Validated HPTLC Method for Stability Studies of Vonoprazan Fumarate

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***Abstract*— Vonoprazan Fumarate is a novel potassium-competitive acid blocker used in the management of gastric ulcers and gastroesophageal reflux disease (GERD). As a recently approved drug, the development of a reliable and stability-indicating analytical method is essential for quality control. The present study aimed to develop and validate a simple, precise, and accurate High-Performance Thin Layer Chromatography (HPTLC) method for the estimation of Vonoprazan Fumarate in bulk and tablet dosage forms. Chromatographic separation was achieved on silica gel 60 F_{254} aluminum plates using a mobile phase of propanol: water: methanol (7:2:1, v/v/v). Densitometric scanning was performed at 262 nm. The method was validated in accordance with ICH Q2 (R1) guidelines for specificity, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification. Linearity was observed over the range of 200–1600 ng/band. Forced degradation studies under acidic, alkaline, oxidative, photolytic, and thermal conditions demonstrated effective separation of the drug from its degradation products, confirming the stability-indicating nature of the method. The validated method is suitable for routine quality control and stability analysis of Vonoprazan Fumarate in pharmaceutical formulations.**

***Index Terms* —Vonoprazan Fumarate; HPTLC; Stability-indicating method; Method validation; Forced degradation; Pharmaceutical analysis.**

I. INTRODUCTION

Vonoprazan fumarate is a novel potassium-competitive acid blocker (PCAB) used in the treatment of stomach ulcers and gastroesophageal reflux disease (GERD). It is chemically 1-(5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl)-N-methylmethanamine fumarate (Fig 1). It is available as 10 mg and 20 mg tablets and was approved by the USFDA in 2023 and CDSCO in 2024. Compared to proton pump inhibitors (PPIs), vonoprazan provides faster, stronger, and more sustained gastric acid suppression, thereby promoting ulcer healing and relieving symptoms such as heartburn and epigastric pain. Long-term PPI therapy has been associated with adverse effects including *Clostridium difficile*-associated infections and osteoporosis-related fractures, making PCABs a promising alternative.

Vonoprazan, a pyrrole derivative, competitively inhibits K^+ binding to the H^+/K^+-ATPase enzyme in gastric parietal cells, suppressing both active and resting proton pumps. It is acid-stable, rapidly absorbed under fasting and fed conditions (Tmax 1.5–2 h), exhibits a half-life of approximately 7.7 h, and accumulates in the canalicular region due to its high pKa (>9). Dose adjustment is generally not required in renal or hepatic impairment, although pharmacokinetic variability may occur based on CYP2C19 genotype, age, sex, and dose. Dietary triggers such as spicy foods, alcohol, and caffeine are advised to be avoided during therapy.

Analytical method validation ensures reliable and reproducible results within a defined range[1–6]. According to ICH Q2 (R1), essential validation parameters include specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness[7]. Stability studies as per ICH Q1A (R2) involve stress testing under hydrolytic, oxidative, photolytic, and thermal conditions to assess degradation behavior and stability-indicating capability[8]. High-Performance Thin Layer Chromatography (HPTLC) is a cost-effective

analytical technique offering high throughput, minimal sample preparation, solvent flexibility, and enhanced sensitivity for qualitative and quantitative analysis[9–10]. Previously reported methods include stability-indicating HPLC[11]. Chromatographic assays using HPLC-DAD and HPTLC with green evaluation[12], RP-HPLC for combination analysis[13], and ecofriendly RP-HPLC methods for multi-drug estimation[14].

II. MATERIALS AND INSTRUMENTS

The vonoprazan fumarate tablets (Vault-10) were brought from Zydus Healthcare Limited. The study was conducted using a CAMAG HPTLC system from Muttens, Switzerland, which included a Camag Linomat V automatic sample applicator, Hamilton syringe (100 µL), Camag HPTLC scanner-3, Camang Vision CATS software, Camag twin trough Chamber of two dimensions 10 x 10 cm, 20 x 10 cm and ultrasonicator and UV cabinet. Precoated silica gel aluminium plate 60 F_{254} (20 cm x 10 cm with 0.2 mm thickness) was the type of HPTLC plate that was employed (E. Merck, Mumbai, India).

