A QbD-Guided Development and Validation of RP-HPLC Method for Quantitative Estimation of Vonoprazan Fumarate

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ABSTRACT

Using a Quality by Design (QbD) approach, a unique, straightforward, sensitive, accurate, and reliable Reverse Phase–High Performance Liquid Chromatography (RP-HPLC) method was created and validated for the measurement of Vonoprazan in bulk medication and tablet dosage forms. Central composite design (CCD) was used in the chromatographic condition optimization process to methodically assess the impact of crucial technique parameters. Using an Agilent Zorbax C18 column (250 mm × 4.6 mm, 5 µm), the chromatographic separation was carried out on an HPLC system with Chromeleon 7.3 software. The wavelength used for detection was 225 nm. The ideal chromatographic conditions included a mobile phase that was supplied at a flow rate of 1.0 mL/min and contained phosphate buffer and acetonitrile in a 60:40 (v/v) ratio. This resulted in a retention period of roughly 5.2 minutes. The developed method was validated using standard metrics criteria, such as linearity, accuracy, precision, specificity, and robustness. The method showed satisfactory linearity with a correlation coefficient (r^2) of 0.9939. Vonoprazan mean recovery of 99.91% confirmed the approach's accuracy, while precision testing showed %RSD values below 2.0%, indicating strong repeatability. The overall findings showed that the suggested approach has good predictability and robustness, which makes it appropriate for routine quantitative investigation of Vonoprazan in pharmaceutical formulations and bulk.

Keywords: RP-HPLC, QbD, Vonoprazan, Method Development, Validation.

1. INTRODUCTION

Vonoprazan, a recent potassium-competitive acid blocker (P-CAB), is a potent substitute for the traditional proton pump inhibitors (PPIs) used to prevent diseases linked to gastric acid secretion. It can exhibit indifferent response to changes in efficacy with variations in metabolism because it directly and reversibly inhibits the H +/K + -ATPase enzyme, which occupies the potassium binding site of gastric parietal cells.

Vonoprazan clinical therapeutic response is since shown through the pharmacological characteristics of Vonoprazan in the treatment of the gastroesophageal reflux disease (GERD), in the treatment of peptic ulcer disease, and the treatment of the Helicobacter pylori infection. [4,5]

A much useful method of optimization of important factors is the central composite design (CCD). CCD entails the optimal and desirable worth of variables that results in the optimal and desirable response and characterizes the circumstances of the procedures that are inaccessible to the rational changes of the settings of factors. It also shows that there exists a mathematical model, between response and the critical variables therefore permits the prediction of response with the least error to be transferred to the response. [6-9]

During the review of the literature, it was established that determination has been carried out on drug using HPLC. [11-16]

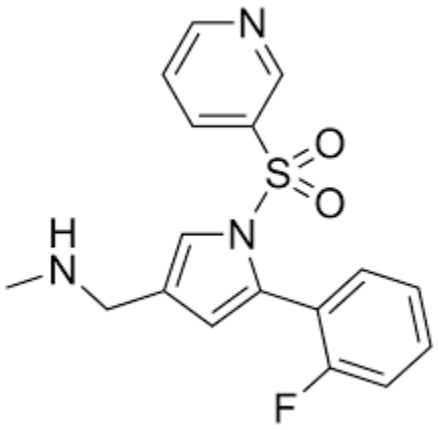

Fig. 1. Chemical structure of Vonoprazan

2. EXPERIMENTAL DESIGN METHODOLOGY

2.1 Instrumental Methodology:

The Agilent Zorbax C18 column (150 mm 4.6 mm 5 mm 5 mm) and the HPLC Thermo Ultimate 3000 with Chromeleon 7.3 software were used for method development and validation. Design Expert software version 11.0 was used to create the design CCD model. Wensar Analytical Balances were used to weigh the samples after they were prepared using (Labman Scientific Instruments).

2.2 Materials:

Arni Analytical Laboratory Nashik provided the samples of Vonoprazan. Thermo Ultimate 3000 provided HPLC-grade solvents such as Acetonitrile, Methanol and HPLC system.

