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Green Assessment-Based Stability-Indicating Rp-Hplc Method Development And Validation For The Determination Of Vonoprazan Fumarate In Bulk And Formulations

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ABSTRACT
A rapid and reliable Green Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the estimation of Vonoprazan Fumarate in pharmaceutical formulations. The method utilized a BDS Hypersil C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of water (pH 3.0) and acetonitrile (95:5, v/v) under isocratic conditions. Detection was performed at 213 nm using a PDA detector, with a flow rate of 0.8 mL/min and a retention time of 3.9 minutes. Linearity was established in the 10–60 µg/mL range, with a recovery of 98.90%. The method demonstrated stability-indicating properties through forced degradation studies. Greenness was assessed using AGREE (0.86), AGREEprep (0.77), and BAGI (75), confirming its environmental and industrial feasibility. The developed RP-HPLC method, validated according to ICH guidelines, is suitable for analyzing Vonoprazan Fumarate in pharmaceutical formulations. Forced degradation studies confirmed its stability-indicating capability. This study highlights the method's balance between efficiency and environmental responsibility.

KEY WORDS: RP-HPLC, Vonoprazan Fumarate, Stability-indicating assay, Validation, Forced Degradation, Greenness evaluation.

INTRODUCTION

Vonoprazan Fumarate is an oral medication that acts as a potassium-competitive acid blocker. It prevents potassium ions from attaching to the proton pump in the stomach, which is responsible for the final step of producing gastric acid in parietal cells. This drug was designed to help with conditions like Gastroesophageal Reflux Disease (GERD), peptic ulcers, and other issues related to excess acid. Compared to the conventional proton pump inhibitors, Vonoprazan Fumarate is more effective at suppressing gastric acid for a longer duration. It also tends to be safer, making it a good choice for those who need extended acid reduction. Currently, Vonoprazan Fumarate is not officially recognized in any standard pharmacopoeia. This highlights the need for a clear and efficient HPLC method to test its safety and quality. RP-HPLC is chosen for this purpose because it is very sensitive and can handle complex mixtures well. It provides accurate, reliable results and is accepted by regulatory agencies, ensuring that pharmaceutical products remain safe and effective over time. As per literature survey researchers utilize various analytical techniques to study the combination of Vonoprazan and Aspirin (1), Techniques like ultraviolet spectrophotometry and fluorimetry are straightforward and useful for measuring quantities, but face challenges in identifying other substances and impurity that might interfere (2). On the other hand, liquid chromatography with mass spectrometry (LC-MS) the method primarily focuses on impurity profiling and does not involve stability testing under various environmental conditions (e.g., temperature, humidity, light exposure). Its main objective is to detect, impurities rather than assess the drug's behavior over time under stress conditions (3). Another method in which simulated gastric fluid (SGF) might introduce issue, like interference from other substances in the fluid, this could affect accuracy of result (4). In Previous studies research on stability of Vonoprazan Fumarate under physiological conditions remains limited, which is vital for assessing its prolonged efficacy and safety (5)(6). Bioanalytical methods using plasma are employed for studying the pharmacokinetics and dynamics of a drug which have limited applicability for Stability Studies (7).

To ensure that the method works well, thorough testing in various environments may be necessary, which constitutes a stability study. High-performance liquid chromatography (HPLC) is another method used to check for related substances

in Vonoprazan Fumarate. It uses a simpler and more stable approach called isocratic elution, in contrast to gradient elution. Each of these methods has its own level of complexity, sensitivity, and cost, giving scientists different options for analyzing drugs. Research shows that other testing methods can be quite expensive, as they require special equipment, costly solvents and specific reagents, along with a lot of upkeep. In contrast, the method is designed to be cost-effective while adhering to the ICH Q1A(R2) guidelines on stability testing, which emphasize the need of stability-indicating methods for evaluating drug stability. The degradation behavior of the drug is a critical aspect of this evaluation. The drug is subjected to various stress conditions, to simulate the effects of prolonged storage. These stress tests allow for the identification of degradation products and ensure that the analytical methods are capable of detecting both the active pharmaceutical ingredient and its byproducts. This approach enables the assessment of the drug's stability, determination of its shelf life, and confirmation of its safety and efficacy throughout storage and use. By optimizing conditions and utilizing cost effective solvents and materials, expenses can be minimized while maintaining reliable and accurate results.

