IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, May 2026
Impact Factor: 8.2

Development and Validation of an Eco-friendly RP-HPLC Method for Vonoprazan: A QbD Approach

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Abstract: *This study presents the development of a robust, eco-friendly reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of vonoprazan, guided by the principles of Quality by Design (QbD). A systematic approach using Design of Experiments (DoE) was employed to identify critical method parameters and optimize chromatographic conditions. The method was validated in accordance with ICH Q2(R1) guidelines, covering specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).*
To assess environmental sustainability, the method was evaluated using green analytical metrics such as Analytical Eco-Scale, AGREE, and GAPI, confirming its eco-friendly nature. The validated method demonstrated high sensitivity, reproducibility, and compliance with regulatory standards, making it suitable for routine analysis of vonoprazan in pharmaceutical formulations..

Keywords: Vonoprazan, Quality by design,Validation, ICH, GAPI, AGREE

I. INTRODUCTION

Vonoprazan is a novel potassium-competitive acid blocker (P-CAB) widely used in the treatment of acid-related gastrointestinal disorders, including gastroesophageal reflux disease and peptic ulcers. Its growing clinical importance has created a demand for reliable, sensitive, and environmentally sustainable analytical methods to ensure quality control in pharmaceutical formulations.

Reverse-phase high-performance liquid chromatography (RP-HPLC) remains one of the most versatile and widely applied techniques for drug analysis due to its accuracy, reproducibility, and adaptability. However, traditional chromatographic methods often involve extensive use of organic solvents, raising environmental and safety concerns. To address these challenges, the integration of **Quality by Design (QbD)** principles into method development provides a systematic framework for identifying critical parameters, optimizing conditions, and ensuring robustness.In recent years, the concept of **green analytical chemistry** has gained prominence, encouraging the adoption of eco-friendly practices in pharmaceutical analysis. Tools such as the **Analytical Eco-Scale, AGREE, and GAPI** allow researchers to evaluate the environmental impact of analytical procedures. By combining QbD with green chemistry metrics, RP-HPLC methods can be developed. that are not only scientifically rigorous but also sustainable

This review highlights the development and validation of an eco-friendly RP-HPLC method for vonoprazan, emphasizing QbD-driven optimization, adherence to ICH guidelines, and the application of green analytical metrics to achieve a balance between analytical performance and environmental responsibility.

Drug Profile- Vonoprazan

Vonoprazan is a novel potassium-competitive acid blocker (P-CAB) developed as an alternative to proton pump inhibitors (PPIs) for the treatment of acid-related gastrointestinal disorders. It was first approved in Japan in 2014 under

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the brand name Takecab and later received FDA approval in 2022 as Voquezna. Unlike PPIs, vonoprazan does not require activation in an acidic environment, which gives it a faster onset of action and more consistent acid suppression.

Molecular weight	345.39 g/mol
Appearance	White, Crystalline powder
Solubility	soluble in water, freely soluble in Methanol,DMSO
Category	potassium-competitive acid blocker medication
IUPAC	(E)-but-2-enedioic acid;1-[5-(2-fluorophenyl)-1-pyridin-3 ylsulfonylpyrrol-3-yl]-N-methylmethanamine

Mechanism of Action

Vonoprazan works by **competitively inhibiting the gastric H^+/K^+-ATPase enzyme**, also known as the proton pump, at the potassium-binding site. This prevents the final step of gastric acid secretion. Its binding is reversible but highly stable, resulting in prolonged acid suppression compared to PPIs. Because it does not depend on acid activation, vonoprazan is effective even in patients who metabolize PPIs rapidly or show poor response to conventional therapy.

Pharmacokinetics

The drug is rapidly absorbed after oral administration, with peak plasma concentrations typically reached within 1.5 to 2 hours. It has a relatively long half-life, allowing once-daily dosing. Vonoprazan is metabolized primarily in the liver, involving cytochrome P450 enzymes, and excreted through urine and feces. Its pharmacokinetic profile contributes to sustained acid suppression over 24 hours.

