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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPTLC METHOD FOR VONOPRAZAN FUMARATE WITH GREEN CHEMISTRY ASSESSMENT

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ABSTRACT
A novel, eco-friendly high-performance thin-layer chromatographic (HPTLC) method was developed and validated for the quantification of Vonoprazan fumarate in bulk and tablet dosage forms. The separation was achieved on silica gel 60 F_{254} plates using a mobile phase comprising methanol, ethyl acetate and formic acid (8.5:1:0.5 v/v/v). Densitometric detection was carried out at 266 nm. The method exhibited excellent linearity over the range of 1000–6000 ng/spot, with high precision and accuracy (99.05 – 100.10 %), as well as acceptable robustness. Specificity was established through forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions, confirming the method's stability-indicating capability. The environmental sustainability of the method was assessed using three complementary tools like. Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE) metric, and Whiteness Index, all of which confirmed its green profile. This validated method is simple, rapid, economical and suitable for routine analysis in quality control settings.

KEYWORDS: Chromatographic analysis, Eco-friendly method, Tablet analysis, Analytical greenness, Stability testing.

INTRODUCTION
Vonoprazan fumarate is a first-in-class potassium-competitive acid blocker (P-CAB) that has significantly advanced the treatment of acid-related gastrointestinal disorders, including gastroesophageal reflux disease (GERD), peptic ulcers and *Helicobacter pylori* infections. Unlike traditional proton pump inhibitors (PPIs), Vonoprazan reversibly and non-covalently inhibits the gastric H^+/K^+-ATPase enzyme by competing with potassium ions. This mechanism confers a faster onset of action, enhanced acid suppression, and prolonged therapeutic effect, even in PPI-resistant patients. It is slightly soluble in water but freely soluble in methanol and dimethyl sulfoxide (DMSO). After its initial regulatory approval by Japan's PMDA in 2014, Vonoprazan was subsequently approved by the U.S. FDA in 2019 and the European Medicines Agency (EMA) in 2020.[1,3]

Despite its expanding clinical application, a validated, robust, and eco-friendly analytical approach for quantifying Vonoprazan fumarate remains underexplored. Most existing methods rely heavily on High-Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC) and Liquid Chromatography–Mass Spectrometry (LC-MS), HPTLC in Combination.[3,5] These techniques, although accurate and sensitive, often involve high operational costs, large solvent consumption, and complex instrumentation; making them less accessible in resource-limited laboratories. Furthermore, many of these methods do not align with the principles of green analytical chemistry (GAC).[6]

In recent years, environmental sustainability has become a priority in pharmaceutical analysis. Tools such as the Green Analytical Procedure Index (GAPI), Analytical GREEnness metric (AGREE), and Whiteness Analytical Chemistry (WAC) have emerged to assess the ecological impact of analytical procedures. However, the greenness of analytical methods reported for Vonoprazan remains largely unassessed.

High-Performance Thin-Layer Chromatography (HPTLC) presents a promising alternative analytical tool due to its simplicity, cost-effectiveness, minimal solvent usage, and high throughput.

This study aims at development and validation of stability-indicating HPTLC method for Vonoprazan

fumarate estimation in bulk and marketed dosage forms. The proposed method is evaluated in accordance with ICH Q2(R1) guidelines and critically assessed using green chemistry metrics (GAPI, AGREE, and WAC), ensuring scientific reliability and environmental compatibility.[7,10]

Instrumentation

Chromatographic analysis was performed using a CAMAG HPTLC system. Sample application was carried out with a Linomat V semi-automatic applicator under nitrogen. Plate development was conducted in a twin-trough glass chamber pre-saturated with mobile phase vapors. Detection and densitometric scanning were performed using a CAMAG TLC Scanner III with WinCATS software. Densitometric scanning was carried out at 266 nm.