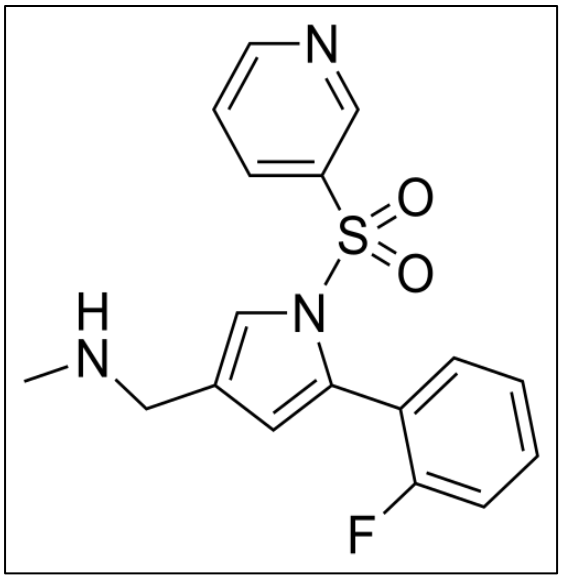

Fig.1 – Chemical Structure of Vonoprazan.

Experimental Conditions

For the development of the HPTLC method, the experimental parameters were carefully adjusted to ensure effective separation of Vonoprazan Fumarate. The choice of solvent was based on the drug's solubility in different solvents. It was found to dissolve in methanol and dimethyl sulfoxide, but not in ethyl acetate, ethyl ether, acetonitrile, propanol, butanol, or ethanol.

Therefore, methanol was selected for preparing and diluting the stock solution. Silica gel 60 F_{254} aluminium plates with a thickness of 200 µm, particle size of 10 – 12 µm, and pore size of 60 Å were chosen as the stationary phase for UV detection at around 254 nm. A 10 µg/mL solution of the drug was analyzed between 200 to 400 nm to identify the suitable wavelength for analysis.

Different combinations of mobile phases were tested using solvents such as water, methanol, ethyl acetate, ethyl ether, propanol, butanol, and ethanol. The system containing propanol, water, and methanol produced consistent peak characteristics. The ratio of these three solvents was further fine-tuned to achieve sharp and well-separated peaks.

TLC chambers of sizes 10 × 10 cm and 20 × 10 cm were tested with saturation times of 10, 20, and 30 minutes. The optimal saturation time was selected based on improved chromatographic performance. Finally, the distance of the solvent front was examined between 7 – 8 cm, and the best distance was chosen to provide proper separation with an acceptable Rf value for Vonoprazan Fumarate.

Preparation Of Standard Solutions

Preparation of standard stock solution: A precise amount of 10 mg of the drug Vonoprazan Fumarate was weighed and dissolved in methanol to make a total volume of 10 ml, resulting in a stock solution concentration of 1000 µg/ml.

Preparation of standard working solution: The stock solution was diluted to achieve a concentration of 100 µg/ml. This dilution was performed using methanol as the solvent.

Preparation of sample solution in the drug matrix (Formulation):

Twenty individual tablets of Vonoprazan Fumarate were accurately weighed, and the average weight was determined. A quantity of tablet powder equivalent to 10 mg of Vonoprazan Fumarate was weighed and transferred to a 10 ml volumetric flask. Methanol was added to the tablet powder, and the drug was extracted by sonication for 5 minutes. The final volume was then brought to 10 ml. The formulation solution was filtered twice using Whatman filter paper. The initial 2 ml of filtrate was

discarded, and the remaining solution was used for the assay analysis of the Vonoprazan Fumarate tablet formulation. The filtrate was further diluted with methanol to obtain a concentration of 100 µg/ml. The prepared solution was analyzed following the established analytical procedure.

Method Validation

The developed HPTLC method was validated in accordance with ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and system suitability.

Specificity: Method specificity was confirmed by peak purity analysis. The purity of Vonoprazan Fumarate was evaluated by comparing UV spectra at peak start (s), peak apex (m), and peak end (e) positions. Since Vonoprazan Fumarate is a salt of fumaric acid, pure fumaric acid was analyzed under identical chromatographic conditions to verify the absence of interference at the drug's retention factor.