2.3 Preparation of solutions:

Determine the average weight by weighing ten tablets. The tablets should be ground into a fine powder. Weigh the powder in a 100 ml volumetric flask to ensure that the weight is as exact as feasible and equivalent to the average weight (tablet weight). Fill the capacity with 100 ml of water and 0.2 ml of orthophosphoric acid, then sonicate for 10 minutes. Use Whatman filter paper number 41 to filter the aforementioned solution. To make 50 ml, add additional water to the 5 ml of filtrate above.

2.4 Preparation of Mobile Phase:

Dilute buffer and Acetonitrile (60:40) v/v.

2.5 Preparation of Buffer:

Make up 0.01M Potassium dihydrogen orthophosphate in water.

2.6 Preparation Standard Solution:

Accurately weigh 20mg of Vonoprazan in 100 ml volumetric flask. Measure 60 ml of water and 0.2 ml of orthophosphoric acid and sonicate it taking 5 minutes. Make up the volume. Continue to add 5 ml water to above solution to make 50 ml solution.

2.7 Chromatographic Conditions:

A mixture of buffer: 60% acetonitrile-containing (v/v; 40%) on Agilent Zorbax C18 column (250 mm x 4.6 mm, 5 mm id) packed with 5 μm particles to separate substances by using 1.0mL/min flow rate through the column, at 218 nm detection wavelength, injected 20 μL into the injection port, and 3:30 minutes run time.

Table 1: A summary of parameters used to optimise the method.

Trials	Column	Mobile phase ratio (v/v)	Flow rate (ml/min)	Retention time (min)	Observation	Inference
1	Agilent Zorbax C_{18} (250 mm × 4.6 mm; 5 µm)	Water: ACN (60:40) v/v	1.0	-	No peak detected	Need to add a buffer
2	Agilent Zorbax C_{18} (250 mm × 4.6 mm; 5 µm)	Buffer: ACN (60:40) v/v	1.0	6.0	Peak detected, but retention time increases	Need to increase buffer and solvent concentration.
3	Agilent Zorbax C_{18} (250 mm × 4.6 mm; 5 µm)	Buffer: ACN (70:30) v/v	1.0	2.6	Peak observed but need to improve and peak shape	Need to increase buffer and solvent concentration.
4	Agilent Zorbax C_{18} (250 mm × 4.6 mm; 5 µm)	Weigh 0.081 g KH_2PO_4, transfer to a beaker, add 60 mL distilled water, sonicate to dissolve, then add 40 mL acetonitrile and sonicate again (Buffer: ACN)(40:60)	1.0	Fumarate 2:45min and Vonoprazan 3:45	Optimized peak observed clearly	-

3. Optimization Design:

The most commonly used design was 'central composite design (CCD)' since it is very efficient and the number of runs can be stored. The CCD with k 'factors must contain 2k factorial runs, 2K axial experiments divided symmetrically at half the value - on each axis of variable and one center point. The 3 crucial factors were designed in order to achieve optimum desiring responses via a rotatable CCD (-1, 0, +1, +) of every variable factor (, -1, 0, +1, +) and 13 random runs consisting of combination of 5 center points. The main task of HPLC analytical process is that the analysis will be made timely and the peaks will be separated adequately. In cases where the chromatographic factors or independent factors influence such responses of the HPLC system is known, then central composite design is applied to optimise the factors where two factorial design is coined to establish rotatability or orthogonality and as a result, an estimation of the second order equation can be derived as expounded by Box and Wilson. It can therefore be applied to obtain the coefficient of a quadratic regression equation that would result in the forecast of nonlinear impacts of variables. The construction of CCD model was made on the basis of Design Expert Copyrighted by Stat-Ease, Inc, Minneapolis, MN, USA. [6-10]

The thirteen trial tests were informed about the CCD matrix and the number of independent variables, and the response value as in Table 2.

Table 2: CCD showing 13 randomized trial runs.