MATERIALS AND METHODS

Materials: Active Pharmaceuticals Ingredient- Vonoprazan Fumarate, Vonogress Tablet 20mg, HPLC grade Acetonitrile and methanol, distilled water, Orthophosphoric acid from Merk Laboratories Mumbai.
Instrumentation: JASCO Extrema lC system-4000 was used with PDA detector consisting quaternary pump for RP-HPLC development. chromatograms were recorded and analyzed through ChromNAV software. UV-Vis Spectrophotometer of Shimadzu UV-1800 UV Probe,

Chromatographic conditions

The analysis of Vonoprazan Fumarate was conducted at 250°C using a BDS Hypersil C18 column (250 x 4.6 mm, 5 µm particle size). The mobile phase composition was maintained constant throughout the analysis. Detection was performed at a wavelength of 213 nm, and the flow rate was set to 0.8 mL/min, with an injection volume of 10 µL. Under these chromatographic conditions, retention time of Vonoprazan Fumarate is 3.9 minutes The total run time was 10 minutes.

Preparation of standard solutions

A precise quantity of 100 mg of Vonoprazan Fumarate was weighed and transferred into a 100 mL volumetric flask. It was dissolved in a solvent mixture of acetonitrile and water in an 80:20 (v/v) ratio, resulting in a standard stock solution with a concentration of 1000 µg/mL. Subsequently, 10 mL of this standard stock solution was diluted using diluent into a 100 mL volumetric flask, resulting in a sub-stock solution with a final concentration of 100 µg/mL.

Preparation of sample solution

Ten tablets were weighed and finely ground into a homogeneous powder. A specific quantity of this powder corresponding to the mass of one tablet, approximately 153 mg based on a concentration of 20 mg per tablet, was accurately measured. This powder was transferred into a 100 mL volumetric flask, and 100 mL of diluted with diluent. The mixture was sonicated for 20 minutes to ensure complete dissolution. The flask was subsequently filled to the calibration mark with the same solvent to prepare the first stock solution. After thorough mixing, the solution was filtered through Whatman No. 1 filter paper. To prepare a solution with a concentration of 100 µg/mL, 1 mL of this stock solution was diluted with diluent to obtain concentration of 10 µg/mL.

Selection of mobile phase

In the pursuit of optimization for achieving a clear and uniform chromatographic peak for the drug. A series of tests were conducted to identify the most suitable mobile phase for the RP-HPLC method. During the selection process for the mobile phase, various parameters were scrutinized, including peak symmetry, peak tailing, peak height, and the number of theoretical plates. These factors were essential in assessing the efficacy of different mobile phases, prompting the execution of multiple trials to ascertain the optimal solution. The results of these evaluations are detailed in Table No.1.

Sr. No.	Mobile Phase Composition	Ratio (v/v)	Remark
1	Methanol: Water	90:10	Peak tailing was observed
2	Methanol: Water	80:20	Peak fronting
3	ACN: Water	10:90	Peak was not resolved
4	ACN: Water	05:95	Peak was symmetric

Table No.1 mobile phase compositions along with their respective ratios and the observed remarks.

Selection of wavelength

The standard solution of Vonoprazan Fumarate of 10 µg/mL was scanned under the UV spectrophotometer between the range of 200nm to 400nm against Water: Acetonitrile (20:80) as a blank solution to determine λ_{max}. For selecting the λ_{max}, standard solution of Vonoprazan Fumarate was scanned in spectrum mode.

