

Development, Optimization and Validation of a Robust RP-HPLC Method for the Estimation of Vonoprazan Fumarate in Bulk Drug and Pharmaceutical Formulation Using an Analytical Quality by Design (AQbD) Approach

Sergin P¹, SivaSubramanian P², Poovizhi M³, Murugesan S⁴, Kavisri R⁵

^{1,3,4,5} Post graduate student, ² Assistant Professor

^{1,2,3,4,5} Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

Abstract: Vonoprazan fumarate is a potassium-competitive acid blocker, and a rapid and robust chromatographic method is needed for its routine quality control. A reversed-phase HPLC (RP-HPLC) method was developed using an Analytical Quality by Design (AQbD) approach. Acetonitrile content, buffer pH and flow rate were studied using a 15-run design of experiments with retention time as the response, and the optimized method was validated according to ICH guidelines. Separation was achieved on an X-Bridge C18 column (2.1 × 100 mm, 3.5 µm) with phosphate buffer (pH 3.0) and acetonitrile (95:5, v/v) at 1.0 mL/min and 230 nm, giving a retention time of about 2.83 min. A quadratic model described the retention time data very well ($R^2 = 0.9999$), and acetonitrile content had the strongest influence. The method was linear over 2–10 µg/mL ($R^2 = 0.9997$), with a mean recovery of 99.97%, a system precision of 0.28% RSD, an LOD of 1.18 µg/mL, an LOQ of 3.58 µg/mL and robustness %RSD values below 2%. The assay of a tablet formulation was 99.93% of the label claim. The method is rapid, specific, accurate, precise and robust, and is suitable for routine quality control of vonoprazan fumarate. Forced degradation studies were not part of this work.

Keywords: AQbD; RP-HPLC; vonoprazan fumarate; design of experiments; response surface methodology; method validation; quality control

INTRODUCTION

Vonoprazan fumarate is a potassium-competitive acid blocker (P-CAB) used in the management of acid-related gastrointestinal disorders, including gastric ulcers and gastroesophageal reflux disease (GERD). Chemically, it is 1-(5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl) methylmethanamine fumarate. Unlike conventional proton pump inhibitors (PPIs), vonoprazan competitively inhibits potassium binding to the gastric H^+/K^+ -ATPase in parietal cells, thereby suppressing both active and resting proton pumps. Its acid stability, rapid absorption, and prolonged acid-suppressive activity contribute to its therapeutic advantages. Vonoprazan is available in 10 and 20 mg tablet strengths and

has gained regulatory approval for the treatment of acid-related disorders [1–4]. The development of reliable analytical methods is essential for assuring the identity, strength, quality, and stability of vonoprazan fumarate in pharmaceutical formulations. Although several analytical techniques, including UV spectrophotometry, fluorimetry, conventional RP-HPLC, and LC–MS-based methods, have been reported for the estimation and characterization of vonoprazan, these approaches may have limitations related to selectivity, matrix interference, solvent consumption, instrumentation requirements, or applicability to routine quality-control analysis [5–8]. Furthermore, the limited availability of comprehensive stability-indicating analytical procedures

emphasizes the need for a simple, reliable, and robust chromatographic method capable of providing consistent analytical performance. Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely employed in pharmaceutical analysis because of its versatility, reproducibility, sensitivity, and suitability for the separation and quantitative determination of drug substances and formulations. However, conventional chromatographic method development frequently relies on trial-and-error or one-factor-at-a-time approaches, which may not adequately evaluate interactions among critical method variables. Consequently, a systematic approach is desirable for developing chromatographic methods with improved robustness and predefined performance characteristics [9–14]. Analytical Quality by Design (AQbD) provides a science- and risk-based framework for systematic analytical method development. The approach begins with defining an Analytical Target Profile (ATP), followed by identification of critical method attributes (CMAs) and critical method parameters (CMPs), risk assessment, and systematic optimization using Design of Experiments (DoE). Evaluation of the relationship between method parameters and analytical responses enables the establishment of an appropriate Method Operable Design Region (MODR), within which the method is expected to provide consistent and reliable performance. AQbD therefore facilitates the development of robust analytical procedures while reducing experimental burden and supporting continual method improvement throughout the analytical lifecycle [15–18]. Analytical method validation is an essential component of method development and demonstrates that the procedure is suitable for its intended purpose. Parameters such as specificity, linearity, range, accuracy, precision, detection limit, quantification limit, and robustness are evaluated in accordance with applicable ICH recommendations. In addition, stability studies under hydrolytic, oxidative, thermal, and photolytic stress conditions are important for establishing the stability-indicating capability of the developed method [19,20]. Therefore, the present study was undertaken to develop, optimize, and validate a robust RP-HPLC method for the estimation of vonoprazan fumarate in bulk drug and pharmaceutical

formulation using an AQbD approach. The method development strategy involved systematic assessment of critical chromatographic variables using risk assessment and DoE, followed by optimization of the chromatographic conditions and evaluation of method performance through validation and stress studies. The developed method is intended to provide a simple, reliable, reproducible, and robust analytical procedure suitable for routine pharmaceutical quality-control applications.

2. Materials and Methods

2.1. Chemicals and Reagents

Vonoprazan fumarate reference standard, vonoprazan fumarate bulk drug, and marketed tablet formulation were used for the analytical study. HPLC-grade acetonitrile and methanol were procured from Merck, Mumbai, India. High-purity water was obtained from a Milli-Q water purification system. Sodium dihydrogen phosphate was used for preparation of the aqueous buffer, while analytical-grade hydrochloric acid, sodium hydroxide, and orthophosphoric acid were used for solution preparation and pH adjustment.