Chromatographic Conditions

The optimized mobile phase was methanol:ethyl acetate:formic acid (8.5:1:0.5, v/v/v). Plates were pre-saturated for 10 minutes and bands (6 mm) were applied at a consistent rate. Development was carried out up to 90 mm, followed by densitometric scanning at 266 nm using a slit dimension of 4 mm × 0.45 mm. Vonoprazan exhibited a consistent Rf value of -0.61.

Preparation of Standard and Sample Solutions

A stock solution (1000 µg/mL) was prepared by dissolving 10 mg of Vonoprazan in 10 ml methanol. Working solutions were made via serial dilutions. For sample analysis, tablet powder equivalent to 10 mg of the drug was extracted with methanol, sonicated (15 min), filtered, and diluted to the required concentration. All solutions were freshly prepared and used immediately.

Forced Degradation Studies

Stability-indicating capacity was evaluated under ICH Q1A(R1) conditions under which drug was subjected to forced degradation under Acidic (0.1 N HCl at 60°C for 1 h); Alkaline (0.1 N NaOH at 60°C for 1 h); Oxidative (30% H_2O_2 at room temperature for 1 h); Thermal (Solid drug at 100°C for 6 h); Photolytic (UV light for 6 h). Samples were neutralized (where necessary), diluted with methanol, and analyzed. Degradation peaks were well-resolved from the parent compound, confirming the method's stability-indicating nature.

Method Validation

The method was validated according to ICH Q2(R1) guidelines for Specificity, Linearity, Precision, Accuracy, LOD, LOQ and Robustness.[11]

Greenness Assessment

Whiteness Index (WI):

A composite score (WI = 0.91) confirmed an ideal balance of analytical quality, environmental greenness and operational sustainability with minimal solvent use and low energy consumption.

Blue–BAGI (Blue Analytical Greenness Index):

High scores were achieved across analytical performance (A), environmental impact (G), and safety/practicality (I), indicating excellent overall greenness.

Modified GAPI (Green Analytical Procedure Index):

Color-coded evaluation showed predominantly green sections for sample preparation and instrumental steps. Yellow indicators were noted for methanol use (Class 2 solvent), and moderate concerns were recorded for waste management due to lack of solvent recycling. Overall, the method exhibited a strong green footprint.

RESULTS AND DISCUSSION

Development and Optimization of HPTLC Method

A simple, stability-indicating High-Performance Thin-Layer Chromatography (HPTLC) method was developed for the quantification of Vonoprazan fumarate (VPZ). Various mobile phases were evaluated to achieve well-resolved peaks. The optimized mobile phase methanol: ethyl acetate: formic acid (8.5:1:0.5, v/v/v) provided a compact band at Rf 0.49 ± 0.02, with minimal tailing.

Linearity and Range

Linearity was established in the concentration range of 1000–6000 ng/spot. A calibration curve was plotted between peak area and concentration, yielding a correlation coefficient (r^2) of 0.992, which indicates excellent linearity and suitability of the developed HPTLC method for quantitative estimation of Vonoprazan Fumarate. The detailed results of the linear regression analysis are summarized in Table 1.

Table 1: Results of linearity of Vonoprazon Fumarate.

Conc (ng/band)	1000	2000	3000	4000	5000	6000
1	6383.70	10384.50	12448.00	14636.80	17983.80	19569.30
2	6542.20	10491.10	12247.40	14982.90	17364.20	19935.50
3	6340.80	10269.20	12044.30	14400.31	17363.60	20399.10
4	6598.70	10499.90	12220.30	14040.00	17334.70	19757.00
5	6605.10	10142.70	12353.10	14121.80	17124.40	19906.00
6	6421.90	10362.30	12732.30	14054.20	17511.30	20170.80
AVG	6482.07	10358.28	12340.90	14222.6	17447.00	19956.28
SD	114.55	136.24	234.98	242.70	290.79	294.80
%RSD	1.77	1.32	1.90	1.71	1.67	1.48

Accuracy and Precision

Recovery studies conducted at 50%, 100%, and 150% levels yield recoveries ranging from 99.05 – 100.10 %, indicating the accuracy of the developed method. Precision was assessed by performing intra-day and inter-day analyses, with %RSD values consistently below 2%, confirming the method's repeatability and intermediate precision. The detailed results are presented in Table 2 (Accuracy) and Table 3 (Precision). A representative densitogram (Fig. 1) illustrates the peak overlay of replicate tracks, further supporting the method's precision and accuracy.