Linearity: Linearity was assessed by spotting working standard solutions in the range of 200–1600 ng/band (2–16 µL). The developed plates were analyzed using a CAMAG HPTLC system, and peak area was plotted against concentration. The calibration curve showed a linear response, and regression analysis was performed to obtain the equation and correlation coefficient.

Accuracy: Accuracy was evaluated by recovery studies at three concentration levels (50%, 100%, and 150%), performed in triplicate. Known amounts of standard drug were added to the pre-analyzed formulation, and percentage recovery was calculated by comparing the measured and added amounts.

Precision: Precision was determined as intraday and interday precision at a concentration of 400 ng/band (4 µL of 100 µg/mL solution). Intraday precision was assessed at three different time intervals on the same day, while interday precision was evaluated over three consecutive days, with six determinations each. Results were expressed as %RSD.

LOD and LOQ: LOD and LOQ were calculated using the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the intercept and S is the slope of the calibration curve.

System Suitability: System suitability was evaluated by analyzing the standard solution six times. Parameters such as retardation factor, resolution, and tailing factor were calculated and found to be within acceptable limits.

Stability Studies for Vonoprazan Fumarate.

Stability studies were conducted in accordance with ICH guidelines to evaluate the stability-indicating capability of the developed method. Vonoprazan Fumarate was subjected to acidic, alkaline, oxidative, photolytic, and thermal stress conditions.

Acidic degradation was performed by dissolving 10 mg of the drug in methanol and treating with 0.1 N, 0.5 N, and 1 N HCl, with exposure times of 0, 2, and 4 h, followed by neutralization with sodium hydroxide and dilution with methanol to obtain 100 µg/mL solutions.

Alkaline degradation was carried out similarly using 0.1 N, 0.5 N, and 1 N NaOH, followed by neutralization with hydrochloric acid and dilution to 100 µg/mL.

Oxidative degradation was assessed using 10%, 20%, and 30% hydrogen peroxide with exposure times of 0, 2, and 4 h, after which solutions were diluted to 100 µg/mL.

Photolytic degradation was studied by exposing methanolic drug solutions to light (60 W) for 4, 8, and 24 hr.

Thermal degradation was evaluated by exposing the drug solutions to 25°C and 45°C for 0, 2, and 4 h. After stress treatment, all samples were diluted to 100 µg/mL with methanol and analyzed chromatographically.

III. RESULTS AND DISCUSSION

Vonoprazan Fumarate exhibited complete solubility in methanol, forming a clear solution with no visible residue; hence methanol was selected as the working solvent throughout the study.

Method development and validation were performed using activated Merck TLC silica gel 60 F254 plates (10×10 cm and 20×10 cm). The drug showed maximum absorbance (λ_{max}) at 262 nm (Fig 2),

which was optimized as the detection wavelength due to its well-resolved peak shape and high sensitivity.

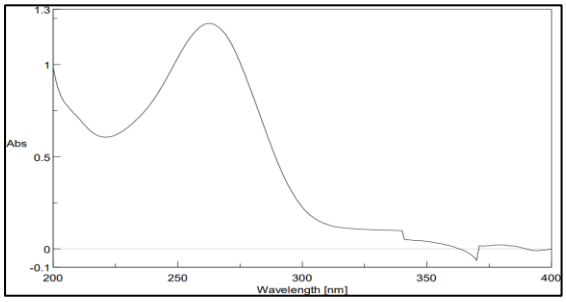

Fig.2 – UV Spectra of Vonoprazan Fumarate.

Several solvent systems were evaluated and the mobile phase comprising Propanol: Water: Methanol (7:2:1, v/v/v) was finalized as it produced sharp and symmetric drug peaks with good resolution (>2). A chamber saturation time of 30 minutes and a solvent front migration distance of 8 cm with band width of 5.4 mm and migration time of 75 min by using deuterium lamp were optimized for best chromatographic performance. The method demonstrated excellent specificity, as indicated by peak purity values of r(s,m)=0.999 and r(m,e)=0.999 with no interference from fumaric acid, confirming the absence of co-eluting impurities.

Linearity was observed in the range of 200–1600 ng/band with an Rf value of 0.510±0.05 (Fig 3 & 4, Table 1) and strong correlation in the calibration plot.

