid. It is a proton pump inhibitor (PPI), which works by reducing the amount of acid produced in the stomach. This class of drugs is commonly prescribed for conditions like gastroesophageal reflux disease (GERD), peptic ulcers, and gastric ulcers, as well as to help in the healing of acid-related damage to the esophagus and stomach lining. Fexuprazan works by inhibiting the proton pump (H^+/K^+ ATPase) in the stomach lining, which is responsible for the final step in acid secretion. This decreases stomach acid production, thereby providing relief from symptoms related to acid reflux, heartburn, and ulcers. Fexuprazan Hydrochloride is indicated in Gastroesophageal reflux disease (GERD) to help manage acid reflux by reducing stomach acid, Peptic and gastric ulcers to aid in the healing of ulcers by creating a less acidic environment and *Helicobacter pylori* eradication which often part of combination therapy to eradicate this bacteria, which is linked to ulcers. Fexuprazan Hydrochloride is administered orally in the form of its hydrochloride salt, and its absorption is optimized when taken before meals. After administration, it undergoes rapid absorption with peak plasma concentrations typically achieved within a few hours. Fexuprazan Hydrochloride's reversible mechanism of action may confer several advantages over older PPIs. These include a faster onset of action and a more consistent pH profile, which could enhance therapeutic outcomes in patients with severe GERD or ulcers [1,2]. Additionally, early studies suggest that Fexuprazan Hydrochloride may present a lower risk of adverse effects commonly associated with PPIs, such as bone fractures, hypomagnesemia, and vitamin B12 deficiency. Furthermore, it may be more effective at maintaining a higher and more stable intragastric pH, potentially leading to better healing outcomes in gastric and duodenal ulcers. The chemical structure of fexuprazan hydrochloride is shown in Figure 1.

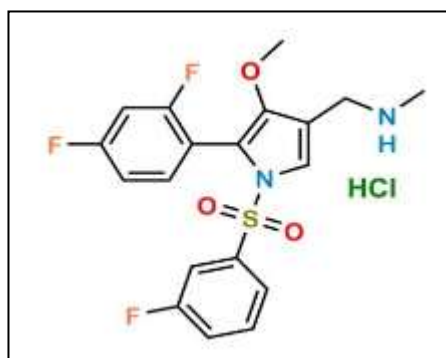


Figure 1. Structure of Fexuprazan Hydrochloride

Fexuprazan acts through reversible and potassium-competitive inhibition of the proton pump, blocking acid secretion irrespective of enzyme activation. Pharmacokinetic studies indicate rapid absorption with peak plasma concentration within 2–3 hours and sustained pharmacodynamic activity. The drug is primarily metabolized by CYP3A4 and shows minimal food interaction [3, 4]. Clinical studies have demonstrated comparable or improved efficacy over conventional PPIs [5].

Analytical approaches are vital to assure the quality and stability of pharmaceutical formulations. Reverse-phase high-performance liquid chromatography (RP-HPLC) is broadly utilized owing to its accuracy, sensitivity, and applicability for pharmaceutical formulations and biological matrices [6]. Method validation as per ICH guidelines ensures reliability and reproducibility [7].

2. MATERIALS AND METHODS

Materials: Fexuprazan hydrochloride reference standard was obtained from a certified pharmaceutical supplier (India). Commercial tablet formulation containing 40 mg of fexuprazan hydrochloride was procured from the local market. Methanol (HPLC grade) was purchased from Merck Ltd. (India). Potassium dihydrogen phosphate and acetonitrile were obtained from Rankem (India). Ultrapure water was prepared using a Milli-Q system. All reagents used were of analytical grade.

Bioanalytical method development of Fexuprazan HCl Using RP-HPLC

Selection of mobile phase

The mobile phase was selected based on the physicochemical properties of Fexuprazan HCl and its chromatographic behavior on a reversed-phase C18 column. Different combinations of aqueous buffers and organic solvents such as methanol and acetonitrile were investigated.

