



Received on 09 June 2026; received in revised form, 15 June 2026; accepted, 20 June 2026; published 01 October 2026

DEVELOPMENT AND VALIDATION OF A GREEN STABILITY-INDICATING RP-HPLC METHOD FOR VONOPRAZAN FUMARATE WITH AGREE-BASED GREENNESS ASSESSMENT

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Keywords:

RP-HPLC, Vonoprazan fumarate, Method development and validation, Forced degradation, Green analytical chemistry

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ABSTRACT: **Aim:** The present study aimed to develop and validate a simple, rapid, accurate, precise, stability-indicating, and environmentally friendly RP-HPLC method for the quantitative estimation of Vonoprazan Fumarate (VPZ) in pharmaceutical dosage forms and to evaluate its greenness using AGREE-based assessment tools. **Study Design:** Experimental analytical method development and validation study based on ICH guidelines. **Place and Duration of Study:** Department of Pharmaceutical Chemistry and Department of Pharmaceutical Analysis, Delonix Society's Baramati College of Pharmacy, Maharashtra, India. The study was carried out during the academic research period of 2025–2026. **Methodology:** Chromatographic separation was performed on C18 column (250 × 4.6 mm, 5 µm) with an isocratic mobile phase of methanol and aqueous phase containing 1% HCl (70:30 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 268 nm. The developed method was validated for specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ) as per ICH requirements. Forced degradation studies were performed under acid, base, oxidative, thermal and photolytic stress conditions to assess the method's ability to indicate stability. AGREE and AGREEprep metrics were used to assess greenness. **Results:** The technique showed a clear symmetric peak at about 3.3 min retention time. The method showed good linearity over the concentration range of 5–25 µg/mL with a correlation coefficient (r²) of 0.9991. The recoveries were in the range 98% to 102% as per accuracy surveys. %RSD readings for intra-day and inter-day precision were 0.63% and 0.98%, respectively. The results revealed that the LOD and LOQ were 1.58 µg/mL and 5.220 µg/mL, respectively. Robustness and ruggedness studies gave acceptable values of %RSD below 2%. The specificity and stability-indicating nature of the method was proved by forced degradation studies. The greenness assessment results were 0.73 and 0.62 for AGREE and AGREEprep, respectively. **Conclusion:** The developed RP-HPLC method is simple, precise, accurate, stability-indicating, and environmentally sustainable, making it suitable for routine quality control analysis of Vonoprazan Fumarate in pharmaceutical formulations.

INTRODUCTION: The development and validation of analytical procedures are essential to the advancement of pharmaceutical research and the assurance of the effectiveness, safety, and quality of therapeutic medicines¹.

Vonoprazan, a potassium competitive acid blocker, represents a significant advancement in treating acid-related conditions, serving as a viable alternative to the most effective proton pump inhibitors.

Its effectiveness in treating *Helicobacter pylori* infection, peptic ulcer disease, and gastroesophageal reflux illness is well-documented². A more potent inhibitor of acid secretion is vonoprazan. It starts working quickly, has less anti-secretory variability, is safer, and is more tolerable

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³⁻⁴. Eighty percent of the protein binding is inactivated through liver metabolites, primarily by (CYP) 3A4, and passed out through urine (67%) and feces (31%). Vonoprazan has a half-life of 7.1 hrs. It is consumed orally. It is used to treat erosive oesophagitis, duodenal ulcers, and stomach ulcers ⁵. When compared to proton pump inhibitors, which necessitate several dose schedules to produce comparable therapeutic results, vonoprazan's quick and sustained acid suppression offers a substantial advantage ⁶.

RP-HPLC, or reverse-phase high-performance liquid chromatography, is a popular analytical way because of its excellent resolution, repeatability, and sensitivity ⁷. Vonoprazan is often formulated as VPZ, a modification that substantially enhances its stability and solubility characteristics. The formulation's components, including the solubilizing agent, buffering agent, pH-modulating agent, and solvent, collectively contribute to the drug's improved solubility and prolonged stability throughout its shelf life ⁸. Currently, VPZ lacks formal recognition in any established pharmacopoeia. This underscores the critical need for a transparent and efficient high-performance liquid chromatography (HPLC) method to evaluate its quality and safety profile. Given its high sensitivity and capacity to manage complex mixtures, reversed-phase HPLC (RPHPLC) is employed for this purpose. By providing accurate and reliable results, and being accepted by regulatory agencies, this method ensures the sustained safety and effectiveness of pharmaceutical products ⁹.