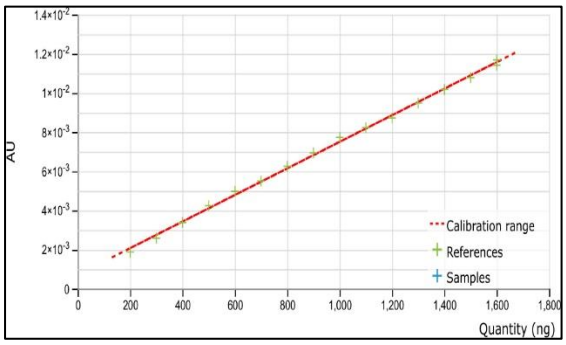

Fig.3 – Calibration curve of Vonoprazan (200-1600 ng/band).

Concentration (ng/band)	Peak area Vonoprazan
200	0.00120
300	0.00188
400	0.00250
500	0.00315
600	0.00373
700	0.00426
800	0.00480
900	0.00537
1000	0.00573
1100	0.00616
1200	0.00654
1300	0.00721
1400	0.00779
1500	0.00830
1600	0.00908

Table 1- Linearity range of Vonoprazan.

The standard densitogram obtained at different concentration.

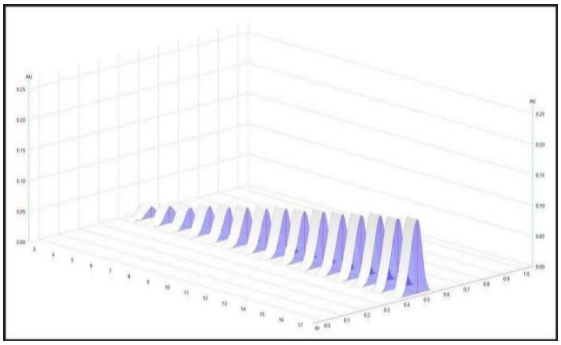

Fig.4 – 3D Densitogram of Vonoprazan calibration standards on precoated TLC plate.

The accuracy studies at 50%, 100% and 150% levels showed acceptable % recovery and low %RSD, in three different intervals and confirming the reliability of the method which is shown (Table 2).

Level (%)	Concentration of standard added (ng/band)	% Recovery	% RSD*
50	200	103.33	1.69
100	400	95.8	0.94
150	600	97.05	1.30

Table 2- Accuracy studies data of Vonoprazan.

Precision results demonstrated low variability for both intraday and interday analyses at 400 ng/band.

For intraday precision, at a concentration of 400 ng/band, the peak area values were 0.00243,

0.00248, and 0.00245. The corresponding percentage relative standard deviation (%RSD) values were 1.01, 0.99, and 1.00, indicating good precision and repeatability of the analytical method at this concentration level.

For interday precision, at a concentration of 400 ng/band, the peak area values were 0.00247, 0.00254, and 0.00253. The corresponding %RSD values were 1.53, 0.49, and 1.43, respectively. These results indicate acceptable precision of the method at this concentration level.

The LOD and LOQ were determined as 28.49 ng/band and 86.36 ng/band, respectively, indicating adequate sensitivity.

System suitability parameters including resolution and tailing factor complied with pharmacopeial limits, ensuring robust method performance (Table 3).

Compound	%RSD* of peak area	Resolution	Tailing factor
Vonoprazan	0.93	7.1	1.49

Table 3-System suitability studies data.

Application of the validated method to marketed tablets showed accurate drug quantification with no significant excipient interference (Fig 5 & Table 4).

Drug	Amount of drug formulation		% Purity	% RSD
	Labelled (mg)	Estimated (mg)		
Vonoprazan	10	9.84	98.4	0.4

Table 4- Result for Formulation analysis.

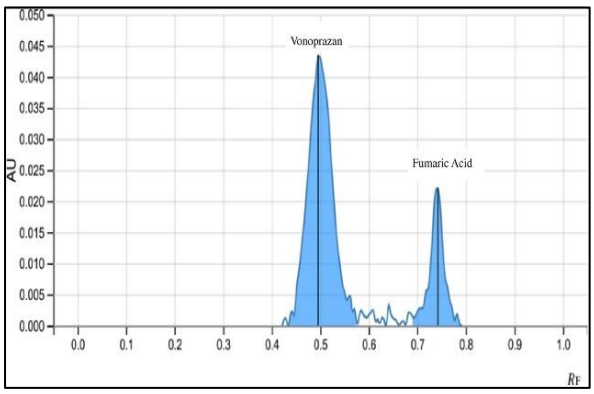

Fig.5 – Densitogram of 10mg Vonoprazan Fumarate formulation.