Methanol :Water	50:50 v/v	Poor resolution
Acetonitrile : Methanol	20:80 v/v	Most Suitable

Optimized Separation Variables for Fexuprazan HCl

Variable	Optimized condition
Column	Thermo Scientific C18 (Octadecylsilane)
Column dimension	250 mm × 4.6 mm
Particle size	5 µm
Bonded phase	Octadecylsilane (C18)
Mobile phase	Methanol (80:20, v/v)
Diluent	Methanol
Flow rate	1.0 mL/min
Column temperature	Ambient temperature
Injection volume	20 µL
Detection wavelength	254 nm
Retention time	Fexuprazan HCl: 5.125 ± 0.3 min

Preparation of Standard Stock Solution

An accurately weighed 10 mg of Fexuprazan HCl working standard was transferred into a 10ml volumetric flask. A 5 ml of the diluent (mobile phase) was added and the solution was sonicated until the drug was completely dissolved.

Preparation of Sub-Stock Solution

An appropriate aliquot 1 ml of the standard stock solution was accurately pipetted into a volumetric flask and diluted with the diluent to obtain a suitable intermediate or sub-stock solution of 100µg/ml.

Preparation of Different Working Solutions

Different working solutions 1 to 5 µg/ml of Fexuprazan HCl were prepared by sub-stock solution using the selected diluent. Appropriate concentrations covering the expected analytical range were prepared for construction of the calibration curve. All solutions were prepared freshly until analysis.

Protein Precipitation Procedure

For preparation of plasma samples, 1 ml of blank plasma was transferred into labelled microcentrifuge tubes. 2ml of Fexuprazan HCl working solution was added to the plasma to obtain the desired concentration. The plasma-drug mixture was vortex mixed thoroughly.

Preparation of Calibration Standards in Plasma

Appropriate aliquots of the Fexuprazan HCl working solution were added to predetermine volumes of blank plasma to prepare a series of calibration standards covering the selected concentration range. Each calibration sample was mixed thoroughly and subjected to the same protein precipitation procedure. The chromatographic response obtained for each concentration was recorded.

Preparation of Blank and Zero Samples

A blank plasma sample containing no drug was processed using the complete sample- preparation procedure to determine interference from endogenous plasma components. A zero sample, consisting of blank plasma, was also processed to distinguish the response of the endogenous components and the analyte.

Linearity and Calibration Curve

Linearity was evaluated by preparing a series of Fexuprazan HCl calibration standards at different concentrations covering the proposed analytical range. Each Linear regression analysis was performed to determine the regression equation and correlation coefficient (r or R^2).

The regression equation was expressed as:

$$Y = mX + c$$

Where: Y = chromatographic response or peak-area ratio, c = intercept

X = concentration of Fexuprazan HCl m = slope of the calibration curve

Chromatographic Analysis

The processed samples were injected into the RP-HPLC system using the optimized chromatographic conditions. The chromatograms were monitored at the selected detection of wavelength. The retention time of Fexuprazan HCl was recorded, and the peak area was used for quantitative determination. The developed method was subsequently evaluated for specificity, precision, accuracy, recovery, sensitivity, and stability according to the intended bioanalytical validation requirements (Loh et al., 2023).

Validation of developed method

The validation parameters as per ICH guidelines are described below:

Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration or the amount of analyte in the sample. The calibration plot was constructed after analysis of five different (from 1 to 5 µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated.

Specificity

Specificity was assessed by analyzing blank, standard, and sample solutions to confirm the absence of interference at the retention time of fexuprazan hydrochloride, demonstrating method selectivity [7].

Accuracy

Accuracy was evaluated using the standard addition method at 80%, 100%, and 120% levels. The percentage

recovery values were within acceptable limits, confirming method trueness [7].

Precision

Precision was assessed in terms of repeatability and intermediate precision. The %RSD values were found to be within acceptable limits, indicating good method reproducibility [7].

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5 µg/ml for Fexuprazan HCl indicates the precision under the same operating condition over short interval time (Vybiralova et al., 2005).

2. Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations.