2.2. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed using a high-performance liquid chromatography system equipped with a quaternary pump, autosampler/injector, and photodiode array (PDA)/UV detector. Chromatographic data acquisition, peak integration, and system suitability calculations were performed using Empower chromatographic data system software. Separation was carried out using a reversed-phase C18 column.

The optimized chromatographic conditions consisted of a C18 stationary phase, phosphate buffer adjusted to pH 3.0 and acetonitrile as the mobile phase in the optimized ratio of 95:5 (v/v). The flow rate was maintained at 1.0 mL/min, the detection wavelength was 230 nm, the injection volume was 10 μ L, and the column temperature was maintained at 30 $^{\circ}$ C. The total chromatographic run time was 10 min. The optimized conditions are summarized in Table 1.

Table 1. Optimized chromatographic conditions.

Parameter	Optimized condition
Column	X-Bridge C18, 2.1 × 100 mm, 3.5 µm
Mobile phase	Phosphate buffer : ACN (95:5 v/v)
Buffer pH	3.0
Flow rate	1.0 mL/min
Detection	230 nm
Column temperature	30 °C
Injection volume	10 µL
Retention time	≈ 2.83 min

2.3. Preparation of Phosphate Buffer

Sodium dihydrogen phosphate (12 g) was accurately weighed and transferred into a suitable volumetric vessel. Approximately 80–90% of the required volume of Milli-Q water was added and the solution was sonicated until complete dissolution. The pH was adjusted to 3.0 using diluted orthophosphoric acid, and the volume was made up to the required volume with Milli-Q water. The buffer was filtered through a 0.45 µm membrane filter and degassed by sonication prior to use.

2.4. Preparation of Diluent and Mobile Phase

The diluent was prepared using phosphate buffer at pH 3.0 and the selected organic solvent in the optimized mobile-phase proportion. The resulting solution was filtered through a suitable membrane filter and degassed before use. The mobile phase was prepared by mixing phosphate buffer (pH 3.0) and acetonitrile in a 95:5 (v/v) ratio. The prepared mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication prior to chromatographic analysis.

2.5. Preparation of Standard Stock and Working Solutions

A standard stock solution of vonoprazan fumarate was prepared by accurately weighing 10 mg of the reference standard and transferring it into a 100 mL volumetric flask. Approximately three-fourths of the required volume of diluent was added, followed by sonication for approximately 10 min to ensure complete dissolution. The solution was then diluted to volume with the diluent and mixed thoroughly. The resulting stock solution contained 100 µg/mL of vonoprazan fumarate. For preparation of the working standard solution, 1.0 mL of the standard stock solution was transferred into a 10 mL volumetric flask and diluted to volume with the diluent. The resulting solution contained 10 µg/mL of vonoprazan fumarate and was used as the 100% nominal concentration.

2.6. Preparation of Sample Solution

For the pharmaceutical formulation, ten vonoprazan fumarate tablets were individually weighed and the average tablet weight was determined. The tablets were finely powdered, and an accurately weighed quantity of the powder equivalent to 10 mg of vonoprazan fumarate was transferred into a 100 mL volumetric flask. Approximately 50 mL of diluent was added and the mixture was sonicated for approximately 25 min to facilitate complete extraction of the drug from the tablet matrix. The solution was allowed to cool to room temperature, when necessary, and diluted to volume with the diluent. The solution was mixed thoroughly and filtered through a suitable 0.45 µm membrane filter, discarding the initial portion of the filtrate. The resulting sample stock solution contained 100 µg/mL of vonoprazan fumarate. A 1.0 mL aliquot of the sample stock solution was subsequently diluted to 10 mL with diluent to obtain a final concentration of 10 µg/mL.

2.7. AQbD-Based Analytical Method Development

The RP-HPLC method was developed using an Analytical Quality by Design (AQbD) approach. The analytical method development strategy involved defining the intended analytical performance, identifying method variables that could potentially influence chromatographic performance, and optimizing the critical chromatographic conditions. The chromatographic variables considered during method development included mobile-phase composition, buffer pH, flow rate, column temperature, and detection wavelength. The principal chromatographic responses considered for evaluating method performance included retention time, peak shape, theoretical plate count, and resolution. Risk-based assessment was used to identify parameters having a potential influence on chromatographic performance. Critical method parameters were subsequently optimized to obtain suitable chromatographic separation and reproducible analytical performance. The optimized chromatographic conditions consisted of phosphate buffer (pH 3.0) and acetonitrile (95:5, v/v), a flow rate of 1.0 mL/min, detection at 230 nm, injection volume of 10 µL, and column temperature of 30 °C. Under these conditions, vonoprazan fumarate was satisfactorily separated and quantified.

2.8. Design of Experiments

A Quality by Design (QbD)-based Design of Experiments (DoE) approach was employed for the systematic optimization of the chromatographic conditions for the determination of vonoprazan fumarate. A three-factor experimental design consisting of 15 experimental runs was selected to evaluate the individual and combined effects of the critical method variables on chromatographic performance. The selected factors were acetonitrile volume (A), pH of the aqueous phase (B), and flow rate (C). These factors were selected based on preliminary method development studies and their expected influence on chromatographic behavior, particularly retention time. The response selected for optimization was retention time (min). The experimental retention time values obtained from the 15 runs ranged from 2.83 to 4.39 min. The factor combinations and the corresponding chromatographic observations are presented in Table 2. The generated experimental data were subsequently subjected to statistical analysis to evaluate the significance of the selected factors and to identify the optimum chromatographic conditions for the RP-HPLC method.