The assay of Vonoprazan tablet formulation with a labelled claim of 10 mg showed an estimated drug content of 9.84 mg, corresponding to a percentage purity of 98.4%. The %RSD value of 0.4 indicates good precision and reproducibility of the method.

Forced degradation studies revealed that Vonoprazan Fumarate was stable under acidic (Fig 6 & 7), oxidative (Fig 10), photolytic (60 W) (Fig 11) and thermal (45°C) (Fig 12) conditions for the tested period, with degradation limited to 15.34%, 8.5%, 7.94% and 6.67% respectively; however, the drug showed significant degradation (25.42%) under basic conditions(Fig 8 & 9), indicating sensitivity toward alkaline hydrolysis.

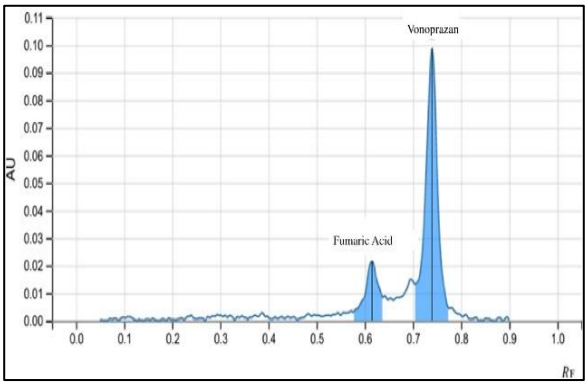

Fig.6 – Densitogram of Vonoprazan Fumarate in acidic condition at 4 hours.

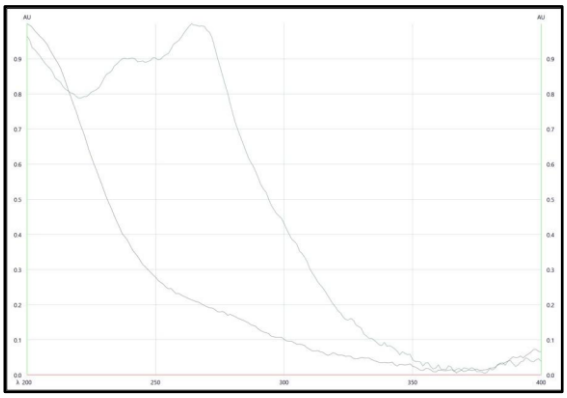

Fig.7 – Spectral match of Vonoprazan and Fumaric acid at Rf value of 0.740 and 0.611 at 4 hours.

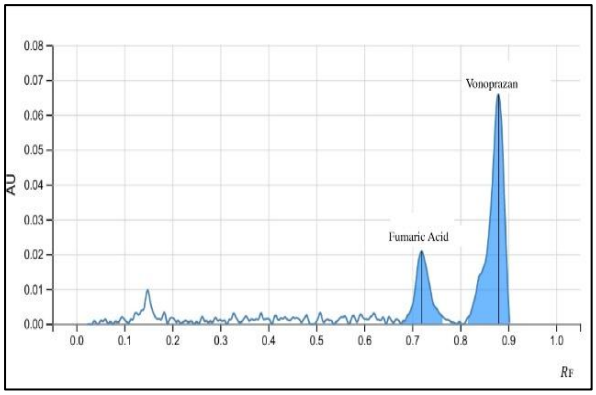

Fig.8 – Densitogram of Vonoprazan Fumarate in basic condition at 4 hours.

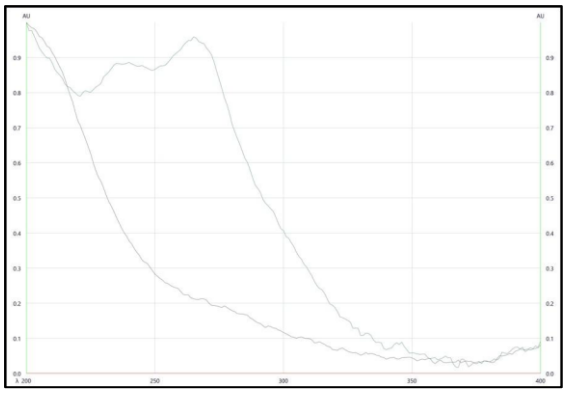

Fig.9 – Spectral match of Vonoprazan and Fumaric acid at Rf value of 0.880 and 0.720 at 4 hours.