Preparation of mobile phase:
The mobile phase composed of water adjusted to pH 3.0 using 0.1% orthophosphoric acid (OPA), and acetonitrile in a 95:5 (v/v) ratio. The diluent (solvent) used consisted a mixture of water and acetonitrile in a 20:80 (v/v) ratio.

Forced degradation studies
Forced degradation studies were conducted per ICH Q1A(R2) guidelines to assess method robustness and stability. (8) The drug was subjected to acidic, alkaline, oxidative, thermal, and photolytic stress conditions. Acidic and alkaline degradation involved treatment with 0.1N HCl and 0.1N NaOH, respectively, followed by neutralization after 24 hours. Oxidative degradation was induced using 30% H_2O_2 for 2 hours. Thermal and photolytic degradation were assessed by exposing the API to 60°C and UV light for 2 hours, respectively. The drug showed greater degradation under alkaline conditions, confirming the method's stability-indicating capability.

METHOD VALIDATION
System suitability
Six distinct of Vonoprazan Fumarate were used to evaluate the technique's applicability. The retention time, peak area, column efficiency, peak symmetry, and theoretical plates were calculated for standard solutions.
Specificity
To confirm that diluent do not interfere with the drug peak, both the standard solution and the sample solution were prepared according to the established method and subsequently analysed.
Linearity
A precisely weighed amount of 10 mg of the drug was transferred into a 10 mL volumetric flask and diluted with solvent to the calibration mark, yielding a concentration of 1000 µg/mL. To assess the drug's linear response, concentrations ranging from 10 to 60 µg/mL were prepared from this stock solution. A calibration curve was constructed by plotting the peak area against the corresponding concentration. The linearity of the response was evaluated, and the correlation coefficient (r^2) and y-intercept were determined from the resulting curve.
Accuracy
The accuracy of the method was determined by spiking with a known amount of drug to result in sample solutions with the following levels of drug 80%, 100% and 120% relative to the working concentration in triplicate according to the method of analysis and analyzed as per the method. The % recovery was calculated.
Precision
A standard solution of 30 µg/ml was prepared and injected 6 times into the system. A chromatogram was recorded and the %RSD of the Peak Area was calculated.
Intermediate precision
The intermediate precision was calculated by measuring the responses of a standard peak on another day of the same solution concentration.
Robustness
The stability of the method was assessed by making small adjustments to parameters such as flow rate, temperature, and wavelength. Through systematic variation of these conditions, the method's reliability and consistency were evaluated to ensure its robustness and accuracy across different settings.
LOD & LOQ
Six sets of linearity concentrations were analysed and LOD & LOQ were calculated using the following equations as per ICH guidelines, based on the response and slope of a regression equation.
Assay
In accordance with ICH guidelines, the assay method must be capable of quantifying the active pharmaceutical ingredient in the presence of excipients, impurities, and degradation products without interference. The assay was performed on tablet formulations. The percentage of assay for the drug content was determined. The results demonstrate that the method is both accurate and specific, enabling precise measurement of the active ingredient in the presence of excipients in the tablet matrix (9).

GREEN ASSESSMENT
The environmental sustainability of the developed RP-HPLC method was assessed using multiple evaluation frameworks, including Analytical Greenness (AGREE), AGREEprep, and the Blue Applicability Grade Index (BAGI). AGREE provided a comprehensive assessment of the method's compliance with green analytical chemistry principles, considering factors such as solvent usage, energy consumption, and waste generation. AGREEprep further optimized sample preparation sustainability, ensuring minimal environmental impact. BAGI evaluated the industrial feasibility and broader applicability of the method in routine analysis.(10)

RESULT

Wavelength of detection

The UV spectrum of Vonoprazan Fumarate (10µg/ml) was scanned between 200-400 nm region on a UV spectrophotometer. The optimal peak response and maximum absorption was obtained when using 213 nm as a wavelength for detection considered as λ_{max} for Vonoprazan Fumarate. Represent in Figure No.1.