2.9. System Suitability

System suitability testing was performed to verify the adequacy and consistency of the chromatographic system under the optimized conditions. The HPLC system was equilibrated with the optimized mobile phase until a stable baseline was obtained. The standard solution of vonoprazan fumarate at 10 µg/mL was injected six consecutive times. Retention time, peak area, theoretical plate count, tailing factor, and resolution, where applicable, were evaluated. The mean, standard deviation, and percentage relative standard deviation (%RSD) were calculated for the replicate injections. The chromatographic system was considered suitable when the predetermined system suitability requirements were fulfilled. The acceptance criteria included %RSD of retention time not greater than 1.0%, %RSD of peak area not greater than 2.0%, theoretical plates of at least 2000, tailing factor not greater than 2.0, and resolution of at least 2.0 between critical peaks.

2.10. Method Validation

The method was validated as per ICH guidelines [9] for specificity, linearity, accuracy, precision, LOD, LOQ and

robustness. Specificity was assessed by comparing the blank chromatogram with that of the standard. Linearity was studied at 20, 40, 60, 80 and 100% of the working concentration (2–10 µg/mL). Accuracy was studied at 80, 100 and 120% levels. System, method, intraday and intermediate precision were evaluated. Robustness was assessed by deliberate changes in flow rate (0.8 and 1.2 mL/min), mobile phase ratio (97:3 and 93:7) and detection wavelength (228 and 232 nm). LOD and LOQ were calculated from the standard deviation of the response (σ) and the slope of the calibration curve (S):

$LOD = 3.3 \sigma / S,$	(1)
$LOQ = 10 \sigma / S.$	(2)

The assay of the tablet formulation was calculated as:

$\text{Assay (\%)} = \left(\frac{\text{Sample area}}{\text{Standard area}} \times \left(\frac{\text{Standard dilution}}{\text{Sample dilution}} \right) \times \text{Potency of standard (\%)} \right)$	(3)
---	-----

2.11. Data Analysis

Chromatographic data were acquired and processed using the chromatographic data system. The analytical results were expressed as mean, standard deviation, and percentage relative standard deviation, as appropriate. Regression analysis was used for evaluation of linearity, while the validation results were assessed against the predefined acceptance criteria for the respective analytical characteristics.

3. Results

3.1. Design of Experiments

The 15 experimental runs and the key chromatographic observations are summarized in Table 2. The retention time of vonoprazan fumarate ranged from 2.83 to 4.39 min across the design. Trial 14 (buffer : ACN 95:5 v/v, pH 3.0, 1.0 mL/min) gave a sharp, symmetrical peak with optimal retention time and was selected as the optimized condition. The chromatograms of Trials 1–15 are shown in Figure B1 (Appendix B).

Table 2. Chromatographic method optimization summary (Trials 1–15). Fixed parameters: X-Bridge C18 column (2.1 × 100 mm, 3.5 µm), 30 °C, 230 nm, injection volume 10 µL.

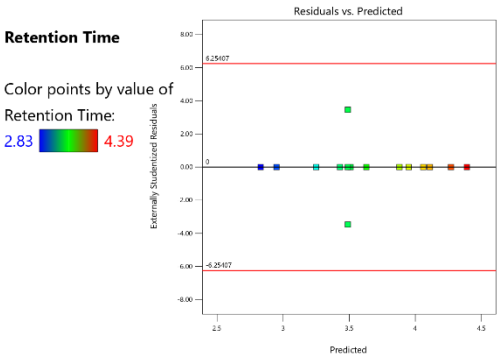
Trial	Buffer : ACN (v/v)	pH	Flow rate (mL/min)	Result / key observation
1	98:2	4.0	1.00	Increased RT, slight peak broadening, poorer symmetry.
2	95:5	4.0	0.75	Increased RT, slight broadening, minor deterioration in symmetry.
3	90:10	3.0	0.75	Decreased RT, sharper peak, improved symmetry, extra peak eluted.
4	95:5	4.0	0.75	Increased RT, slight broadening, minor symmetry reduction.
5	95:5	5.0	0.75	Increased broadening, reduced symmetry; higher pH unfavorable.
6	98:2	4.0	1.00	Improved sharpness and symmetry, separation not optimal.
7	98:2	4.0	0.50	Increased RT, slight peak broadening, minor reduction in symmetry.
8	95:5	3.0	0.50	Decreased RT, sharper peak, improved symmetry; high RT due to low flow.
9	95:5	5.0	1.00	Decreased RT, peak broadening, slight deterioration in symmetry.
10	95:5	5.0	0.50	Increased RT, slight broadening, tailing; prolonged run time.
11	95:5	4.0	0.75	Decreased RT, improved peak symmetry, reduced peak broadening.
12	98:2	5.0	0.75	Increased RT, slight broadening, minor deterioration in peak symmetry.
13	90:10	4.0	0.50	Earlier elution, sharper peak, improved symmetry; RT not optimal.
14 (optimized)	95:5	3.0	1.00	Sharp, symmetrical peak, optimal RT and performance (selected condition).
15	90:10	4.0	1.00	Earlier elution, sharp peak; lower RT compared to Trial 14.

3.2. Statistical Analysis of the Model

The DoE/RSM results are summarized in Table 3. A quadratic model was fitted to retention time. The model was highly significant ($p < 0.0001$) and the lack of fit was not significant ($p = 1.0000$). The R^2 , adjusted R^2 and predicted R^2 values were 0.9999, 0.9998 and 0.9998, respectively, the adequate precision was 302.09 and the CV was 0.1753%. In the normal plot of residuals, the points followed the straight line closely, supporting normality of the residuals (Figure 1a). The residuals were randomly scattered within the ± 6.25 bounds without any pattern, indicating a good model fit (Figure 1b).