Table 2: Results of Accuracy of Vonoprazon Fumarate.

Sr. No.	Amount of marketed formulation added (ng/band)	Amount of API added (ng/band)	Area	Total amount of drug (ng/band)	Amount recovered	% Recovery	Average Area	S.D	R.S.D.
50%	2000	1000	12238.90	3000	2992.43	99.75	100.10	0.49	0.49
	2000	1000	12251.10	3000	2997.12	100.10			
	2000	1000	12310.20	3000	3019.83	100.66			
100%	2000	2000	14642.80	4000	3916.30	97.91	99.05	1.02	1.03
	2000	2000	14848.40	4000	3995.31	99.88			
	2000	2000	14793.60	4000	3974.25	99.36			
150%	2000	3000	17232.50	5000	4911.57	98.23	99.66	1.24	1.25
	2000	3000	17499.60	5000	5014.22	100.28			
	2000	3000	17524.30	5000	5023.71	100.47			

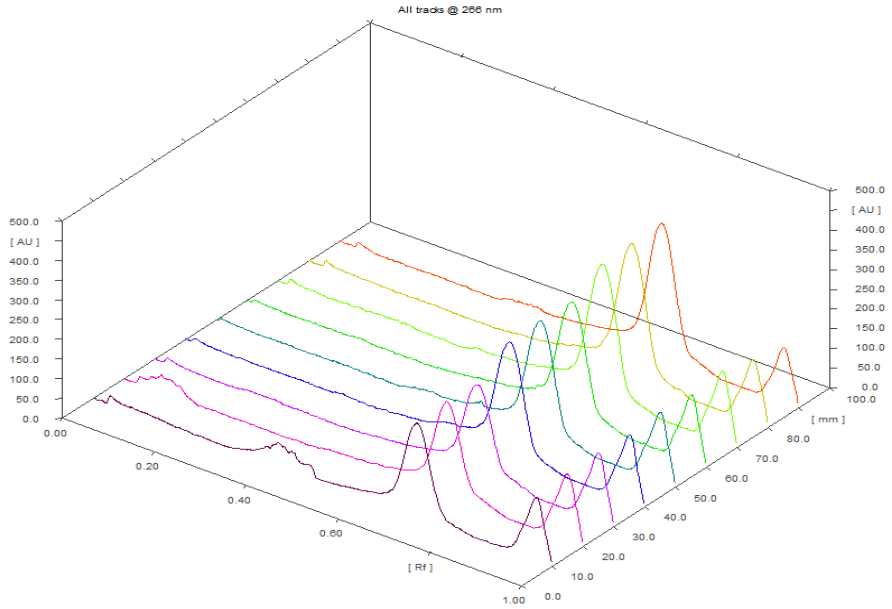


Fig 1: Densitogram of Precision Study.

Table 3: Intraday and Interday Precision Results of Vonoprazan Fumarate (n = 3)

Theoretical Concentration (ng/spot)	Intraday Peak Area	Amount Recovered (ng/spot)	% Recovery	Interday Peak Area	Amount Recovered (ng/spot)	% Recovery
2000	9515.70	1945.85	97.29	9528.10	1950.61	97.53
	9606.90	1980.90	99.04	9564.20	1964.49	98.22
	9711.00	2020.91	101.05	9639.60	1993.47	99.67
Mean ± %RSD	–	–	99.77 ± 1.11	–	–	98.48 ± 1.09
4000	14931.90	4027.40	100.69	14694.30	3936.09	98.40
	14816.40	3983.01	99.58	14976.70	4045.54	101.14
	14746.60	3956.19	98.90	14479.10	3853.83	96.33
Mean ± %RSD	–	–	99.72 ± 1.51	–	–	99.58 ± 1.41
6000	20151.60	6033.44	100.56	20146.80	6031.59	100.53
	19947.30	5954.92	99.25	19908.20	5939.89	99.00
	19981.30	5967.99	99.47	19833.80	5911.30	98.52
Mean ± %RSD	–	–	99.76 ± 0.70	–	–	99.35 ± 1.05