Over the length of the pharmaceutical product's lifecycle, each of these elements ensures the method's reliability and consistency. In recent years, the use of Design of Experiments (DoE) and Quality by Design (QbD) approaches has increased in method development, with the goal of improving understanding and control of methods ¹⁰. Furthermore, regulatory bodies like the US FDA and EMA strongly recommend using stability-indicating assays to assess the integrity of active pharmaceutical ingredients (APIs) in their formulations during the drug approval process ¹¹. The idea of "green analytical chemistry" (GAC) forces analytical chemists to take safety, health, and environmental concerns into account.

The greenness of Analytical processes is a complex, multivariate parameter that is difficult to measure ¹²⁻¹³.

MATERIALS AND METHODS:

Material: Vonoprazan fumarate API gifted by Cipla Ltd., Vocab tablet 10mg, HPLC grade methanol, HPLC grade water, HCL.

Instruments: Double beam UV-vis Spectrophotometer (Shimadzu 1900i), HPLC System (JASCO), Sonicator (LABQUEST BY BOROSIL), Weighing balance (WENSAR), pH meter(AE MAX), Vaccume filter (BORO 017), UV chamber, Hot air oven (REMI).

Method Development:

Preparation of Mobile Phase: The mobile phase was a mixture of methanol and aqueous phase of 1% hydrochloric acid in the ratio of 70:30 (v/v). The aqueous phase was prepared by adding 0.1mL of hydrochloric acid to 500 mL of purified water and mixed well. The individual mobile phase components were filtered through a 0.45µ m membrane filter and degassed by sonication for 15 minutes before their use. The prepared mobile phase was mixed in the required proportion, equilibrated with the chromatographic system and used for analysis. Fresh mobile phase was prepared every day to ensure consistent chromatographic performance.

Preparation of Standard Stock Solution: 25 mg of pure med (VPZ) was weighed exactly and transferred into a 25 mL volumetric flask. It was sonicated for 5-10 minutes to dissolve in methanol: water (pH 3) then diluted to the volume to prepare stock solution of 1000 µg/mL and filtered through Whatman paper or 0.45 µm membrane filter.

Preparation of Sample Solution: Accurately weigh 10 mg of API and dissolve in 100 mL volumetric flask in methanol: water (70:30, pH 3). Sonicate for 15–20 min, cool and dilute to volume, filter through 0.45 µm filter and then dilute to 5–25 µg/mL for RP-HPLC analysis.

Selection of Detection Wavelength: The detection wavelength was determined by scanning a diluted standard solution across the ultraviolet spectrum, specifically from 200 to 400 nm. The wavelength of 268 nm was carefully chosen for subsequent

analysis, as it exhibited the highest absorbance λ_{max} , demonstrating both exceptional sensitivity and accuracy¹⁴.

Selection of Mobile Phase and Chromatographic Conditions:

The chromatographic conditions were optimized for the column, mobile phase, pH and flow rate for better resolution. The C18 column with methanol: water (1% HCl, 70:30 v/v, pH 3) resulted in a sharp and symmetric peak. Detection at 268 nm provided a good sensitivity and the method was found to be reproducible and system suitable for routine analysis of RP-HPLC¹⁵.

Optimization of Mobile Phase: During method development, various chromatographic parameters such as column type, flow rate, wavelength and pH were optimized.

A C18 column, flow rate of 1.0 mL/min, detection wavelength of 268 nm and mobile phase pH 3 (adjusted with 1% HCl) provided sharp symmetrical peak with acceptable retention time and good chromatographic performance. Thus, these conditions were selected for further validation studies.

TABLE 1: OPTIMIZED PARAMETERS

Sr. no.	Mobile phase	Ratio	RT	Peak shape	Observation
1	Methanol: Water	75:25	Very low	distorted	Very fast elute poor peak resolution, slight peak distortion
2	(1%HCL)	60:40	Moderate	Slightly asymmetric	Improved peak shape but inadequate resolution and symmetry
3		50:50	high	symmetric	Better peak symmetry but increased retention time and broadening
4		70:30	3.3	Sharp and symmetric	Sharp, well defined symmetric peak with good resolution and acceptable retention time

Method Validation:

Specificity: We tested the RPHPLC method's specificity by injecting blank, placebo, std, and trial solutions. There was no interfering at the analyte's rt, and the peak purity was within acceptable limits. This shows that the method is specific and can accurately measure the drug even when other substances are present¹⁶.