Table 3. DoE/RSM statistical results for retention time.

Model statistic	Result
Model	Quadratic
Model p-value	< 0.0001
Lack of fit	$p = 1.0000$

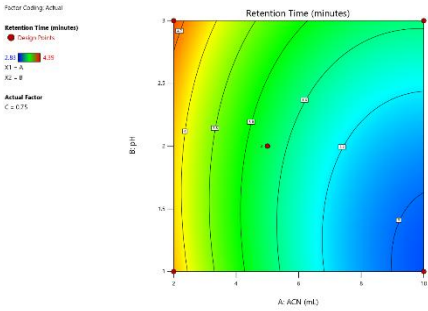


(b)

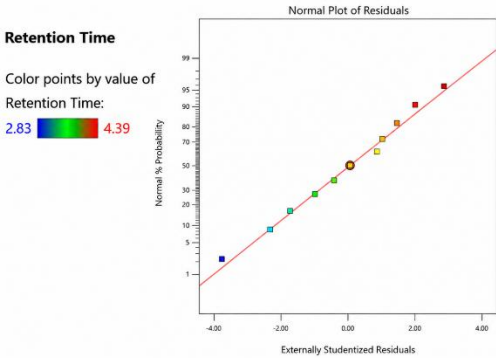
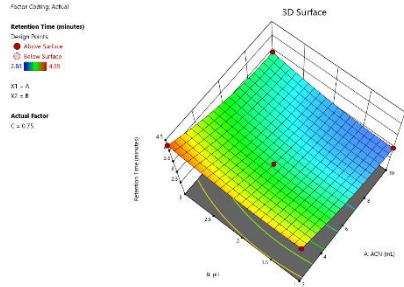
Figure 1. Diagnostic plots of the model for retention time: (a) normal plot of residuals; (b) residuals vs. predicted values.

3.3. Effect of Factors and Response Surface Analysis

The order of influence of the factors on retention time was ACN content > flow rate > pH. An increase in ACN content decreased the retention time, and an increase in flow rate also decreased the retention time, whereas pH showed a comparatively positive effect. The 2D contour plot (flow rate held at 0.75 mL/min) showed retention time ranging from about 3 min at high ACN content to about 4.2 min at low ACN content and higher pH (Figure 2a). The 3D surface plot showed the same relationship, with retention time decreasing as ACN content increased (Figure 2b).



(a)



(a)

(b)

Figure 2. Response surface plots of retention time as a function of ACN volume (A) and pH (B) at a flow rate of 0.75 mL/min: (a) 2D contour plot; (b) 3D surface plot.

3.4. Optimization and Confirmation

The numerical optimum was ACN 5 mL (buffer : ACN 95:5 v/v), pH 3.0 and flow rate 1.0 mL/min, with a predicted retention time of 2.83 min. The prediction interval for the mean was 2.815–2.845 min and that for an individual observation was 2.808–2.852 min. With the X-Bridge C18 column (2.1 × 100 mm, 3.5 µm) at 30 °C and detection at 230 nm, the observed retention time was approximately 2.83 min (Table 1).

3.5. System Suitability

System suitability was evaluated from replicate injections of the standard solution (Table 4; chromatograms in Figure B1). The retention time was 2.802–2.816 min, the tailing factor was about 3.30 (acceptance ≥ 2.0) and the plate count was at least 2794.

(acceptance $N \geq 2000$). The peak area %RSD was 0.28% (acceptance NMT 2.0%). The chromatographic system was therefore suitable for the analysis.

Table 4. System suitability parameters for vonoprazan fumarate.

RT (min)	USP plate count	Tailing	USP resolution
2.802	2802	1.22	3.297
2.808	2815	1.21	3.315
2.809	2794	1.23	3.286
2.809	2808	1.22	3.304
2.813	2799	1.21	3.291
2.816	2811	1.22	3.309

3.6. Method Validation

3.6.1. Specificity

The blank chromatogram showed no interfering peak at the retention time of vonoprazan fumarate, and no significant interference was observed (Figure 3).

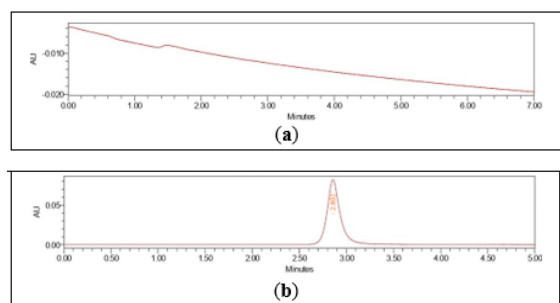


Figure 3. Chromatograms for specificity: (a) blank; (b) standard solution of vonoprazan fumarate.

3.6.2. Linearity

The method was linear over 2–10 µg/mL (20–100% levels) with a correlation coefficient (R^2) of 0.9997 (Table 5, Figure 4), showing an excellent linear response over the studied range. The chromatograms at each level are given in Figure B2.

Table 5. Linearity data for vonoprazan fumarate.

Level	Conc. (µg/mL)	Peak area
20%	2	752470
40%	4	1542967
60%	6	2305200
80%	8	3085855
100%	10	3800357

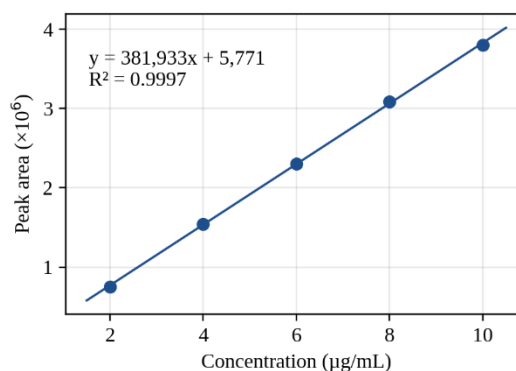


Figure 4. Linearity of vonoprazan fumarate over 2–10 µg/mL.