Specificity and Stability-Indicating

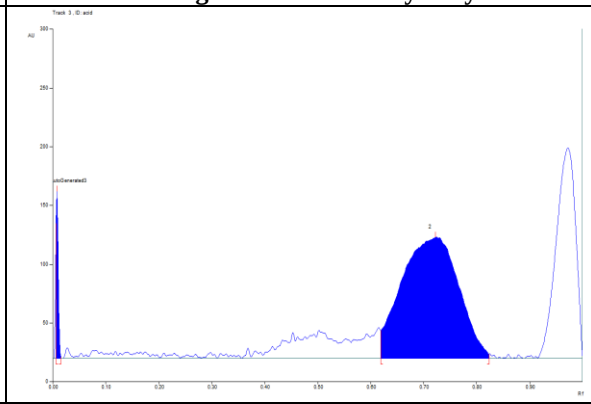
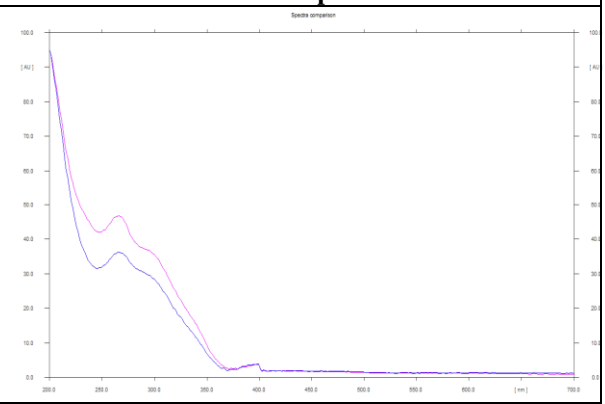
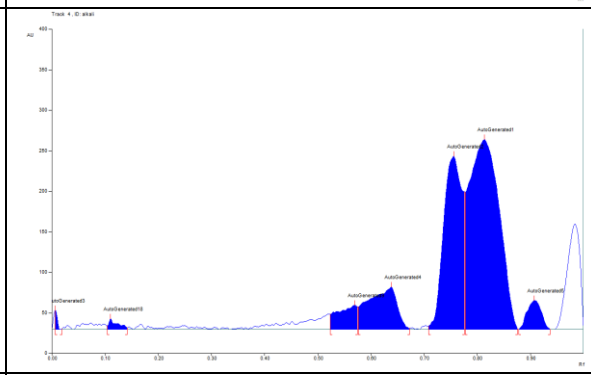
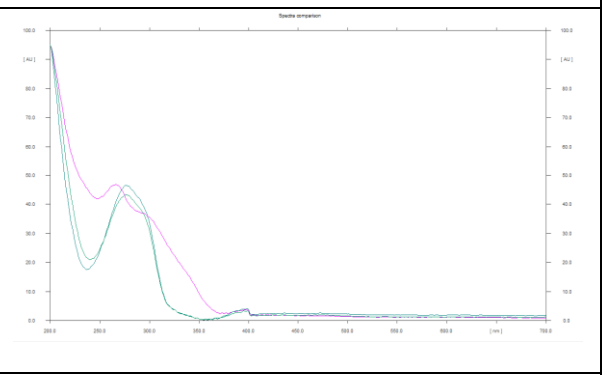
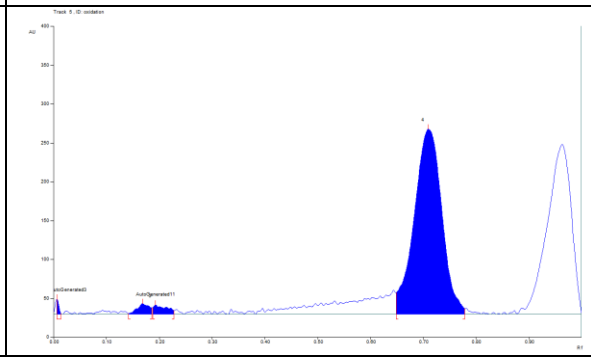
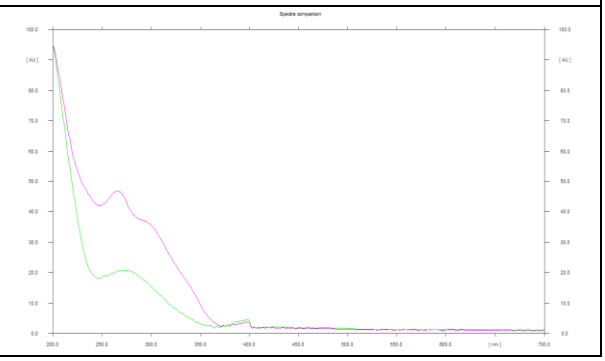
The drug was subjected to various stress conditions, including acidic, alkaline, oxidative, and thermal degradation. Significant degradation was observed under alkaline condition. The method was able to resolve the parent drug peak from its degradation products without interference, with well-separated peaks observed at distinct Rf values, confirming its specificity. The absence of overlapping peaks at the Rf of Vonoprazon in stressed samples demonstrates the method's capability to selectively quantify the intact drug even in the presence of its degradation products.