The Rf value in acid hydrolysis was found at 0.74 for Vonoprazan & in alkali condition at 0.88 for which was confirmed by the spectral scan of the drug peak of Vonoprazan for its Rf shift.

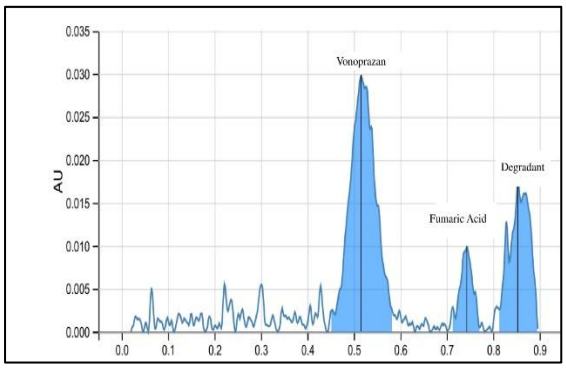

Fig.10 – Densitogram of Vonoprazan Fumarate in oxidative condition at 4 hours.

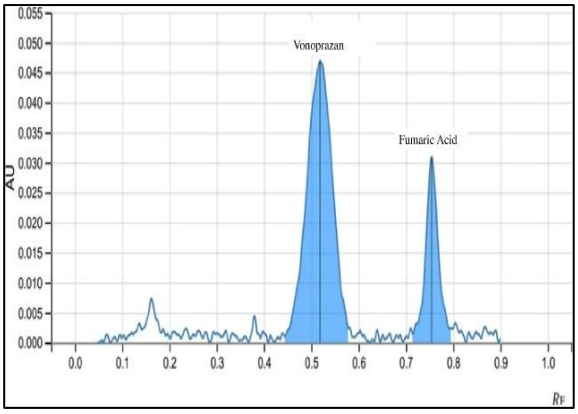

Fig.11 – Densitogram of Vonoprazan Fumarate in photolytic condition at 24 hours.

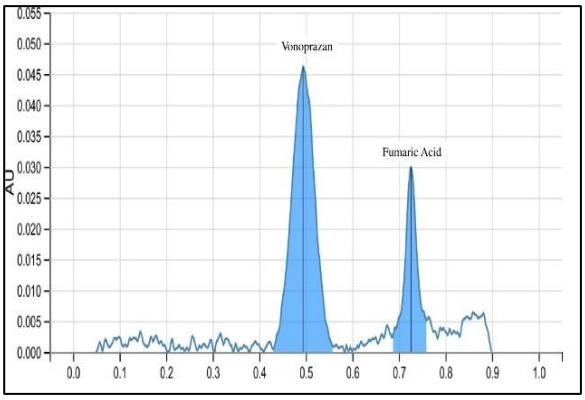

Fig.12 – Densitogram of Vonoprazan Fumarate in thermal condition at 4 hours.

Overall, the developed HPTLC method proved to be specific, precise, accurate, and stability-indicating for routine estimation of Vonoprazan Fumarate in pharmaceutical formulations.

DISCUSSION

A simple, precise and accurate HPTLC method was developed for the estimation of Vonoprazan Fumarate in tablet dosage form. Separation was achieved on a pre-coated silica gel 60 F254 aluminum plate using a mobile phase of propanol: water: methanol (7:2:1, v/v/v) with detection at 262nm. The retention factor values for Vonoprazan and Fumaric acid were 0.510 ±0.05 and 0.745 ± 0.03, respectively and it was validated according to ICH guidelines. Linearity was established over 200 – 1600ng/band for Vonoprazan with correlation coefficients of 0.9990. Recovery ranged from 95.8–103.33%, confirming accuracy and precision (%RSD) was below 2%. LOD and LOQ were

28.49ng/band and 86.36ng/band for Vonoprazan. The method successfully quantified drug in marketed formulations Vault® 10 (tablets) with assay results within acceptable limits. Forced degradation studies demonstrated the stability-indicating nature of the method, showing degradation under acid hydrolysis, base hydrolysis, oxidation, photolysis and thermal degradation, with degradation ranging from 6.5% to 25.5%. The drug was stable till 4 hours under acid hydrolysis, oxidation, photolysis and thermal degradation and where as in base hydrolysis it was found to be unstable.