Run	%Acetonitrile	Buffer	Flow rate	Rt Vonoprazan (min)
1	75	25	1	7.273
2	70	30	1.2	6.081
3	60	40	0.7	5.644
4	60	40	1	5.229
5	60	40	1	5.233
6	50	50	1.2	4.844
7	46	54	1	5.106
8	60	40	1	5.218
9	50	50	0.8	5.376
10	70	30	0.8	6.661
11	60	40	1	5.274
12	60	40	1	5.241
13	60	40	1.2	5.106

Table 3: Critical factors and levels used in CCD.

Name	Low (-1)	Medium (0)	High (+1)
Independent factor			
A: Acetonitrile %	30	40	75
C: Flow rate	0.8	1	1.2
Dependent factor			
R1	Retention time of Vonoprazan		

Table 4:Experimental results and optimized conditions

Parameters	Description
Program	Isocratic
Mobile phase	Phosphate buffer: Acetonotrile (60:40 v/v)
Wavelength	225 nm
Inoculation volume	20 µL
Flow rate	1 mL/min

4. Method validation

The International Council for Harmonization's guidelines, notably ICH Q2(R2), which address system appropriateness, specificity, linearity, detection limit (DL), quantitation limit (QL), accuracy, precision, and robustness, were followed in the development and validation of the analytical method. In order to ensure that there was no interference from excipients, specificity was assessed by comparing the HPLC chromatograms of pure active ingredients with those of prepared samples, including commercial formulations, blanks, and placebos. The relative standard deviation (%RSD) of peak areas and retention periods was used to express precision, which shows how repeatable the process is under the same circumstances. In order to verify consistency of results, intermediate precision was evaluated by doing the analysis under comparable circumstances (same laboratory, analyst, equipment, and brief time interval).

Recovery experiments at three concentration levels (80%, 100%, and 120%) were used to assess accuracy, showing how closely measured values matched the actual value. Plotting concentration against peak area to create calibration curves allowed for the establishment of linearity over the 80–120% range. Vonoprazan stability in standard and sample solutions kept in firmly sealed volumetric flasks at room temperature for a full day was also assessed, confirming that there had been no appreciable degradation during the study period.

5. Results and discussions

Lastly, two critical factors percentage of acetonitrile and flow rate—likely to influence the selected responses were optimized. Spectral analysis of the drug in the range of 200–400 nm revealed a maximum absorbance (λmax) at 225 nm, and therefore chromatographic detection was set at 225 nm. The method was performed using an Agilent Zorbax C18 column with a flow rate of 1.0 mL/min and a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 60:40 (v/v). While keeping other parameters constant, both variables were optimized within the design space (sweet spot). The two variables—acetonitrile concentration and flow rate—were investigated at five levels using a Central Composite Design (CCD), yielding thirteen experimental runs. A quadratic regression model created with Design-Expert software was used to characterize the connection between factors and responses. Analysis of variance (ANOVA) was used to further confirm the model's significance and appropriateness. The resultant F-value and P-value ($p < 0.0001$) showed a statistically significant effect of the independent variables on the answer, meeting the acceptance condition ($p \leq 0.05$).