Robustness

The robustness of an analytical method is a measure of its ability to remain unaffected by small, but deliberate change in method parameters and provides an indication of to remain normal during usage. As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected (Sher et al., 2010). The ratio of mobile phase was change from, methanol: acetonitrile (80:20 % v/v) to (85:15 % v/v).

Detection Limit and Quantitation Limit

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve, indicating adequate sensitivity of the method [7].

The detection limit (DL) may be expressed as:

$$DL=3.3 \sigma / S$$

Where, σ is the standard deviation of the response, S is the slope of the calibration curve. The slope S might be determined from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, based on the standard deviation of the blank and the calibration curve. A signal-to-noise ratio between 10 or 10:1 is generally considered acceptable for estimating the quantitation limit. The quantitation limit (QL) may be expressed as: $QL=10 \sigma / S$

Where, σ is the standard deviation of the response, S is the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, based on the standard deviation of the blank and the calibration curve. The LOQ level is usually confirmed by injecting standards which have an acceptable percent relative standard deviations (% RSD) not more than 10 %.

Analysis of marketed formulation

The developed RP-HPLC method was applied for the quantitative analysis of Fexuprazan HCl in marketed tablet formulations. A quantity of the powdered tablets equivalent to 1 mg of Fexuprazan HCl was accurately weighed and transferred into a 10 mL volumetric flask. Approximately 5 mL of the selected diluent/mobile phase was added, and the mixture was sonicated for about 10–15 min with intermittent shaking to ensure complete extraction of the drug. The volume was then made up to the mark with the same diluent and mixed thoroughly. The resulting solution was filtered through Whatman filter paper No. 41 to remove insoluble excipients. An appropriate aliquot of the clear filtrate was further diluted with the diluent to obtain a final concentration of 1 µg/ml.

The prepared sample solution was transferred to an HPLC vial and 20 µl was injected into the RP-HPLC system under the optimized chromatographic conditions. The chromatogram was recorded and the peak area corresponding to Fexuprazan HCl was noted.

3. RESULTS AND DISCUSSION

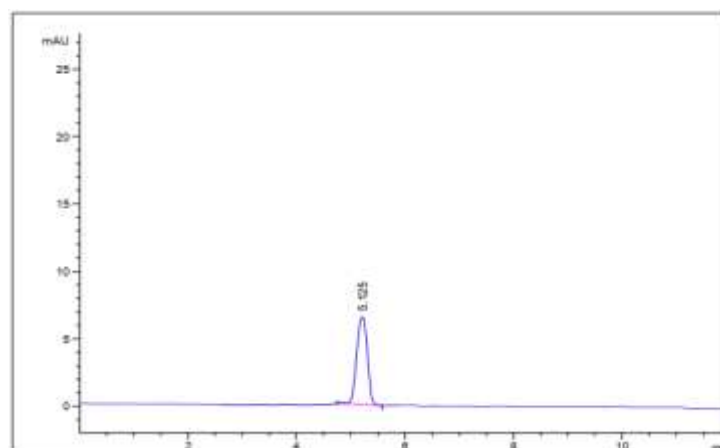
Results of Bioanalytical developed method by RP-HPLC

Results of Selection of mobile phase

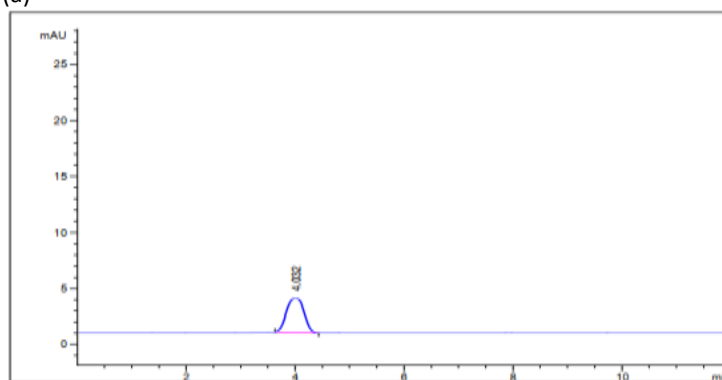
The selection of a suitable mobile phase was performed by evaluating different solvent combinations to obtain satisfactory chromatographic separation of Fexuprazan HCl.