3.6.3. Accuracy

The mean recovery was 99.93%, 100.00% and 99.99% at the 80, 100 and 120% levels, respectively, with an overall mean recovery of 99.97% (Table 6). The chromatograms of the accuracy samples are given in Figure B3.

Table 6. Accuracy (recovery) of vonoprazan fumarate.

Accuracy level	Mean recovery (%)
80%	99.93
100%	100.00
120%	99.99
Overall	99.97

3.6.4. Precision

The precision results are given in Table 7. The system precision was 0.28% RSD and the method precision was 0.24% RSD. The intraday precision was 0.13% RSD, and the

intermediate precision over three days was 0.24%, 0.07% and 0.07% RSD for retention time, peak area and assay, respectively. The individual injection data are given in Appendix A (Table A1) and the chromatograms of the repeatability study in Figure B4.

Table 7. Precision of the method.

Precision parameter	%RSD
System precision	0.28
Method precision	0.24
Intraday precision	0.13
Intermediate precision – RT	0.24
Intermediate precision – area	0.07
Intermediate precision – assay	0.07

3.6.5. LOD and LOQ

The LOD and LOQ were calculated from the calibration curve over 1–1000 µg/mL (Figure 5; data in Table A4) using Equations (1) and (2). With $\sigma = 269.52$ and $S = 752.08521$, the LOD was 1.18 µg/mL and the LOQ was 3.58 µg/mL, corresponding to 0.118% and 0.358% of the 1000 µg/mL level (Table 8). The chromatograms at the studied levels are given in Figure B5.

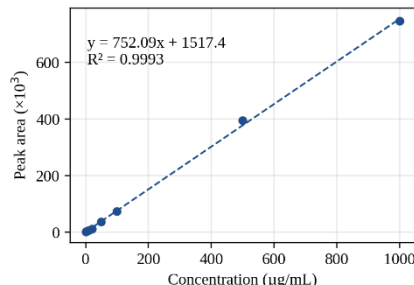


Figure 5. Calibration curve used for LOD and LOQ determination (1–1000 µg/mL).

Table 8. Sensitivity of the method.

Parameter	Concentration (µg/mL)	% of 1000 µg/mL
LOD	1.18	0.118
LOQ	3.58	0.358

3.6.6. Robustness

Deliberate changes in flow rate (0.8 and 1.2 mL/min), mobile phase ratio (97:3 and 93:7) and wavelength (228 and 232 nm) gave %RSD values between 0.05% and 0.62%, all below 2% (Table 9). The individual injection data are given in Table A3 and the chromatograms in Figure B6.

Table 9. Robustness of the method.

Variation	Mean RT (min)	%RSD (RT)	Mean area	%RSD (area)
Flow 1.2 mL/min	2.101	0.05	511800	0.11
Flow 0.8 mL/min	3.707	0.05	806115	0.03
Buffer : ACN 93:7	2.440	0.33	693660	0.60
Buffer : ACN 97:3	3.332	0.51	668278	0.23
Wavelength 228 / 232 nm	--	0.62	--	--

Wavelength: only the %RSD of 0.62% is reported.

3.7. Assay of the Formulation

The assay of the tablet formulation was calculated using Equation (3). The amount found was 9.9927 mg against a label claim of 10 mg, giving a mean assay of 99.93% (acceptance 90.00–110.00%). The replicate standard and sample injections are given in Table 10 and Appendix A (Table A2), and the chromatograms in Figure B7.

Table 10. Assay of vonoprazan fumarate tablets (n = 6).

Solution	Mean RT (min)	%RSD (RT)	Mean area	%RSD (area)
Standard	2.8187	0.36	728435.2	0.16
Sample	2.8608	0.09	733747.5	0.24

Table A1. Repeatability and intermediate precision of vonoprazan fumarate.

Day	Inj.	RT (min)	Peak area	Assay (%)
Day 1	1	2.867	689945	99.42
	2	2.867	689865	99.41
	3	2.868	690027	99.43
	4	2.873	691681	99.67
	5	2.880	691760	99.68
	6	2.887	690843	99.55
Day 2	1	2.871	690215	99.46
	2	2.869	690342	99.48
	3	2.874	690185	99.45
	4	2.876	690521	99.50
	5	2.878	690398	99.49
	6	2.888	690612	99.52
Day 3	1	2.873	690105	99.44
	2	2.875	690278	99.47
	3	2.875	690351	99.48
	4	2.879	690485	99.50
	5	2.882	690562	99.51
	6	2.889	690694	99.53
Mean		2.8762	690492.72	99.49
SD		0.00696	516.32	0.0744
%RSD		0.2418	0.0748	0.0748

Table A2. Replicate standard and sample injections for the assay.

Inj.	Std RT (min)	Std area	Sample RT (min)	Sample area
1	2.802	728271	2.857	733575
2	2.817	729114	2.859	736745
3	2.819	727455	2.861	731646
4	2.820	728903	2.861	734648
5	2.820	726812	2.863	733421
6	2.834	730056	2.864	732450
Mean	2.8187	728435.2	2.8608	733747.5
SD	0.01019	1177.21	0.00256	1790.65
%RSD	0.36	0.16	0.09	0.24

Table A3. Individual injection data of the robustness study.