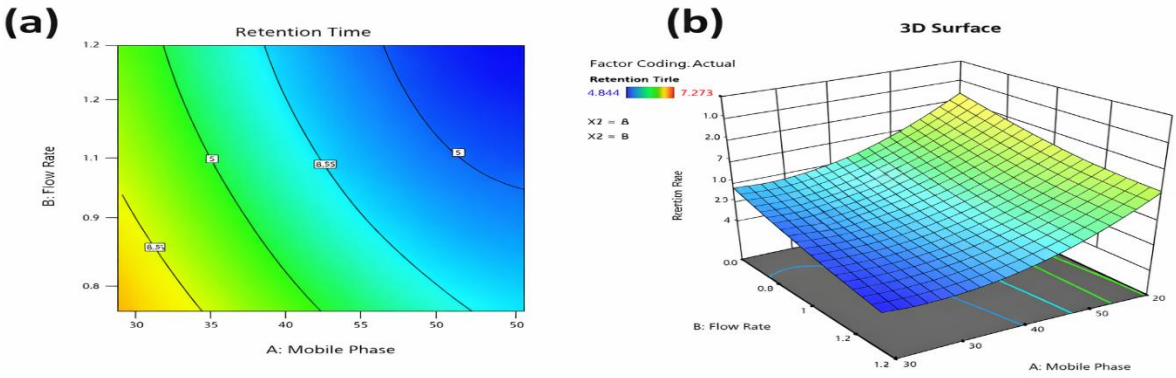

Fig. 2. 2D and 3D plots display correlation between all the dependent variable against both the independent variables.

Fig. 2, the 2D and 3D plots in the 2D and 3D plot show relationship between each of the dependent variable and each of the two independent variables. The plots were evident that retention time would decrease with an increase of flow rate and mobile phase acetonitrile concentration.

Table 5. Statistical parameters obtained from ANOVA.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.84	5	1.17	142.25	< 0.0001	significant
A-Mobile Phase	3.90	1	3.90	474.87	< 0.0001	
B-Flow Rate	0.4384	1	0.4384	53.37	0.0002	
AB	0.0006	1	0.0006	0.0701	0.7988	
A^2	1.50	1	1.50	182.94	< 0.0001	
B^2	0.0231	1	0.0231	2.81	0.1378	
Residual	0.0575	7	0.0082			
Lack of Fit	0.0557	3	0.0186	41.12	0.0018	significant
Pure Error	0.0018	4	0.0005			
Cor Total	5.90	12				

Table No.5 The Model F-value of 142.25 means that the model is significant. The probability of getting this large an F-value as a result of noise is only 0.01%. P-values below 0.0500 show that model terms are not negligible.

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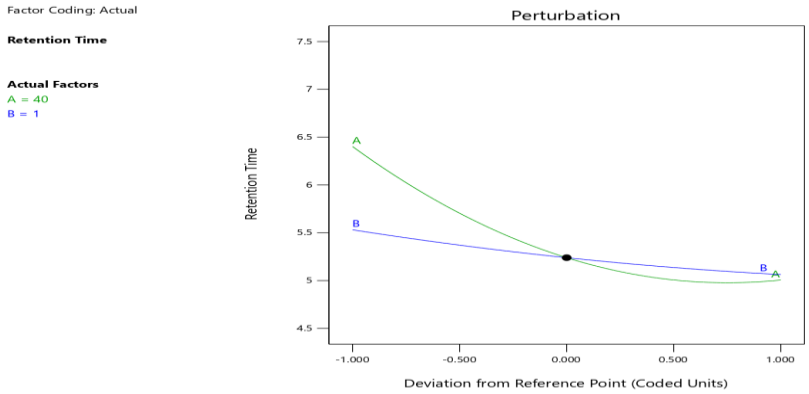

Fig 3.Perturbation plot show effect of factor on responses.

In **Fig 3** show for % ACN and flow rate had most significant negative effect on retention time of Vonoprazan as decreases retention time with increase in flow rate and mobile phase acetonitrile concentration.