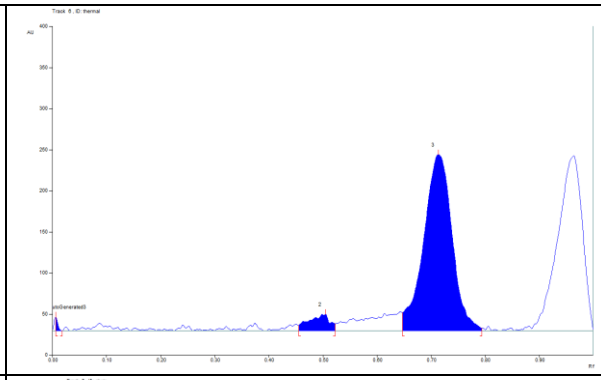
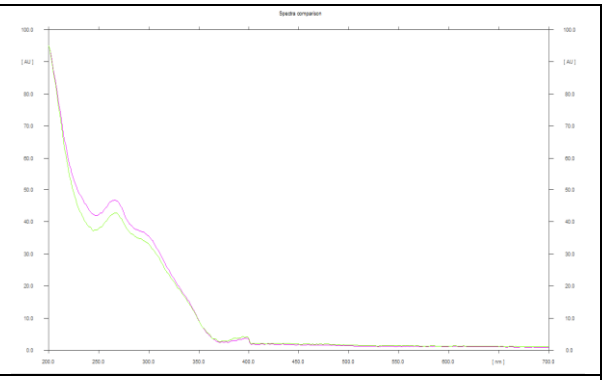
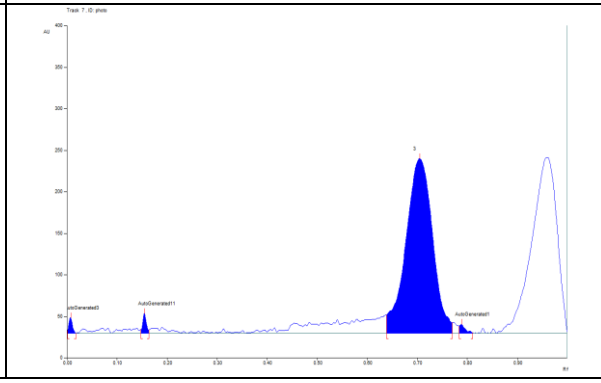
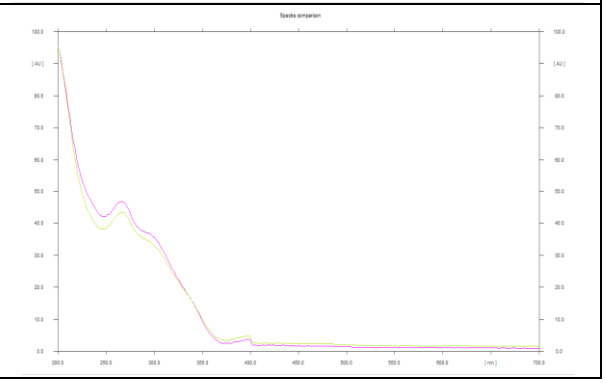
The results of the forced degradation studies are compiled in Table 4, providing a comparative summary of degradation behavior under each condition. Additionally, Table 5 present representative densitograms and in situ UV spectra of Vonoprazon under stress conditions, supporting the visual and spectral confirmation of degradation product separation and ensuring the method's stability-indicating performance.