Condition	Injection	RT (min)	Peak area
Flow rate 1.2 mL/min	1	2.100	511838
	2	2.101	511194
	3	2.102	512368
	Mean (SD)	2.101 (0.001)	511800 (587.37)
	%RSD	0.05	0.11
Flow rate 0.8 mL/min	1	3.705	805841
	2	3.708	806252
	3	3.708	806252
	Mean (SD)	3.707 (0.00173)	806115 (237.06)
	%RSD	0.05	0.03
Buffer : ACN 93:7	1	2.432	691681
	2	2.441	690843
	3	2.448	698455
	Mean (SD)	2.4403 (0.00802)	693659.7 (4164.48)
	%RSD	0.33	0.60
Buffer : ACN 97:3	1	3.316	669396
	2	3.331	668952
	3	3.350	666487
	Mean (SD)	3.3323 (0.017)	668278.3 (1545.13)
	%RSD	0.51	0.23

Table A4. Calibration data used for the determination of LOD and LOQ (1–1000 µg/mL).

Conc. (µg/mL)	Peak area
1	2414
2	3708
10	8078
20	12957
50	36686
100	73872
500	394628
1000	745556

Figure B1. Chromatograms of the six replicate standard injections for system suitability.

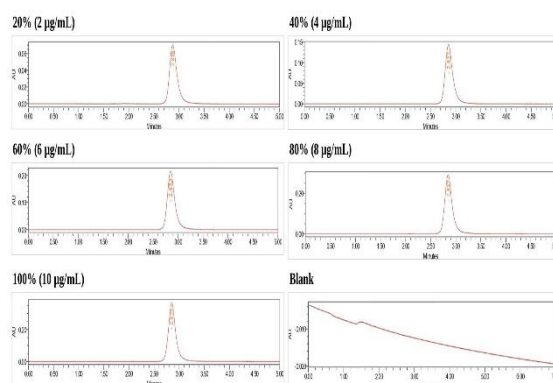


Figure B2. Chromatograms of vonoprazan fumarate at the linearity levels (20–100%) and blank.

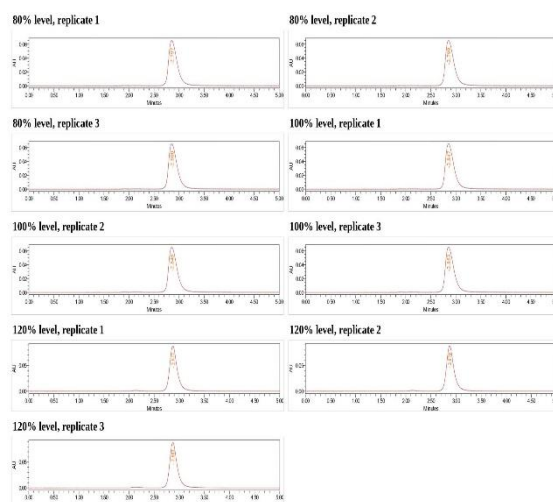


Figure B3. Chromatograms of vonoprazan fumarate at the accuracy levels (80, 100 and 120%).

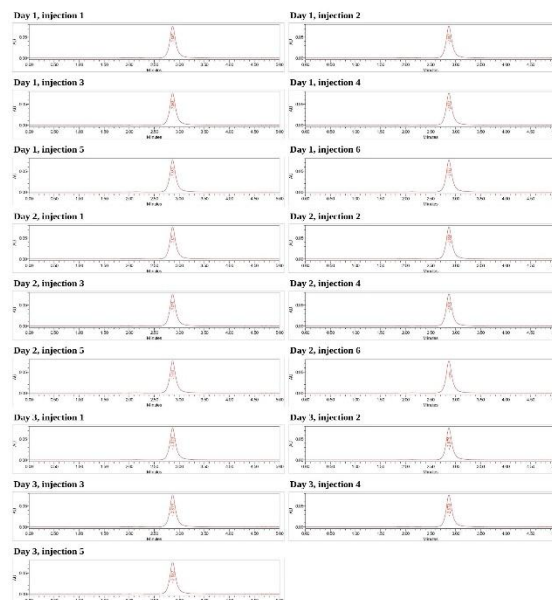
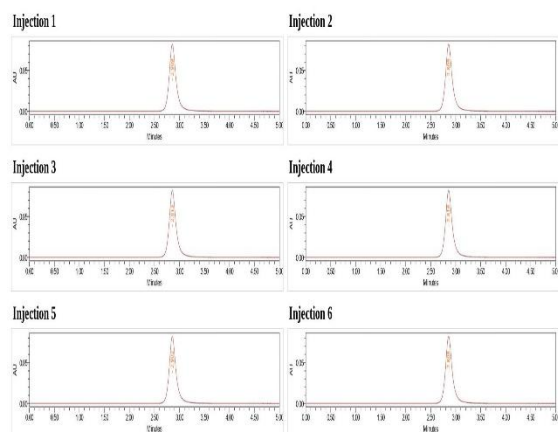


Figure B4. Chromatograms of the repeatability and intermediate precision study (Days 1–3).

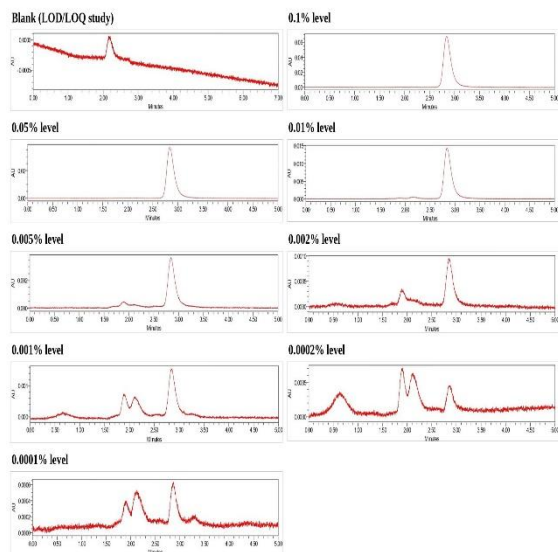


Figure B5. Chromatograms of the blank and of vonoprazan fumarate at the levels studied for LOD and LOQ (percentage of the 1000 µg/mL level).