Table 3.1: Selection of mobile phase

Mobile Phase	Ratio	Retention Time
Methanol : water	50:50 v/v	Not Found
Acetonitrile : Methanol	20:80 v/v	Most Suitable



(a)



(b)

Figure 2: a) Trial Graph of Fexuprazan HCl in Methanol: Water (50:50 v/v)
b) Trial Graph of Fexuprazan HCl in Acetonitrile: Methanol (20:80 v/v)

Results of Linearity and Calibration Graph

The mean AUC values obtained for the concentrations of 1, 2, 3, 4 and 5 mg/ml were approximately 655.58, 1314.62, 1936.47, 2645.27 and 3252.98, respectively. The calibration curve demonstrated a strong linear relationship between Fexuprazan HCl concentration and AUC. The correlation coefficient ($R^2 = 0.999$) indicated excellent linearity over the selected concentration range. The regression equation was obtained as $Y = 653.07X + 1.3836$, where Y represents the AUC and X represents the concentration of Fexuprazan HCl.

Table 3.2: Linearity of Fexuprazan HCl

Standard Concentration $\mu\text{g/ml}$	Area under Curve (AUC)						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
1	655.453	645.854	668.745	640.332	670.336	648.785	654.918
2	1305.652	1310.66	1322.44	1320.34	1310.66	1318.996	1314.790
3	1932.269	1952.46	1945.22	1940.63	1920.48	1927.774	1936.472
4	2645.454	2640.34	2645.59	2635.46	2638.77	2665.998	2645.268
5	3242.657	3255.7	3260.46	3250.75	3285.46	3222.854	3252.978
Correl Coeff (r^2)							0.999
Slope (m)							653.07
Intercept (c)							1.3836

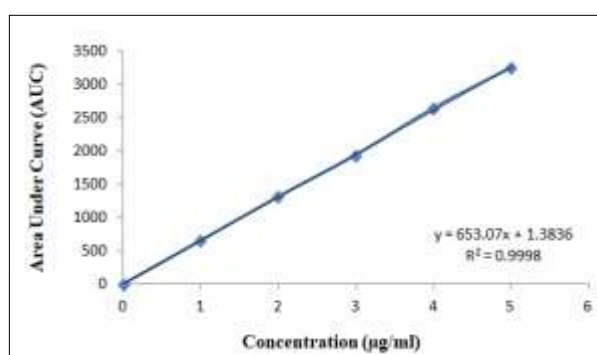


Figure 3: Calibration Curve of Fexuprazan HCl

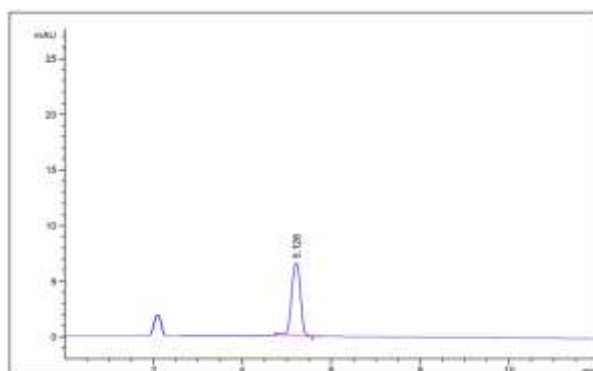


Figure 4: Chromatogram of Fexuprazan HCl

Results of System suitability parameters

The system suitability study was performed to assess the performance and reproducibility of the developed RP-HPLC method for Fexuprazan HCl. Six replicate injections of the standard solution were analyzed under the optimized chromatographic conditions. The retention time of Fexuprazan HCl was consistent, ranging from 5.122 to 5.126 min, with a mean retention time of 5.124 ± 0.002 min and a %RSD of 0.031%, indicating excellent reproducibility of the chromatographic retention.