Linearity: The linearity of the RPHPLC method was tested with the range of 5–25 $\mu\text{g/mL}$ of standard solutions by triplicate injection. The calibration curve of concentration versus mean peak area was found to be highly linear with r^2 value close to 1, indicates the method is suitable for accurate quantifications¹⁷⁻¹⁸.

Range: The current method determined the range to be 5 to 25 $\mu\text{g/mL}$, as the drug exhibited satisfactory linearity, stable peak area response, and acceptable system suitability parameters within this concentration range. The method showed both precision and accuracy across the entire range of values, demonstrating its usefulness for routine quantitative analysis of the drug¹⁹.

Precision: Precision, which indicates the consistency and agreement of results obtained from

repeated evaluations of the same sample, is expressed as %RSD. This encompasses repeatability, intermediate precision, and duplicability, as outlined by ICH guidelines. In the context of RP-HPLC, precision is evaluated through multiple injections a %RSD value under 2% suggests confirming the precision of the method and its suitability for routine use.

Intraday Precision: The precision within a single day was assessed by making six identical injections of a 20 $\mu\text{g/mL}$ standard solution on the same day. The %RSD of the peak areas remained below 2%, thereby demonstrating the method's commendable repeatability and consistency.

Interday Precision: Inter-day precision was assessed through the examination of six replicate injections of a 20 $\mu\text{g/mL}$ standard solution, conducted across three successive days. The relative standard deviation (%RSD) values obtained each day remained below 2%, demonstrating the method's good reproducibility and reliability²⁰.

Accuracy: Accuracy was evaluated by recovery studies at 80%, 100%, and 120% levels. The % recovery was within 98–102% with %RSD less

than 2%, establishing the method's accuracy and reliability without interference from other components²¹.

System Suitability: Following the attainment of system equilibrium, a std solution, exhibiting an absorption of 20 µg/mL, was formulated and afterward subjected to analysis. A total of six injections were performed, and both the retention time and peak area were meticulously documented. The system's reliability was confirmed, as evidenced by a %RSD of 2% or less. These findings, in conjunction with the observed values for theoretical plates and the tailing factor, further substantiated the system's proper functionality.

Limit of Detection: Following the system's equilibration, three injections of a 10 µg/mL solution were made. The analyte showed a consistent response, with a signal-to-noise ratio of about 3:1. This confirmed the method's sensitivity and helped to establish the limit of detection. The 10 µg/mL solution, injected three times, consistently produced a noise to signal ratio of about 1:3. This confirmed the limit of detection.

Limit of Quantification: After the solution had reached equilibrium, three injections of 10 µg/mL were made. The %RSD was within limits, and the signal-to-noise ratio was about 10:1, which confirmed the limit of quantification (LOQ).

Robustness: The method's robustness was assessed through minor alterations to the flow rate (0.8 mL/min), the wavelength (265 nm), and the mobile phase creation (72:28). A 20 µg/mL solution was analyzed in triplicate under each set of conditions. The results demonstrated no significant deviations, and the %RSD values remained within suitable thresholds, thereby confirming the method's reliability and consistent performance.

Ruggedness: Ruggedness was assessed by examining a 20 µg/mL solution across various parameters, including different analysts, days, and instruments. The %RSD values fell within acceptable ranges, thus suggesting the method's reproducibility and consistency under typical variations.

Assay of Marketed Formulation: Twenty tablets of Vocab® tablets containing 10 mg of Vonoprazan

Fumarate were weighed and powdered finely. A quantity of powder equivalent to 10 mg of Vonoprazan Fumarate was weighed accurately and transferred to a 100 mL volumetric flask. About 70 mL of mobile phase was added and sonicated for 15 min to ensure complete extraction of the drug. The volume was made up to the mark with the mobile phase and filtered through 0.45 µm membrane filter. An appropriate dilution was made using mobile phase to get a concentration within the linearity range. The resulting solution was injected into the RP-HPLC system under optimized chromatographic conditions and the chromatograms were recorded.