IV. CONCLUSION

A simple, precise, accurate, and robust HPTLC method was successfully developed and validated for the quantitative estimation of Vonoprazan Fumarate in tablet dosage form. The method complied with ICH Q2(R1) validation parameters, demonstrating excellent linearity, recovery, and precision. The LOD and LOQ values confirmed the sensitivity of the method, while the assay results of marketed formulations were within acceptable limits, indicating its applicability for routine quality control analysis. Additionally, the forced degradation studies confirmed that the proposed method is stability-indicating, capable of effectively separating the drug from its degradation products under various stress conditions. Therefore, this developed HPTLC method is reliable for the routine analysis and stability testing of Vonoprazan Fumarate in pharmaceutical formulations.

Conflict of interest: It is hereby declared that there is no conflict of interest among authors. Acknowledgement: The authors are grateful to The SNR Sons Charitable Trust, Coimbatore for providing the necessary facilities to carry out the research work.

REFERENCES

[1] https://www.Drugs.com. Vonoprazan Uses, Side Effects & Warnings [Internet]. Drugs.com. 2024. Available from: https://www.drugs.com/mtm/vonoprazan.html

[2] Hori Y, Imanishi A, Matsukawa J, Tsukimi Y, Nishida H, Arikawa Y, et al. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamine Monofumarate (TAK-438), a Novel and Potent Potassium-Competitive Acid Blocker for the Treatment of Acid-Related Diseases. Journal of Pharmacology and Experimental Therapeutics. 2010 Jul 12;335(1):231–8.

[3] PubChem. Vonoprazan Fumarate [Internet]. Nih.gov. PubChem; 2025 [cited 2025 Dec 8]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Vonoprazan-Fumarate

[4] Voquezna (vonoprazan) FDA Approval History [Internet]. Drugs.com. Available from: https://www.drugs.com/history/voquezna.html

[5] 1mg.com. Vault 10mg Tablet [Internet]. 1mg. 2025 [cited 2025 Dec 8]. Available from: https://www.1mg.com/drugs/vault-10mg-tablet-1017269

[6] Center for Drug Evaluation and Research Application Number: 218710orig1s000 proprietary name review(s) [Internet]. [cited 2025 Dec 9]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2025/218710Orig1s000NameR.pdf

[7] European Medicines Agency. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology Step 5 Note for Guidance on Validation of Analytical Procedures: Text and Methodology [Internet]. 1995. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r1-validation-analytical-procedures-text-methodology-step-5-first-version_en.pdf.

[8] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. Journal of Pharmaceutical Analysis [Internet]. 2014 Jun;4(3):159–65. Available from: https://www.sciencedirect.com/science/article/pii/S2095177913001007.

[9] B. Ramu, Kishore Babu Chittela. High Performance Thin Layer Chromatography and Its Role Pharmaceutical Industry: Review [Internet]. 2018 [cited 2025 Dec 8]. Available from:https://api.semanticscholar.org/CorpusID:139983134.

[10] HPTLC method development and validation: Strategy to minimize HPTLC method development and validation: Strategy to minimize methodological failures methodological failures. Available from: https://www.jfda-

online.com/cgi/viewcontent.cgi?article=2038&context=journal.

[11] 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. Journal of Pharmaceutical and Biomedical Analysis. 2018 Feb 5;149:133-42.

[12] 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 Nov 16;18(1):230.

[13] P. Kanaga, Analytical Method Development And Validation Of RP – HPLC For The Simultaneous Estimation Of Vonoprazan And Domperidone In Pharmaceutical Dosage Form, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 9, 323-331.

[14] Ali NA, El-Gindy AE, Wahba ME, Mostafaa AE. Ecofriendly chromatographic method for the separation and quantification of vonoprazan fumarate, a novel potassium competitive acid blocker with amoxicillin and clarithromycin which are effective in the treatment of Helicobacter pylori. Acta Chromatographica. 2025 Jan 30.