The mean AUC was found to be 3252.980 ± 20.676 , with a %RSD of 0.636%, demonstrating good repeatability of the detector response. The number of theoretical plates ranged from 2950 to 2985, with a mean value of 2966.667 ± 11.810 , indicating satisfactory column efficiency. The mean tailing factor was 1.150 ± 0.023 , with a %RSD of 1.983%, indicating a symmetrical and acceptable peak shape.

Table 3.3: System suitability parameters of Fexuprazan HCl

System suitability Parameter 	RT	AUC	. of theoretical plates	Tailing factor
Rep-1	5.123	3242.66	2950	1.18
Rep-2	5.126	3255.7	2965	1.17
Rep-3	5.122	3260.46	2985	1.13
Rep-4	5.124	3250.75	2974	1.15
Rep-5	5.126	3285.46	2963	1.12
Rep-6	5.124	3222.85	2963	1.15
Mean	5.124	3252.980	2966.667	1.150
S.D.	0.002	20.676	11.810	0.023
% R.S.D.	0.031	0.636	0.398	1.983

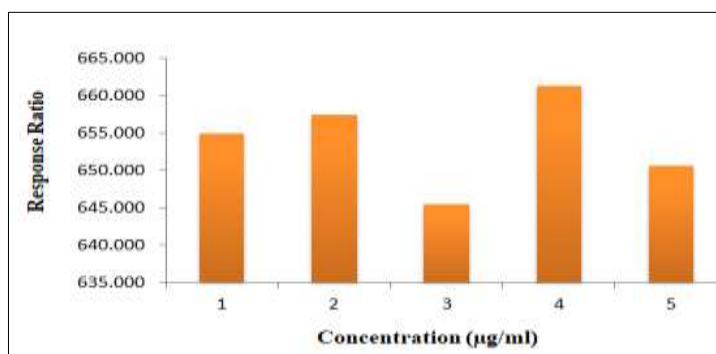
Results of Validation of developed method

Results of Linearity

The response ratio study for Fexuprazan HCl was performed over the concentration range of 1–5 mg/mL. The response ratio was calculated by dividing the mean AUC by the corresponding concentration of Fexuprazan HCl. The response ratios obtained were 654.918, 657.395, 645.491, 661.317, and 650.596 for 1, 2, 3, 4, and 5 mg/mL, respectively. The mean response ratio was found to be 653.943 ± 6.121 , indicating only minor variation in the chromatographic response across the selected concentration range. The calculated %RSD was 0.936%, which demonstrates good consistency and reproducibility of the detector response.

Table 3.4: Response ration data for linearity of Fexuprazan HCl

Replicates	Concentration (μ g/ml)	Mean AUC	Response Ratio
Rep-1	1	654.918	654.918
Rep-2	2	1314.79	657.395
Rep-3	3	1936.472	645.491
Rep-4	4	2645.268	661.317
Rep-5	5	3252.978	650.596
Mean			653.943
SD			6.121
%RSD			3.492



Results of Specificity

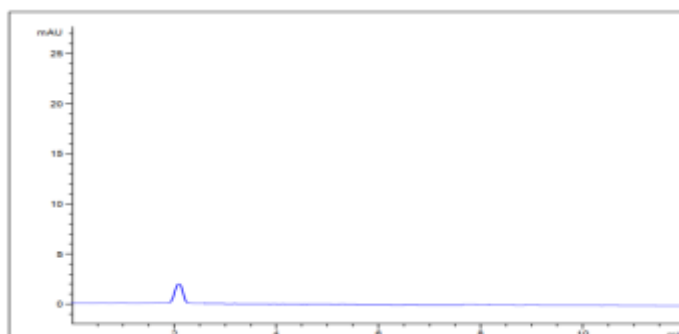


Figure 6: Chromatogram of blank diluents and Chromatogram of Fexuprazan HCl

Results of Accuracy

Recovery study of Fexuprazan HCl (80% Level)

The recovery study of Fexuprazan HCl was carried out at the 80% level to evaluate the accuracy of the developed RP-HPLC method. The study was performed at three concentration levels, with three replicate determinations at each level. The percentage recovery obtained for the three replicate samples at the 1 mg/ml concentration was 97.50– 100.00%, with a mean recovery of 98.75%. At 2 mg/ml, the recovery ranged from 98.75– 99.38%, giving a mean recovery of 99.17%. At 3 mg/ml, the recovery ranged from 98.33– 99.58%, with a mean recovery of 99.03%.