Stability Indicating Study²²⁻²³: Stability-indicating methods (SIMs) are analytical techniques designed to identify and quantify degradation products, impurities and the active pharmaceutical ingredient (API) within drug substances or products, while avoiding interference. These methods ensure the safety, efficacy, and quality of pharmaceuticals throughout their shelf life by isolating the API from excipients, process-related impurities, and potential degradants under stress conditions, including heat, light, acid, base, or oxidation.

Acid Degradation: 1 mL of stock solution was added to the solvent and 0.1 N HCl and kept for 15 min then neutralized with 0.1 N NaOH and diluted to 10 mL. After filtration, analysis by RP-HPLC showed significant degradation by additional peaks and decreased peak area at ~3.3 min.

Base Degradation: One millilitre of the standard stock solution, three millilitres of solvent and one millilitre of 0.1 N NaOH were combined. The mixture was then allowed to stand for fifteen minutes at room temperature. The solution was then neutralised with the addition of 1 mL of 0.1 N HCl and diluted to a final volume of 10 mL. It was analysed by RP-HPLC after filtration. The data showed a significant decrease with more peaks and a smaller peak area at about 3.3 minutes of retention time.

Oxidative Degradation: Oxidative degradation was performed by mixing 1 mL of the standard stock solution with 3 mL of a solvent and 1 mL of 3% hydrogen peroxide. This mixture was then kept at room temperature for 15 minutes. The volume

was made up to 10 mL, filtered, and analyzed by RP-HPLC. The results showed a moderate amount of breakdown, with some small extra peaks and a slight decrease in the peak area around a retention time of about 3.3 minutes.

Thermal Degradation: Thermal degradation of the drug sample was achieved by subjecting it to 110°C for a duration of three hours within a hot air oven. Following cooling, the sample underwent dissolution, dilution, filtration and subsequent analysis *via* RP-HPLC. The analytical outcomes indicated a degree of degradation, characterized by the emergence of minor additional peaks and a slight reduction in peak area, specifically at an approximate retention time of 3.3 minutes.

Method Greenness Evaluation²⁴⁻²⁵: The environmental effect of the new method was assessed using the 12 principles of green chemistry. Specifically, the GAPI, analytical eco-scale, and AGREE tools were used for this evaluation.

The method used a Methanol and water (1%HCL) as the mobile phase, requiring 25mL of methanol for each injection water and Methanol served as the solvents. During sample preparation, a total of 60 mL of methanol was utilized as a solvent. Penalty points were calculated for each reagent, solvent and instrument in the analytical eco-scale evaluation. Furthermore, the AGREE and GAPI tools were employed to evaluate the toxicity of the solvents, instruments, and sample preparation techniques, utilizing a color-coded pictogram to represent greenness levels, ranging from green to red.

RESULTS AND DISCUSSION:

Selection of Detection Wavelength: It was noted that each drug demonstrated appreciable absorbance at 268 nm.

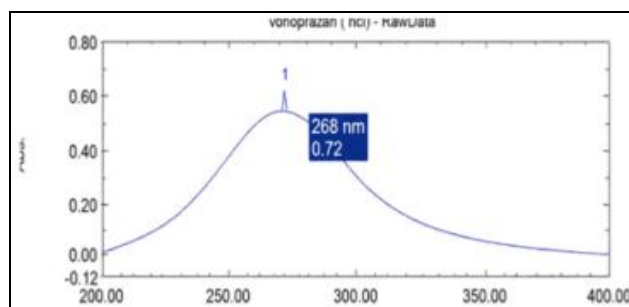


FIG. 1: λ MAX OF VPZ268

Chromatogram and System Suitability Parameters of Drug:

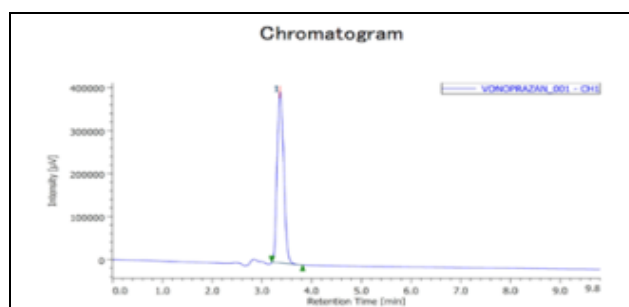


FIG. 2: STANDARD CHROMATOGRAM OF VPZ

Method Validation:

Linearity: These results were then suitably diluted with mobile phase to produce solutions with VPZ concentrations ranging from 5 to 25 μ g/mL. Correlation coefficient (r^2) for calibration curve of (VPZ) 0.9991.