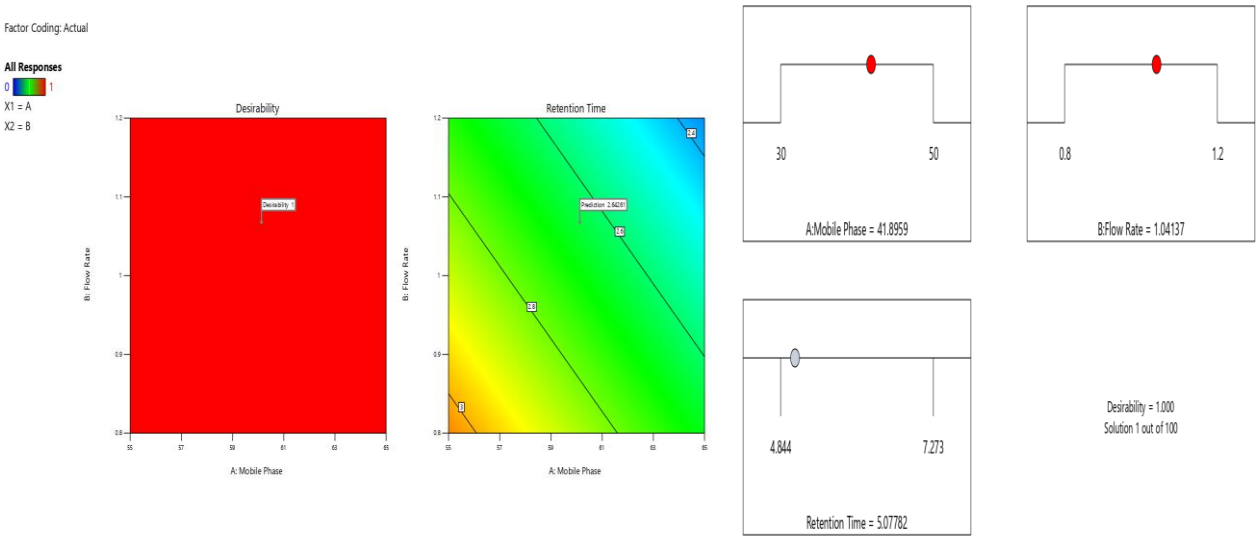

Fig 4. Graphical illustration of the accepted constraints to be used in determination of global desirability and the acquired optimal condition. desirability and obtained optimal condition.

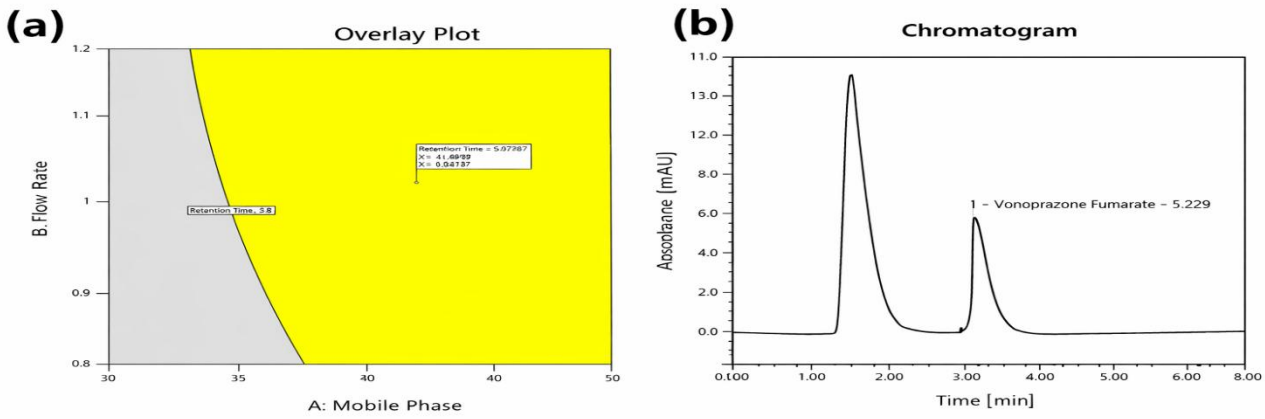

Fig 5. (a) Overlay plot showing the sweet spot where the desired response meet (b) Optimized chromatogram of Vonoprazan.

6. Validation Parameter

a. System suitability

A system suitability test showed that the percent relative standard deviation (%RSD) of peak areas was less than 2.0 and that the theoretical plates were greater than 2000. The tailing factor was less than 2.0, and the resolution factor was greater than 2.0. This data proved that the systems were operating satisfactorily for quantitative analysis. It was shown that the chromatographic system had adequate system suitability, as shown in Table 6.

Table 6: System Suitability Result.