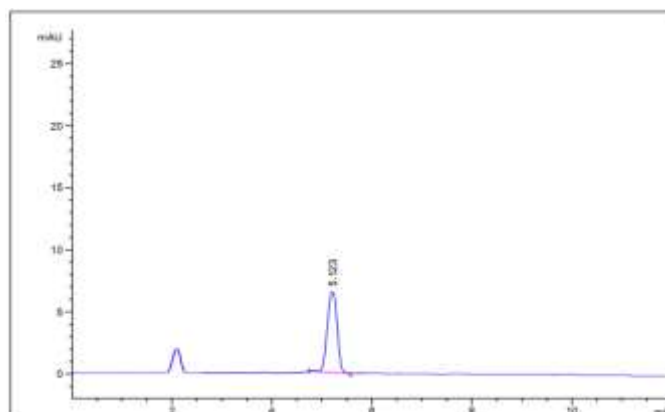


Table 3.5: Recovery study of Fexuprazan HCl (80% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
1	0.8	0.78	0.79	0.8	97.50	98.75	100.00	98.75
2	1.6	1.58	1.59	1.59	98.75	99.38	99.38	99.17
3	2.4	2.36	2.38	2.39	98.33	99.17	99.58	99.03
MEAN SD								98.98
% RSD								0.212
								0.214

Recovery study of Fexuprazan HCl (100% Level)

The study was carried out at three concentration levels, with three replicate determinations at each level. At the 1 mg/ml concentration, the percentage recoveries were 99.00%, 98.00%, and 96.00%, with a mean recovery of 97.67%. At the 2 mg/ml concentration, the recoveries were 99.00%, 98.00%, and 99.00%, giving a mean recovery of 98.67%. At the 3 mg/ml concentration, the recoveries were 99.33%, 98.67%, and 99.00%, with a mean recovery of 99.00%.

Table 3.6: Recovery study of Fexuprazan HCl (100% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
1	1	0.99	0.98	0.96	99.00	98.00	96.00	97.67
2	2	1.98	1.96	1.98	99.00	98.00	99.00	98.67
3	3	2.98	2.96	2.97	99.33	98.67	99.00	99.00
MEAN								98.44
SD								0.694
% RSD								0.705

Recovery study of Fexuprazan HCl (120% Level)

The study was carried out at three concentration levels, with three replicate determinations at each level. At the 1 mg/ml sample concentration, the percentage recoveries were 98.33%, 99.17%, and 97.50%, with a mean recovery of 98.33%. At the 2 mg/ml concentration, the recoveries were 98.33%, 99.17%, and 98.75%, giving a mean recovery of 98.75%. At the 3 mg/ml concentration, the recoveries were 99.72%, 99.17%, and 99.44%, with a mean recovery of 99.44%.

Table 3.7: Recovery study of Fexuprazan HCl (120% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% Conc. Found			Mean % conc
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
1	1.2	1.18	1.19	1.17	98.33	99.17	97.50	98.33
2	2.4	2.36	2.38	2.37	98.33	99.17	98.75	98.75
3	3.6	3.59	3.57	3.58	99.72	99.17	99.44	99.44
MEAN								98.84
SD								0.561
% RSD								0.568

Results of Precision

Repeatability of Fexuprazan HCl

The repeatability study of Fexuprazan HCl was performed at five concentration levels to evaluate the consistency and precision of the developed RP-HPLC method. Five replicate determinations were carried out at each concentration, and the concentration found for each replicate was recorded. The mean concentrations obtained at 1, 2, 3, 4, and 5 mg/ml were 0.976, 1.974, 2.966, 3.962, and 4.918 mg/ml, respectively. The corresponding mean percentage values were 97.60%, 98.70%, 98.87%, 99.05%, and 98.36%, respectively.