Table 4: Summary of Forced Degradation Study Results under Various Stress Conditions.

Stress Condition	% Assay	% Degradation	Degradation Product Observed	Interpretation
Acid	94.34	5.66	No	Moderately stable under acidic conditions
Base			Yes	Significant degradation in basic conditions
Oxidation	84.87	15.13	No	Highly susceptible to oxidative degradation
Thermal	96.61	3.39	No	Stable
Sunlight	92.80	7.20	No	Stable

Table 5: Densitograms and in Situ UV Spectra of Vonoprazan Fumarate under Stress Conditions.

	Densitogram after Base Hydrolysis	In situ UV spectrum
Acid Hydrolysis		
Base Hydrolysis		
Oxidative Degradation		

Thermal Degradation		
Photolytic (Sunlight) Degradation		

Assay

The developed method was applied to a commercial tablet formulation. The assay results showed drug content of 99.46 ± 0.54 %, indicating the method's applicability for routine quality control.

Limit of Detection and Limit of Quantitation

The detection and quantitation limits of method were calculated from calibration curves. The equations used were LoD = 3.3 × σ/S and LoQ = 10 × σ/S, respectively where, σ = the standard deviation of y-intercept. S = slope of the calibration curve. The limits of detection and quantification were 227.60 and 689.70 ng/spot, respectively.

Robustness

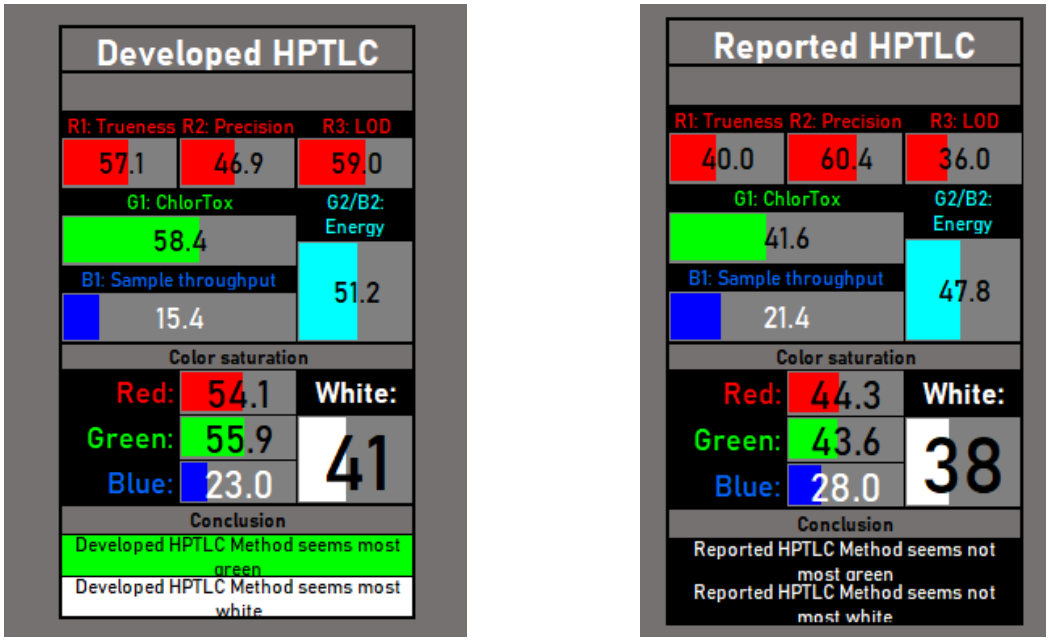
Robustness of developed method was analysed by small but deliberate changes in mobile phase volume (± 0.2 ml), saturation time (± 2 min) and detection wavelength (± 1 nm). One factor at a time was varied to study effect on peak area of drug. As the % RSD values were less than 2, the method is robust.