Clinical Indications

Vonoprazan is indicated for the treatment and maintenance of erosive esophagitis, symptomatic relief of gastroesophageal reflux disease (GERD), and as part of combination therapy for Helicobacter pylori eradication. In H. pylori treatment, it is used alongside antibiotics such as amoxicillin and clarithromycin. Its superior acid suppression enhances antibiotic stability and effectiveness, improving eradication rates compared to PPI-based regimens.

Dosage and Administration

Typical adult doses include **20 mg once daily for healing erosive esophagitis** (up to 8 weeks), followed by **10 mg once daily for maintenance therapy**. For non-erosive GERD, 10 mg daily for 4 weeks is recommended. In H. pylori eradication regimens, vonoprazan is combined with antibiotics, with dosing adjusted according to the specific protocol.

Safety and Precautions

Vonoprazan is generally well tolerated, but long-term use may carry risks similar to PPIs, including **bone fractures, vitamin B12 deficiency, hypomagnesemia, and increased susceptibility to gastrointestinal infections such as *Clostridioides difficile***. It is contraindicated in patients with hypersensitivity to the drug and should not be co-administered with rilpivirine-containing products due to drug interactions. Monitoring is advised for patients on prolonged therapy.

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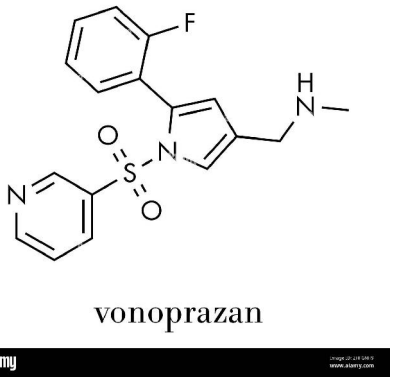
Structure of Vonoprazan :



vonoprazan

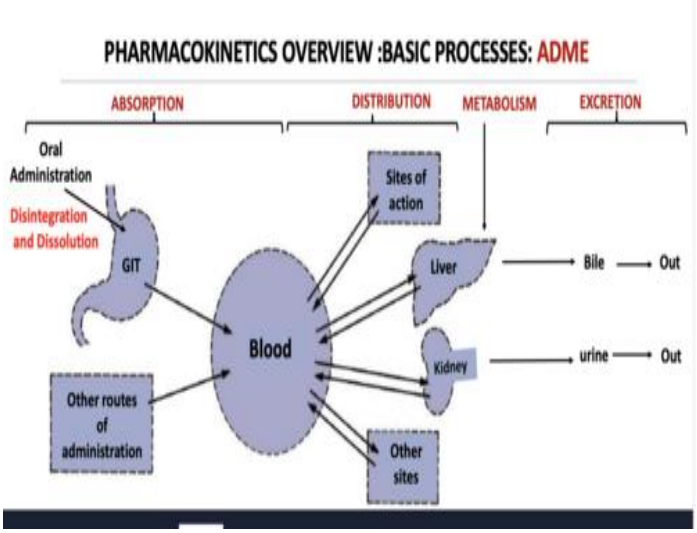
Chemical Formula: $C_{17}H_{16}FN_3O_2S$.

Pharmacodynamics :

Vonoprazan is a **potassium-competitive acid blocker** that exerts its pharmacodynamic effects by directly inhibiting gastric H^+,K^+-ATPase, the proton pump responsible for acid secretion in the stomach. Unlike traditional proton pump inhibitors (PPIs), vonoprazan does not require activation in an acidic environment. Instead, it competes with potassium ions at the binding site of the enzyme, producing rapid and reversible inhibition. This mechanism allows vonoprazan to achieve strong acid suppression within hours of administration, a significant advantage over PPIs that often require several days for full effect.