Table 3.8: Repeatability of Fexuprazan HCl

Conc. Rep.	Concentration found (µg/ml)					Mean
	1	2	3	4	5	
Replicate-1	0.99	1.95	2.99	3.98	4.95	
Replicate-2	0.98	1.98	2.96	3.96	4.98	
Replicate-3	0.97	1.99	2.95	3.94	4.78	
Replicate-4	0.96	1.96	2.97	3.97	4.92	
Replicate-5	0.98	1.99	2.96	3.96	4.96	
MEAN	0.976	1.96	2.99	3.92	4.92	
% MEAN	97.60	98.00	99.67	98.00	98.40	98.33
SD	0.011	0.018	0.015	0.015	0.080	0.028
% RSD	0.012	0.019	0.015	0.015	0.081	0.028

Day-to-day variation of Fexuprazan HCl

The day-to-day variation study of Fexuprazan HCl was performed over three consecutive days to evaluate the intermediate precision and reproducibility of the developed RP-HPLC method. The drug was analyzed at five concentration levels ranging from 1 to 5 mg/ml, and the concentration found was recorded on each day. The mean concentrations obtained for 1, 2, 3, 4, and 5 mg/ml were approximately 0.980, 1.973, 2.967, 3.960, and 4.903 mg/ml, respectively. The corresponding mean percentage values were approximately 98.00%, 98.67%, 98.89%, 99.00%, and 98.07%.

Table 3.9: Day-to-day variation of Fexuprazan HCl

Conc. Rep.	Concentration found (µg/ml)					Mean
	1	2	3	4	5	
Day-1	0.99	1.95	2.99	3.98	4.95	
Day -2	0.98	1.98	2.96	3.96	4.98	
Day -3	0.97	1.99	2.95	3.94	4.78	
MEAN	0.98	1.96	2.99	3.92	4.92	
% MEAN	98.00	98.00	99.67	98.00	98.40	98.41
SD	0.010	0.021	0.021	0.020	0.108	0.036
% RSD	0.010	0.021	0.021	0.020	0.110	0.036

Robustness of Fexuprazan HCl

The robustness study of Fexuprazan HCl was performed to evaluate the ability of the developed RP-HPLC method to remain unaffected by small variations in analytical conditions. The study was carried out at five concentration levels ranging from 1 to 5 mg/ml, with five replicate determinations at each level. The mean concentrations obtained were approximately 0.934, 1.942, 2.978, 3.962, and 4.970 mg/ml for 1, 2, 3, 4, and 5 mg/ml, respectively. The corresponding mean percentage values were 93.40%, 97.10%, 99.27%, 99.05%, and 99.40%, with an overall mean of 97.64%. The results showed good consistency at the higher concentration levels, while slightly greater variation was observed at the 1 mg/mL level.

Table 3.10: Robustness of Fexuprazan HCl

Conc. REP.	Concentration (µg/ml)					Mean
	1	2	3	4	5	
Replicate-1	0.95	1.98	2.98	3.98	4.95	
Replicate-2	0.85	1.85	2.95	3.95	4.98	
Replicate-3	0.96	1.96	2.96	3.95	4.95	
Replicate-4	0.98	1.93	2.99	3.96	4.99	
Replicate-5	0.93	1.99	3.01	3.97	4.98	
MEAN	0.93	1.94	2.98	3.96	4.97	
% MEAN	93.40	97.10	99.27	99.05	99.40	97.64

SD	0.05	0.06	0.02	0.01	0.02	0.03
% RSD	0.05	0.06	0.02	0.01	0.02	0.03

Results of detection limit and quantitation limit

Table 3.11: LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Fexuprazan HCl	0.10	0.35