Greenness and Sustainability Evaluation

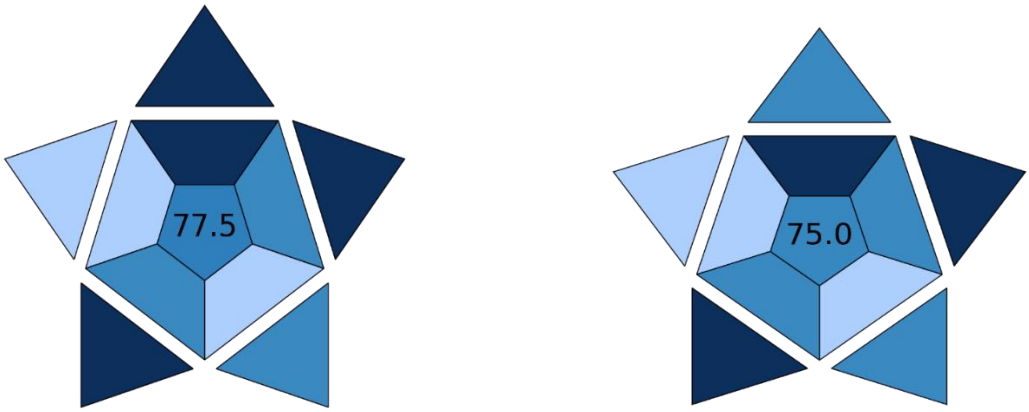
The greenness and sustainability of the developed HPTLC method were comprehensively evaluated using three established green assessment tools:

- Whiteness Index (WI): This index provides a holistic measure that combines environmental impact, analytical efficiency, and operator safety. The developed method showed a higher WI score compared to the reported method, indicating a more balanced and sustainable analytical procedure.
- BAGI (Blue Analytical GREEnness Index): The developed method achieved an AGREE score of 77.5, reflecting strong adherence to green analytical chemistry principles, with improvements in solvent usage and overall energy efficiency.
- Mo–GAPI (Modified Green Analytical Procedure Index): The GAPI pictogram demonstrated fewer red zones in the developed method, signifying reduced environmental and occupational hazards across various procedural steps.

The visual comparison of greenness profiles between the developed and reported HPTLC method is presented in Fig. 2, which includes:
(a) Whiteness Index evaluation, (b) BAGI comparison, and (c) Mo–GAPI pictogram outputs. These results collectively confirm the environmentally benign and sustainable nature of the proposed HPTLC method.



a)



b)

c)

Fig. 2: Comparative greenness profiles of developed (a1, b1, c1) and reported (a2, b2, c2) HPTLC methods using a) Whiteness Index, b) BAGI, and c) Mo–GAPI evaluation tools.

DISCUSSION

The developed HPTLC method for Vonoprazan fumarate offers a simple, precise, and environmentally conscious analytical approach for quantifying the drug in both bulk and marketed formulations. The optimized mobile phase—Methanol: Ethyl acetate: Formic acid (8.5:1:0.5, v/v/v) produced sharp, symmetrical peaks with a consistent Rf value of 0.71 ± 0.02, demonstrating satisfactory chromatographic behavior and reproducibility.