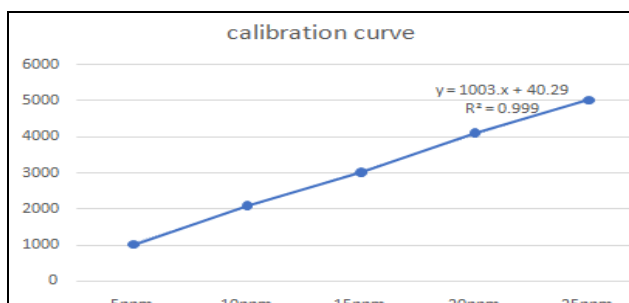


FIG. 3: CALIBRATION CURVE OF VPZ

Accuracy: The result of accuracy shown in Table 2.

TABLE 2: ACCURACY STUDY OF VPZ

Level	Theoretical conc. (μ g/ml)		Area	Practical Conc. (μ g/ml)	% Recovery	Average	Std Dev	% RSD
	Sample	Std.						
80 %	10ppm	8ppm	1927262	7.86	98.25	1961443	31728.54	1.61
			1989955	8.11	101.37			
			1967111	8.02	100.25			
100 %	10ppm	10ppm	2161251	10.16	101.9	2127452	30068.14	1.41
			2103674	9.89	98.86			
			2117431	9.95	99.52			
120 %	10ppm	12ppm	2405252	11.89	99.13	2426320	19684.62	0.81
			2429467	12.02	100.13			
			2444241	12.09	100.74			

Precision: Six injections of the std solution were made in order to evaluate precision. The result of precision report in **Table 3**. % RSD was found to be 0.63% for VPZ

TABLE 3: INTRADAY PRECISION STUDY OF VPZ

Conc.	No. of replicates						SD	%RSD
	1	2	3	4	5	6		
20ppm	4834997	4884718	4900364	4901969	4835668	4857790	30692.75	0.63

Interday Precision: The statistics for day 1 interday precision for VPZ is presented in **Table 4**. % RSD was found to be 0.98% for VPZ.

TABLE 4: INTERDAY PRECISION STUDY OF VPZ

Conc.	No. of replicates						SD	%RSD
	1	2	3	4	5	6		
20ppm	5261213	5150960	5302217	5366368	5353079	5363996	52223.81	0.98

Specificity: Specificity in formation shown in **Table 5** which indicates that there is no interference from VPZ in sample mixture.

TABLE 5: SPECIFICITY STUDY OF VPZ

Concentration	Pure Sample peak area	Sample peak area
20µg/ml	4074456	4095647

Limit of Detection and Quantification: The LOD for VPZ was confirmed to be 1.58 µg/ml. The LOQ for VPZ was confirmed to be 5.220 µg/ml. The obtained LOD and LOQ results a represented in **Table 6**.

TABLE 6: LOD AND LOQ STUDY OF VPZ

Parameters	VPZ
Slope	1003.2
LOD	1.58
LOQ	5.220

Robustness: The findings of the robustness study are reported in **Table 7**. The % RSD was noticed to be 0.62-1% for VPZ.

TABLE 7: ROBUSTNESS STUDY OF VPZ

Sr. no.	Factor	Level	Peak Area*±SD	%RSD
1	Change in Flow rate ± 0.2 ml/min	0.8	3539132 ± 21983.09	0.62
2	Variation in Detection Wavelength (nm) ± 2 nm	265	4085935 ± 40630.23	1.00
3	Alteration in Mobile Phase ratio ± 5%	72:28	3666059 ± 27704.03	0.76

Ruggedness: The found results for ruggedness are summarized in **Table 8**. The % RSD was noted to be 0.75-1.20% for VPZ.

TABLE 8: RUGGEDNESS STUDY OF VPZ

Sr. no.	Factor	Level	PeakArea*±SD	% RSD
1	Alteration in analyst	Analyst 1	3597248 ± 42339.45	1.18
		Analyst 2	3790112 ± 45399.53	1.20
2	Variation in day	Day 1	3710923 ± 31018.04	0.83%
		Day 2	3614006 ± 27400.81	0.75%

System Suitability:

TABLE 9: SYSTEM SUITABILITY STUDY OF VPZ

Injection	RT	No. of theoretical plates	Tailing factor
1	3.367	3445	1.11
2	3.358	3317	1.14
3	3.344	3498	1.10
4	3.339	3486	1.12
5	3.321	3419	1.11
6	3.332	3442	1.12
Mean	3.344	3434.50	1.11
SD	0.017	65.63	0.015

Assay of Marketed Formulation: (Vocab Tablet 10mg).