Sr. No.	AreaArea (mAU·s)	Retention Time (min)	Tailing Factor (Tf)	Theoretical Plate (Tp)
1	7.122	5.229	1.41	5541
2	7.135	5.229	1.35	5478
3	7.131	5.524	1.35	5578
4	7.134	5.233	1.45	6521
5	7.163	5.218	1.41	6521
6	7.158	5.274	1.32	5412
Average	**7.141**	**5.285**	1.382	5841.83
STDEV	**0.016**	**0.119**	**0.049**	**529.20**

RSD	0.23	2.25	3.55	9.06
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NLT 2000

a. Specificity

In the observed retention time of the analytes in the chromatograms, no additional or co-eluting peaks were observed. Therefore, the mountains were regarded as pure and this is evidence of the particularism of the approach. No interference in Standard and Sample was observed as a result of Blank.

Table 7. Specificity results for Vonoprazan

Sr. No.	Vonoprazan	Retention Time (min)
1	Blank	0.000
2	Standard Solution	5.376
3	Sample Solution	5.356

b. Precision

We assessed the method's precision through intraday and interday studies. This involved analyzing six replicates of three quality control samples on a single day for intraday precision, and repeating the process over three consecutive days for interday precision. Both showed a percent coefficient of variation of ≤7.1% (Table 8, Table 9).

Table 8. Intraday precision result for Vonoprazan.

Standard Solution	Area (mAU·s)		
Conc. / No. Of Inj.	5 ppm	10 ppm	15 ppm
1	1.780	3.563	5.345
2	1.783	3.566	5.348
3	1.781	3.564	5.346
4	1.784	3.567	5.349
5	1.782	3.565	5.347
6	1.781	3.565	5.346
Average	**1.782**	**3.565**	**5.347**
STDEV	**0.001**	**0.001**	**0.001**
RSD (%)	**0.06**	**0.04**	**0.02**

Table 9. Interlay precision result for Vonoprazan.

Standard Solution	Area (mAU·s)		
Conc. / No. Of Inj.	5 ppm	10 ppm	15 ppm
1	1.781	3.564	5.346
2	1.783	3.566	5.348
3	1.782	3.565	5.347
4	1.780	3.563	5.345

5	1.784	3.567	5.349
6	1.782	3.565	5.347
Average	**1.782**	**3.565**	**5.347**
STDEV	**0.0014**	**0.0015**	**0.0014**
RSD (%)	**0.08**	**0.04**	**0.03**

c. Accuracy

Accuracy of the developed method was evaluated by recovery studies at different concentration levels. The percentage recovery of Vonoprazan for each injection at all concentration levels was found to be within acceptable limits. The obtained recovery values were 99.96%, 100.18%, 99.60%, and 99.91%, indicating that the method is accurate and reliable. All values complied with the predefined acceptance criteria (98–102%), confirming the suitability of the method for the determination of Vonoprazan assay. Add table no 10

Table 10: Accuracy result for Vonoprazan.

Sr. No.	Level	mg of drug spiked	Area (mAU·s)	mg of drug Recovered	% Recovery
1	80%	0.016000	5.804	0.015994	99.96
2	100%	0.020000	7.271	0.020037	100.18
3	120%	0.024000	8.674	0.023903	99.60
Average					99.91
Std.dev					0.30
%RSD					0.30

d. Linearity

It made 5 injections and the calibration curves were found to be linear between 80 and 120%. The obtained results are within the range and the coefficient of correlation of the standard curve is 0.9939 thus, the method is linear within a certain range. Fig 7 and summarized in Table 11.