The method exhibited excellent linearity (r^2 = 0.992) across the concentration range of 1000–6000 ng/spot. Specificity was confirmed by the clear resolution of the drug from formulation excipients and degradation

products, establishing the method's stability-indicating capability.

Forced degradation studies revealed significant degradation under alkaline stress, moderate degradation under acidic and oxidative conditions, and relative stability under thermal and photolytic conditions. These results reflect the inherent chemical sensitivity of Vonoprazan fumarate and confirm the method's suitability for stability monitoring.

Accuracy was demonstrated through recovery studies at 50%, 100%, and 150% levels, with recoveries ranging from 99.05 – 100.10 %. Precision, evaluated through both intra- and inter-day experiments, yielded %RSD values < 2, indicating excellent reproducibility. Robustness was confirmed as minor changes in method parameters did not significantly impact performance.

From a sustainability perspective, the method was evaluated using green analytical chemistry tools. The GAPI pictogram indicated predominantly green zones with score of 83. Whiteness Index of 41 and BAGI score of 77.5 further validated the method's eco-friendliness and efficiency. Compared to conventional HPLC methods, this HPTLC approach reduces solvent use, minimizes waste, and shortens analysis time—making it a cost-effective and environmentally preferable alternative.

Collectively, these findings confirm the method's alignment with ICH Q2(R1) validation parameters and green analytical chemistry principles, making it a practical choice for routine quality control of Vonoprazan fumarate.

CONCLUSION

A novel, stability-indicating HPTLC method was successfully developed and validated for the quantitative estimation of Vonoprazan fumarate in bulk and tablet dosage forms. The method demonstrated excellent performance characteristics like linearity, specificity, accuracy, precision, robustness, and system suitability in accordance with ICH Q2(R1) guidelines.

Forced degradation studies confirmed its stability-indicating nature, while green chemistry assessment tools (GAPI, AGREE, and Whiteness Index) emphasized its environmental sustainability. The method's simplicity, low cost, minimal solvent usage, and high throughput make it well-suited for routine pharmaceutical quality control, especially in settings emphasizing eco-friendly practices.

Statements and Declarations

Ethical Approval.

Not applicable. This study did not involve human participants or animals.

Conflict of Interest

The authors declare that there is no conflict of interest.

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REFERENCE

1. Sakurai Y, Mori Y, Okamoto H, Nishimura A, Komura E, Araki T, Shiramoto M. Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects-a randomised open-label cross-over study. Alimentary pharmacology & therapeutics, 2015; 42(6): 719-30.
2. Jenkins H, Sakurai Y, Nishimura A, Okamoto H, Hibberd M, Jenkins R, Yoneyama T, Ashida K, Ogama Y, Warrington S. Randomised clinical trial: safety, tolerability, pharmacokinetics and pharmacodynamics of repeated doses of TAK-438 (vonoprazan), a novel potassium-competitive acid blocker, in healthy male subjects. Alimentary pharmacology & therapeutics, 2015; 41(7): 636-48.
3. Shaji J, Chacko AJ, Chacko JB. Development and validation of a stability-indicating HPLC method for Vonoprazan Fumarate. J Appl Pharm Sci, 2022; 12(10): 91–99.
4. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis, 2002 Jun 15; 28(6): 1011-40.
5. Moneim MM, 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 chemistry, 2024; 18(1): 230.
6. Gałuszka A, Migaszewski ZM, Konieczka P, Namieśnik J. Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC Trends in Analytical Chemistry, 2012; 37: 61-72.
7. Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta, 2018; 181: 204-9.
8. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Analytical chemistry, 2020; 92(14): 10076-82.
9. Tobiszewski M. Whiteness of analytical chemistry: Balancing analytical performance and environmental impact. TrAC Trends Anal Chem., 2020; 120: 115652.
10. Nowak PM, Kobyłka M. Blue Analytical Chemistry: Towards sustainable analytical methods. Anal Bioanal Chem., 2021; 413(21): 5343–5350.
11. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1), 2005 Nov; 1(20): 05.