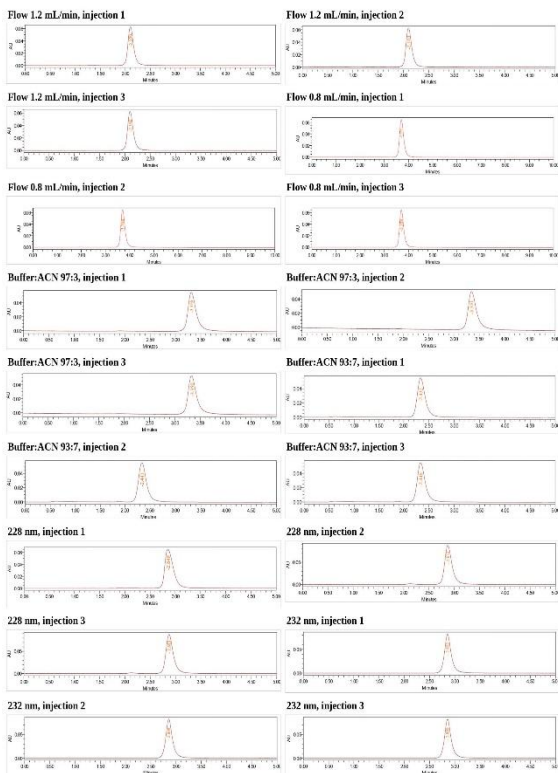


Figure B6. Chromatograms of the robustness study: flow rate (1.2 and 0.8 mL/min), mobile phase ratio (97:3 and 93:7) and wavelength (228 and 232 nm).

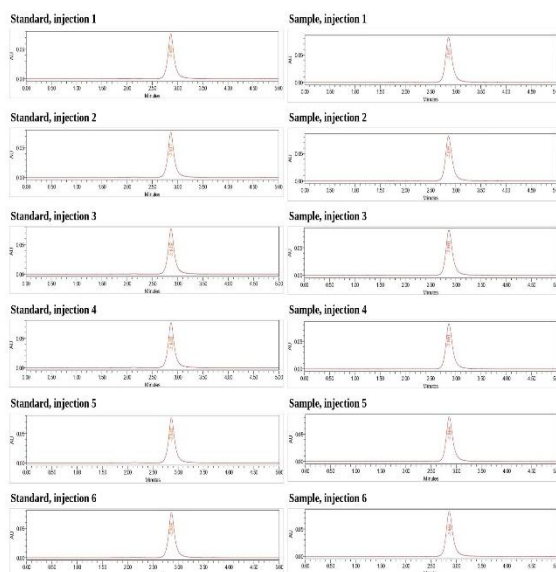


Figure B7. Chromatograms of the standard and sample injections used for the assay.

4. Discussion

Applying AQbD replaced trial-and-error development with a structured study of ACN content, pH and flow rate. The quadratic model fitted the retention time data very well ($R^2 = 0.9999$, adequate precision 302.09, non-significant lack of fit), and the residual plots did not indicate any violation of model assumptions. ACN content had the strongest influence on retention time, followed by flow rate and pH, which is consistent with the behaviour expected in reverse-phase chromatography, where a stronger mobile phase and a faster flow shorten retention. The optimized method uses a small proportion of organic solvent (5% ACN) and a 2.1 mm i.d. column, and vonoprazan fumarate elutes in about 2.83 min. This short run time supports rapid routine analysis. Compared with the reported UV method (LOD 1.557 µg/mL, LOQ 4.719 µg/mL) [1], the present method gave an LOD of 1.18 µg/mL and an LOQ of 3.58 µg/mL. Table 11 summarizes the validation results. All results were within the acceptance criteria, and the method was successfully applied to the tablet formulation.

Table 11. Overall results of the developed method.

Parameter	Result
Optimized RT	≈ 2.83 min
Linearity range	2–10 µg/mL
R ²	0.9997
Accuracy	99.97%
System precision	0.28% RSD
Method precision	0.24% RSD
LOD / LOQ	1.18 / 3.58 µg/mL
Robustness (%RSD)	0.05–0.62
Assay	99.93%

The present study did not include forced degradation studies, so the stability-indicating capability of the method has not yet been established. Future work may include forced degradation under acidic, alkaline, oxidative, thermal and photolytic conditions, LC-MS/MS characterization of degradation products, long-term and accelerated stability studies, application to other marketed formulations and batches, and a formal greenness assessment using the Analytical Eco-Scale, NEMI and AGREE tools.

5. Conclusions

A QbD-based RP-HPLC method was successfully developed for vonoprazan fumarate. DoE and RSM provided a systematic approach to chromatographic optimization, and ACN content showed the strongest influence on retention time. The optimized method gave a rapid retention time of about 2.83 min and demonstrated specificity, linearity, accuracy, precision, sensitivity and robustness. It was successfully applied to a pharmaceutical formulation and is suitable for routine quality-control analysis and assay determination.