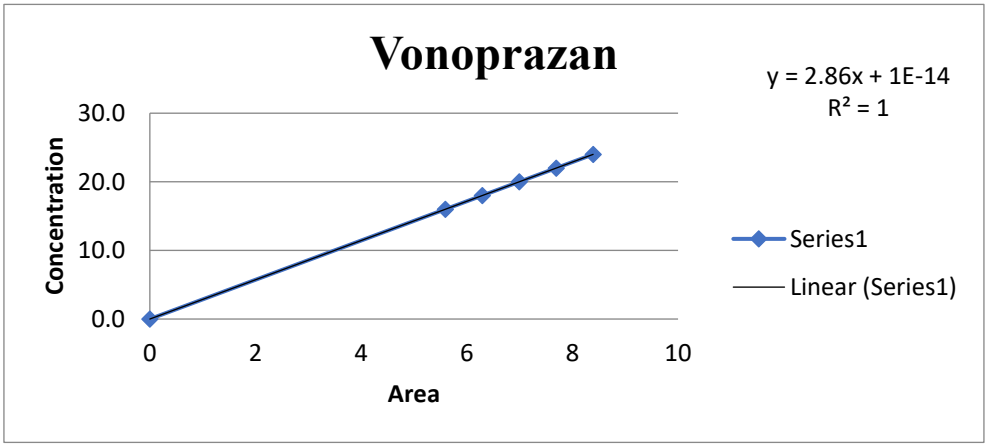

Fig 7: Calibration curve for Vonoprazan.

Table 11: Linearity result for Vonoprazan.

Sr. No.	Concentration in ppm	Concentration of Solution	Diluted to	Area (mAU·s)
1	16.0	80	50	5.594
2	18.0	90	50	6.294
3	20.0	100	50	6.993
4	22.0	110	50	7.692
5	24.0	120	50	8.392

e. Detection Limit, Quantification Limit, and Solution Stability

The detection limit was 0.00808µg/mL, and the quantification limit was 0.02448 µg/mL.

% RSD was found to be less than 2, hence the Vonoprazan 20 mg sample solutions are stable up to 24 hours at room temperature.

Table 12: Solution stability results for Vonoprazan.

Sr. No.	Hours	Area (mAU·s)	% Assay	%Assay Difference
1	Initial	6.569	100.31	-
2	8 Hour	6.564	99.93	0.38
3	24 Hour	6.522	99.29	1.02

f. Robustness:

By purposefully altering important chromatographic parameters, such as flow rate (±10%) and mobile phase composition (±10% organic solvent), the robustness of the new analytical approach was confirmed. Under these various circumstances, standard and sample solutions were produced in accordance with the optimal process and analysed. The findings showed that even small, deliberate adjustments had no discernible impact on peak area or retention duration. The method's reproducibility and dependability were demonstrated by the %RSD values achieved under all situations, which were within acceptable bounds. Because minor changes in

analytical conditions did not negatively impact the method's performance, it was determined to be resilient (see Table 13).

Table 13 Robustness Value for Vonoprazan.

Parameters	Rt (min)	% Estimation	Mean	SD	%RSD
Flow (±0.9 mL/min)	7.328 7.321	99.98	7.313	0.0050	0.07
Flow (±1.1 mL/min)	7.038 6.998	99.85	7.018	0.0283	0.40
Mobile Phase 1	7.294 7.302	99.60	7.298	0.0057	0.08
Mobile Phase 2	6.846 6.854	100.12	6.850	0.0057	0.08

Table 14: Validation results of Vonoprazan.

Parameters	**Vonoprazan Fumarate**
System Suitability	Within Acceptable limit.
Specificity	There was no interference observed in Standard & Sample due to Blank
Linearity	
Range (µg/mL)	80-120 %
Y-Intercept	0.000
Slope	2.8600
Correlation coefficient	0.9939
Accuracy	
% Recovery	99.91
Precision	
System Precision	RSD - 0.22 %
Method Precision	% Assay -100.31
Intermediate precision	% Assay – 100.37
LOD (µg/mL)	0.00808

LOQ (µg/mL)	0.02448
Stability of solution	RSD < 2%
Robustness	Within Acceptable limit.

7. Conclusion

It entailed application of QbD strategy in order to develop an effective and potent RP-HPLC method to ascertain Vonoprazan. The multivariate regression analysis was applicable and could investigate the major impacts of two factors on the retention of Vonoprazan. The chromatographic conditions were optimized using CCD that looks into the interactions and quadratic effects of the significant variables on the response of interest that is selected. The validation was done in relation to ICH Q2R2 criterion. This is not a complicated process, powerful and can be effectively administered on the Vonoprazan which is correct and precise.

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Informed consent: None

Data availability: None

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