TABLE 10: ASSAY STUDY OF VPZ

Injection	Peak area
1	5643280
2	5673889
3	5619472
Mean	5645547
SD	27220.5
%RSD	0.48

Stability Indicating Study²⁶: Stressors such as acidic, basic, thermal, photolytic, and oxidative

environments were applied to the medication. A 1 mL sample of the filtered stock solution was transferred to a 10 mL volumetric flask for the forced deterioration investigations. The volume was then adjusted to 10 mL using the appropriate diluent.

The drug demonstrated enhanced degradation when subjected to alkaline conditions. Sample solutions, each at a concentration of 20 µg/mL, were prepared for each stress condition and then exposed to the corresponding stressors.

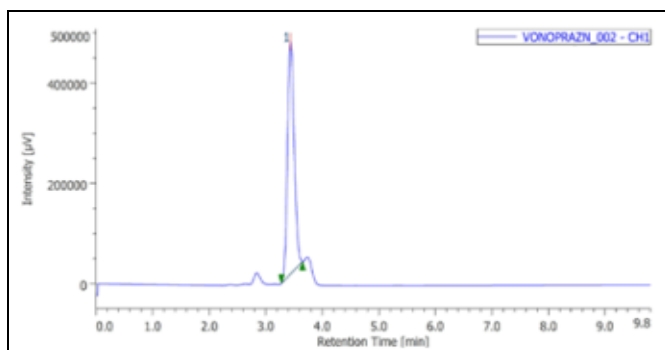


FIG. 5: ACID DEGRADATION

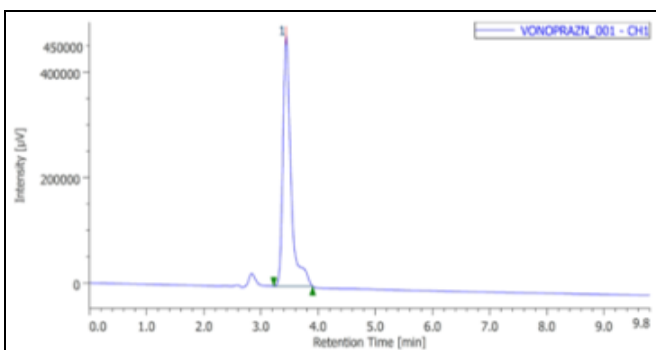


FIG. 6: BASIC DEGRADATION

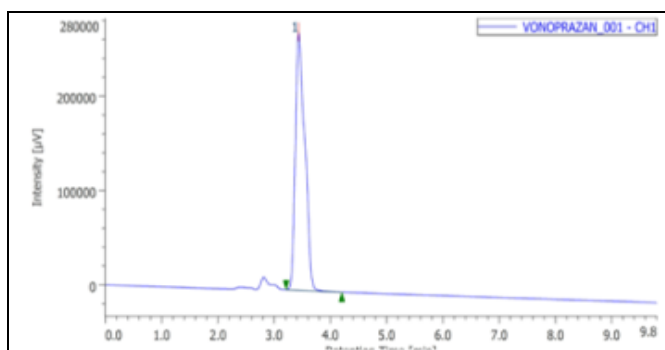


FIG. 7: OXIDATION DEGRADATION

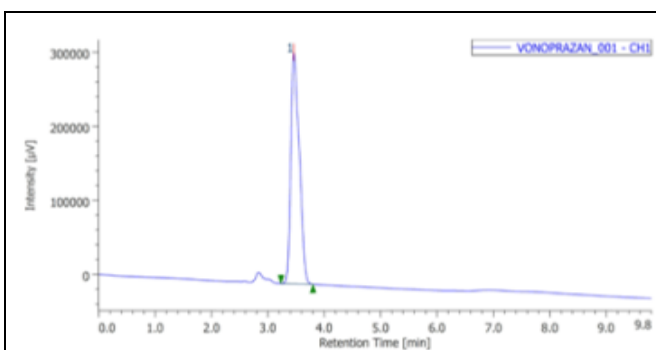


FIG. 8: THERMAL DEGRADATION

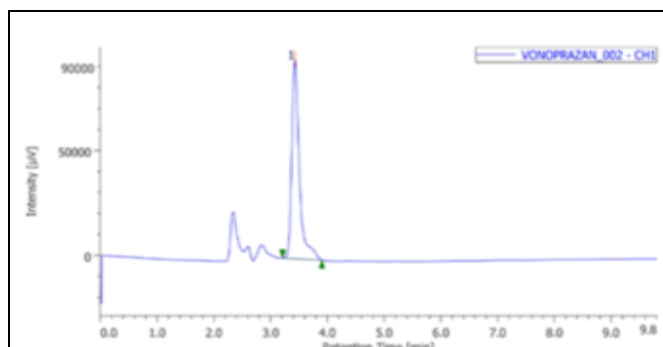


FIG. 9: PHOTOLYTIC DEGRADATION

TABLE 11: DEGRADATION STUDY OF VPZ

Sr. no.	Degradation Condition	%Degradation
1	Acidic degradation	7.16
2	Basic degradation	11.43
3	Oxidative degradation	5.35
4	Thermal degradation	8.78
5	Photolytic degradation	6.59

Greenness Analysis: With an AGREE score of 0.73 and AGREEprep score of 0.62, the approach showed moderate greenness, demonstrating a balance between practicality, sustainability, and efficiency for pharmaceutical quality control.

Agree: The sustainability of the analytical methods is evaluated by applying the 12 principles of green analytical chemistry considering waste, energy utilisation and reagent toxicity. The HPLC method developed in this study showed good greenness with excellent analytical performance, including reduced waste generation, energy consumption and solvent consumption as demonstrated by its AGREE score of 0.73²⁷.

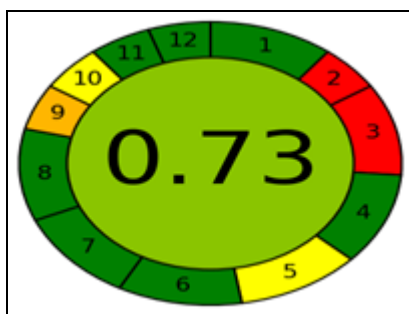