Acknowledgments: The authors sincerely acknowledge the support and facilities provided by the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, for carrying out this research work. The authors are grateful to the laboratory and technical staff for their assistance with the analytical experiments, HPLC analysis, method development, optimization, validation studies, and instrumental analysis. The authors also acknowledge the support provided for the use of laboratory facilities and analytical instruments required for the successful completion of this research work.

Author Contributions: Sergin P Conceptualization, methodology, analytical method development, AQbD study design, optimization, investigation, validation, data collection and analysis, interpretation of results, and writing—original draft. Methodology, experimental investigation, HPLC analysis, system suitability studies, data collection, and manuscript editing. Sivasubramanian P: Supervision, project administration, validation, critical review. Kavisri R, Murugesan S, S Poovizhi M: Data analysis, literature review, manuscript editing, and critical review. All authors contributed to the interpretation of the results, critically reviewed the manuscript, and approved the final version.

Conflicts of Interest: The authors declare that they have no conflict of interest related to this research work, its findings, or its publication.

Appendix A

Table A1 gives the repeatability and intermediate precision data (three days, six injections per day), Table A2 gives the replicate injections used for the assay, Table A3 gives the individual injection data of the robustness study and Table A4 gives the calibration data used for the determination of LOD and LOQ.

Appendix B

Chromatograms recorded during method development and validation are given in Figures B1–B7. Each chromatogram is shown with retention time on the x-axis (minutes) and detector response (AU) on the y-axis.

References

- [1]. Echizen H. The first-in-class potassium-competitive acid blocker, vonoprazan fumarate: pharmacokinetic and pharmacodynamic considerations. Clin Pharmacokinet. 2016;55(4):409-18.
- [2]. Otake K, Sakurai Y, Nishida H, Fukui H, Tagawa Y, Yamasaki T, et al. Characteristics of the novel potassium-competitive acid blocker vonoprazan fumarate (TAK-438). Adv Ther. 2016;33(7):1140-57.
- [3]. Sugano K. Vonoprazan fumarate, a novel potassium-competitive acid blocker, in the management of gastroesophageal reflux disease: safety and clinical evidence to date. Therap Adv Gastroenterol. 2018;11:1-14.

- [4]. Clinical pharmacokinetics of potassium competitive acid blockers: a systematic review and meta-analysis. [journal, vol/issue as available]; 2025.
- [5]. Luo Z, Liu A, Liu Y, Wang G, Chen X, Wang H, et al. Development of a stability-indicating HPLC method for simultaneous determination of ten related substances in vonoprazan fumarate drug substance. *J Pharm Biomed Anal.* 2018;149:133-42.
- [6]. Ajmeera M, Goli S, Pallavi A. Stability-indicating RP-HPLC-DAD method for aspirin and vonoprazan using analytical quality by design and comprehensive greenness assessment. *SSRN [preprint]*; 2025.
- [7]. Development and validation of an eco-friendly RP-HPLC method for vonoprazan: a QbD approach. *Chromatographia.* 2025.
- [8]. Moneim MMA, Hamdy MM. Chromatographic assay of recently approved co-formulation of vonoprazan fumarate with low dose aspirin: AGREE, complex MoGAPI, and RGB 12-model assessments. *BMC Chem.* 2024;18(1):230.
- [9]. Salem YA, Elsabour SA, El-Masry AA. Validated chromatographic approach for determination of two ternary mixtures in newly approved formulations for *Helicobacter pylori* eradication: assessment of greenness profile and content uniformity. *BMC Chem.* 2024;18(1):111.
- [10]. Salva C, Galla R. The development and validation of a stability-indicating RP-UPLC method for the simultaneous estimation of clarithromycin, amoxicillin, and vonoprazan in a physical mixture. [journal as available]; 2024.
- [11]. Anusha K, Sowjanya G. A reliable RP-UPLC-TUV method for simultaneous estimation of clarithromycin, amoxicillin, and vonoprazan in co-packed pharmaceutical dosage forms: method development and validation with stability indicating properties. *Int J Appl Pharm.* 2024;16(2):351-7.
- [12]. Method development and validation for estimation of vonoprazan by RP-HPLC method in bulk and tablets dosage form. *Egypt J Chem.* 2023.
- [13]. Saleh AM. UV spectrophotometric method development and validation of vonoprazan fumarate in bulk and pharmaceutical dosage form; green profile evaluation via eco-scale and GAPI tools. *Egypt J Chem.* 2022.
- [14]. Govindan I, Kailas AA, Abutwaibe KA, Annadurai T, Naha A. Analytical quality by design driven stability-indicating HPLC method for estimation of vonoprazan fumarate. *Int J Appl Pharm.* 2025-26.
- [15]. Ettre LS. M.S. Tswett and the invention of chromatography. *LCGC North Am.* 2003;21(5):458-67.
- [16]. Martin AJP, Synge RLM. A new form of chromatogram employing two liquid phases. *Biochem J.* 1941;35(12):1358-68.
- [17]. van Deemter JJ, Zuiderweg FJ, Klinkenberg A. Longitudinal diffusion and resistance to mass transfer as causes of non-ideality in chromatography. *Chem Eng Sci.* 1956;5(6):271-89.
- [18]. Recent applications of analytical quality-by-design methodology for chromatographic analysis: a review. *J Pharm Biomed Anal.* 2024.
- [19]. Nano-scale analytical insights for determination of vonoprazan and aspirin in a recently approved combined preparation utilizing nucleophilic substitution reaction, along with evaluation approaches for both greenness and whiteness. *Microchem J.* 2023.
- [20]. Chemometric-assisted green spectrophotometric strategies for the simultaneous determination of vonoprazan and aspirin in co-formulated tablets: RGB12 and EPPI-based sustainability assessment. *Anal Chem Lett.* 2026.