The drug demonstrates **high potency**, with an inhibition constant (Ki) in the nanomolar range, and accumulates in gastric parietal cells, ensuring sustained activity. A single dose can maintain intragastric pH above 4 for nearly 24 hours, providing consistent acid control throughout the day and night. This prolonged suppression is particularly beneficial in conditions like gastroesophageal reflux disease (GERD), erosive esophagitis, and in combination therapy for *Helicobacter pylori* eradication.

Pharmacodynamically, vonoprazan is not influenced by genetic polymorphisms of CYP2C19, which often reduce the effectiveness of PPIs in rapid metabolizers.



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Metabolism

Vonoprazan undergoes **hepatic metabolism primarily via cytochrome P450 enzymes**, with **CYP3A4** being the major pathway. Minor contributions come from **CYP2B6, CYP2C19, and CYP2D6**, but unlike proton pump inhibitors, vonoprazan's pharmacodynamics are not significantly affected by CYP2C19 genetic polymorphisms.

Its metabolites are mainly excreted in urine and faeces, with unchanged drug also detectable. This metabolic profile ensures more predictable acid suppression across different patient populations, making vonoprazan a reliable alternative to PPIs.

Absorption:

Vonoprazan is absorbed rapidly after oral administration, with peak plasma concentrations (Tmax) reached within about 1–2 hours, and it shows high bioavailability and sustained gastric acid suppression due to strong binding to H+/K+-ATPase. Its absorption is not significantly affected by food, and concentrations in the stomach remain far higher than plasma levels for over 24 hours, supporting once-daily dosing .

Route of Elimination:

Vonoprazan is eliminated mainly through **hepatic metabolism**, where **CYP3A4** is the dominant enzyme, with minor roles from CYP2B6, CYP2C19, CYP2C9, and CYP2D6, plus a non-CYP sulfotransferase pathway (SULT2A1). All metabolites formed are inactive. After metabolism, about **67% of the dose is excreted in urine** and around **31% in feces**, reflecting both renal and biliary clearance. Its elimination half-life of **7–9 hours** supports once-daily dosing and consistent acid suppression.

Half-Life:

Vonoprazan has a mean terminal half-life of about 7–9 hours in healthy adults, which allows for sustained plasma levels and effective once-daily dosing. This relatively long half-life, combined with strong gastric mucosa retention, ensures prolonged acid suppression compared to conventional PPIs.

Clearance:

Vonoprazan is primarily cleared through hepatic metabolism, mainly via CYP3A4, with contributions from other CYP enzymes and sulfotransferases, and is excreted in both urine (~67%) and feces (~31%). Its clearance is stable and less affected by genetic polymorphisms compared to traditional PPIs.

Validation of Method

Method validations are carried out as per the ICH guide-lines with the help of following parameters, respectively.LinearityThe capacity of the analytical process to produce test find-ings that are exactly proportionate to the analyte concen-tration in the sample, within a certain range, is termed as linearity. A minimum of five distinct concentrations were prepared, and three replicates of each concentration were then made to achieve linearity. The oncentration uses for the performance for linearity use in the range of 50–150%. Least squares linear regres-sion analysis was used to calculate the slope, intercept, and regression coefficient values. The obtained correlation coef-ficients showed good linearity and fell between 0.9992 and 0.9999. The linearity was estimated by plotting the drug concentration against peak area.System Suitability TestSystem suitability testing is necessary to confirm the high performance of the chromatographic system. The relative standard deviation (%RSD) was calculated for retention time, peak area, response factor (peak area/concentration), theoretical plates, tailing factor, and signal-to-noise ratio (S/N ratio) . The precision of the response factors, in particular, provides a normalized measure of detector performance and is a critical indicator of method readiness. The signal-to-noise ratio was determined by comparing the measured signal from the analyte peak to the amplitude of the background noise, measured from a blank injection over a region adjacent to the analyte retention time.