Results of analysis of marketed formulation

The developed RP-HPLC method was applied for the quantitative analysis of Fexuprazan HCl in the marketed tablet formulation. The formulation had a label claim of 40 mg, while the amount of drug found was 39.84 mg, corresponding to a mean assay of 99.60%. The obtained assay value indicates that the formulation contained an amount of Fexuprazan HCl close to its labelled claim. The standard deviation was found to be 0.145, with a %RSD of 0.149%, indicating very low variation among the replicate measurements and demonstrating good precision of the analytical method. The assay result of 99.60% also indicates satisfactory drug content and confirms the suitability of the developed RP-HPLC method for the quantitative estimation of Fexuprazan HCl in marketed tablet formulations. The method provided accurate, precise, and reproducible results for tablet analysis.

Table 3.12: Analysis of tablets sample

S. No.	Parameter	% Assay
1.	Label Claim (%)	40mg
2.	% Found	39.84
3.	Mean	99.60
4.	S. D.	0.145
5.	% RSD	0.149

SUMMARY AND CONCLUSION

The present study was undertaken for the development and validation of an RP-HPLC method for the quantitative estimation of Fexuprazan HCl and its application to the analysis of marketed tablet formulation. The work included preformulation characterization of the drug, optimization of chromatographic conditions, preparation of standard and sample solutions, and evaluation of important analytical method validation parameters.

The melting point of the standard drug was found to be 174°C, while the sample exhibited a melting range of 174–176°C, indicating good agreement with the standard. During chromatographic method development, different mobile-phase compositions were investigated. Methanol:water (50:50, v/v) showed poor resolution, whereas methanol:acetonitrile (80:20, v/v) provided a satisfactory chromatographic response. The optimized method employed a Thermo C18 column (250 × 4.6 mm, 5 µm), methanol:acetonitrile (80:20, v/v) as the mobile phase, methanol as diluent, a flow rate of 1.0 mL/min, ambient temperature, an injection volume of 20 µL, and detection at 254 nm. Under these conditions, Fexuprazan HCl showed a well-defined peak at approximately 5.125 min. The developed method demonstrated satisfactory linearity over the selected concentration range of 1–5 mg/mL, with an R² value of 0.999. The regression equation was reported as Y = 653.07X + 1.3836, indicating a strong linear relationship between Fexuprazan HCl concentration and chromatographic response. System suitability studies demonstrated good reproducibility of the chromatographic system. The mean retention time was 5.124 ± 0.002 min, with a %RSD of 0.031%. The mean AUC was 3252.980 ± 20.676, while the mean theoretical plate count was 2966.667 ± 11.810. The mean tailing factor was 1.150 ± 0.023, indicating satisfactory column efficiency and peak symmetry.

The accuracy of the method was evaluated through recovery studies at 80%, 100%, and 120% levels. The

overall mean recoveries were approximately 98.98%, 98.44%, and 98.84%, respectively, demonstrating good agreement between the added and recovered amounts of Fexuprazan HCl. The robustness study showed that the method maintained satisfactory performance across the evaluated concentration levels, with an overall mean result of approximately 97.64%. The sensitivity of the method was assessed by determining the limit of detection and limit of quantification. The LOD was found to be 0.10 mg/mL, while the LOQ was 0.35 mg/mL, demonstrating adequate sensitivity for the detection and quantitative estimation of Fexuprazan HCl.

The method was successfully applied to a marketed tablet formulation, where the drug content was found to be 99.60% of the labelled claim, with a low %RSD of 0.149%. Therefore, the developed RP-HPLC method can be considered simple, accurate, precise, sensitive, reproducible, and suitable for the quantitative estimation and routine quality-control analysis of Fexuprazan HCl in pharmaceutical tablet formulations.

Declarations:

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Author Contributions: Tarun Acharya contributed to method development, data interpretation, and manuscript drafting. Dr. Govind Nayak supervised the study and critically reviewed the manuscript. All authors approved the final version.

Competing Interests: The authors declare that they have no competing interests.

Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval Not applicable: This study did not involve human participants, human tissues, animals, or clinical investigations.

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