FIG. 10: PICTOGRAM OF AGREE

AGREEPrep: A sustainability metric, employing a 0–1 scale, evaluates ten impact categories, including solvent use, waste generation, energy consumption, and sample size; results are presented as a pictogram. The method under investigation achieved a score of 0.62, thereby recommending both commendable efficiency and economic feasibility. The green pictogram, which emphasizes reduced solvent usage, negligible waste production, and effective source allocation, validates its appropriateness for environmentally conscious routine analysis²⁸.

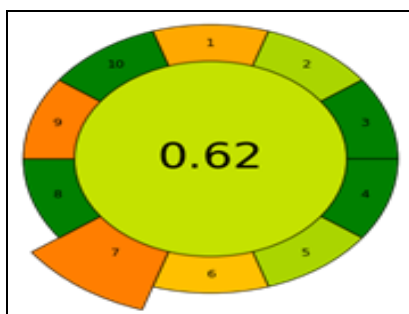


FIG. 11: PICTOGRAM OF AGREEPREP

CONCLUSION: The developed RP-HPLC method for VPZ was simple, rapid, precise and accurate and fulfilled the ICH validation criteria with excellent linearity, sensitivity and specificity. It had short retention time, good peak symmetry and stability-indicating ability in forced degradation studies. The method was also acquired to be good in terms of greenness with lesser solvent

consumption and waste generation, thus making it reliable, economical and suitable for routine pharmaceutical analysis.

ACKNOWLEDGEMENT: I would like to express my deep appreciation to my research supervisor for his advice and help in this study. I am also thankful to my department instructors, friends and co-workers for their support and cooperation. I would like to express my sincere gratitude to my family for their constant support which made it possible for me to do this research.

CONFLICTS OF INTEREST: Nil

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How to cite this article:

Bendgude P, Baokar S and Patil RN: Development and validation of a green stability-indicating RP-HPLC method for vonoprazan fumarate with agree-based greenness assessment. Int J Pharm Sci & Res 2026; 17(10): 3070-78. doi: 10.13040/IJPSR.0975-8232.17(10).3070-78.

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