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Accuracy

The accuracy of the approach determines the degree of cor-relation between the actual value of a measurement and its result. A comparison of test results obtained with a known value is normally employed to establish accuracy, which should be attained over the reportable range of an analytical procedure. In accordance with ICH norms, a recovery study was conducted to confirm the accuracy.

Precision

There are three precision levels: repeatability, intermediate-ate precision, and reproducibility. In the method, pre-cision test was performed on six different sample preparation of Vonoprazan in 15 µg mLconcentration, and calculated the %RSD of all data.Intra-day precision: The intra-day variation of Vono-prazan is happens on the day 1. The relevant regression equations were used to determine the concentrations, mean recovery percentages, and relative standard deviations.Inter-day precision (Day 2): The inter-day precision was carried out by the different person on the different system for the analysis of the Vonoprazan.

Robustness

Depending on the type of method under investigation, the evaluation of robustness should be considered during the development phase. It should show an analysis's reliability in terms of deliberate changes in technique parameters. The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, modi-fications in method parameters and provides an indication of its reliability during normal usage . The stability of standard and sample solutions was rigor-ously evaluated to define a practical analytical window and ensure the reliability of the method throughout a typical workflow. This assessment is critical to prevent inaccura-cies that may arise from the decomposition of the analyte after preparation . To establish a comprehensive stability profile, solutions were analyzed over an extended period at ambient temperature .

Specificity :

The analytical technique should be specific for every stage of development. Once all the anticipated components such as degradants, excipients/sample matrix, and sample blank peaks are identified, the method should be capable of definitively assessing the functional analyte of interest . The specificity of the approach was demonstrated using peak purity analysis. The approach was shown to be specific since the total peak purity index for Vonoprazan and related pol-lutants was higher than the single point criteria that the peak purity was crossed. Each factor may be painted green to signify the greenness of the method or left blank to signify a lack of greenness. The waste volume, pH, and compounds with specific attributes are few of the significant factors that the greenness profile considers .

Result and Discussion

Accuracy :

Accuracy in method development refers to the closeness of a measured value to the true value or the accepted refer-ence standard. The purpose of accuracy is to verify that the method provides results that are both correct and true, with-out significant deviation from the real value . By spiking the API at 50%, 100%, and 150% levels to the placebo, the method's accuracy was determined. With a recovery in the range of 98–101.8% and a recovery percentage RSD of not more than 2, the devised approach was shown to be accu-rate. The average recovery was adequate and well within the allowed ranges. The findings are listed in Table

Accuracy levels	Amount of Vono-prazan (mg)	% Recovery	% RSD
Level I (50%)	12.502	101.8	0.12
Level II (100%)	25.290	98.8	0.29
Level III (150%)	37.540	98.0	0.00

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Precision:

The degree of consistency or reproducibility of the results obtained by applying a procedure to a set of measurements under identical conditions is referred to as precision. It is essential for guaranteeing the correctness and depend-ability of the analytical procedure since it shows how well the approach can yield the same outcome when used again. Six individual samples (n = 6) of the same amount were examined to ascertain intra-day (repeatability) precision. On same day, analysis was carried out for a short period of time. Inter-day precision was conducted on six different samples by another individual with a different sample but the same concentration.

Solution stability

Solution suitability was investigated over an extended period of 72 h to rigorously define the practical working window and understand the degradation kinetics of Vonoprazan in the diluent. demon-strate that the Vonoprazan solution exhibited excellent sta-bility over 24 h, with a negligible change in assay value of only -0.65% from the initial reading. This confirms that solutions remain stable for a full day, which is sufficient for routine analytical work. A significant decrease of 2.1% was observed at the 48-h mark, exceeding the typical accept-ance criterion of NMT 2.0% .

Component Time	Vonoprazan % Assay	% Difference
0 h	100.2	NA
15 h	100.7	0.40
24 h	99.55	0.35
48 h	98.10	0.60
72 h	95.70	0.43

II. CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Vonoprazan in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Vonoprazan in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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