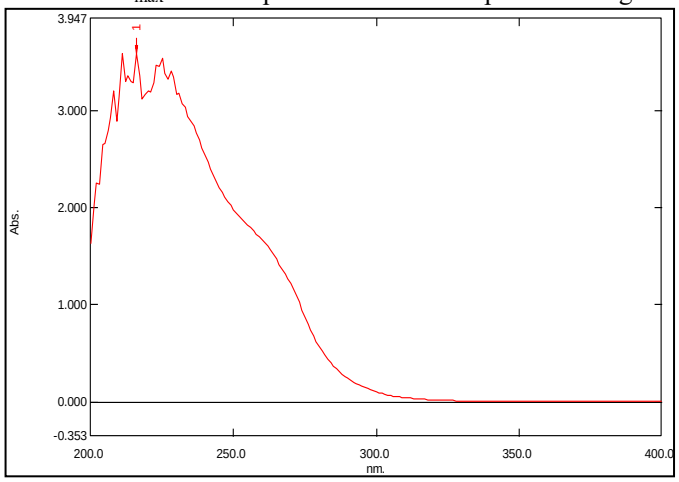

Figure 1. λ max of Vonoprazan Fumarate (213nm)

System suitability parameters

To ensure the reliability of the HPLC system, system suitability tests were conducted as part of the method validation process. For each validation parameter, six duplicate injections of a standard solution at a concentration of 30 µg/mL were performed. The system suitability criteria yielded several important results: the retention time was 3.9 minutes, indicating the duration required for the sample to pass through the chromatographic system. The theoretical plates value was determined to be 15,680, demonstrating the column's efficiency in separating the components. The method's linearity was confirmed to be within the concentration range of 10 to 60 µg/mL, indicating reliable results within this range. The detection limit (LOD) was found to be 5.33 µg/mL, representing the smallest detectable concentration, while the quantification limit (LOQ) was 16.16 µg/mL, marking the lowest concentration that can be quantified with reliability. Regression analysis of the calibration data yielded a y-intercept of 510,977, a slope of 12,683, and a correlation coefficient of 0.9989, confirming a strong linear relationship. Figure 2 illustrates the standard chromatogram data for Vonoprazan Fumarate of mobile phase ACN: Water 5:95 v/v.

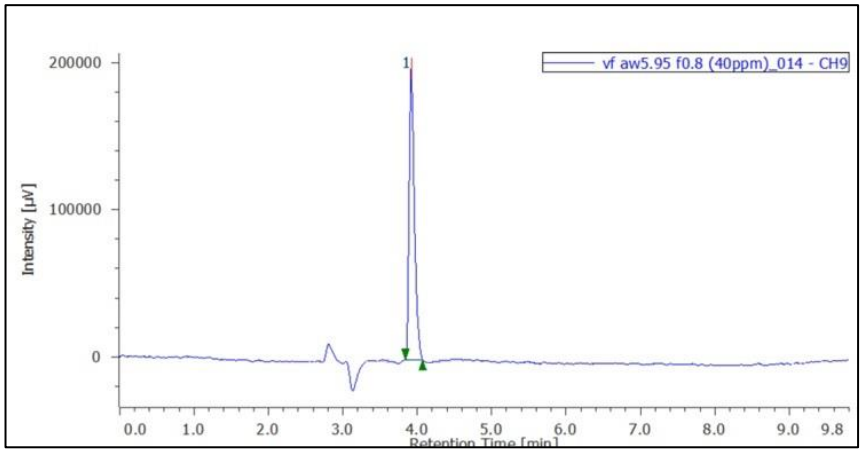

Figure 2. Standard chromatogram of Vonoprazan

Method Validation

Linearity

To evaluate the predictable behavior of Vonoprazan Fumarate, several diluted solutions were prepared from a standard stock, with concentrations ranging from 10 to 60 µg/mL. The resulting calibration data were used to derive the equation: y=12,683x+510,977 with an r^2 value of 0.9989, indicating a strong linear relationship. Figure No.3 presents the calibration curve for Vonoprazan Fumarate as obtained by HPLC.

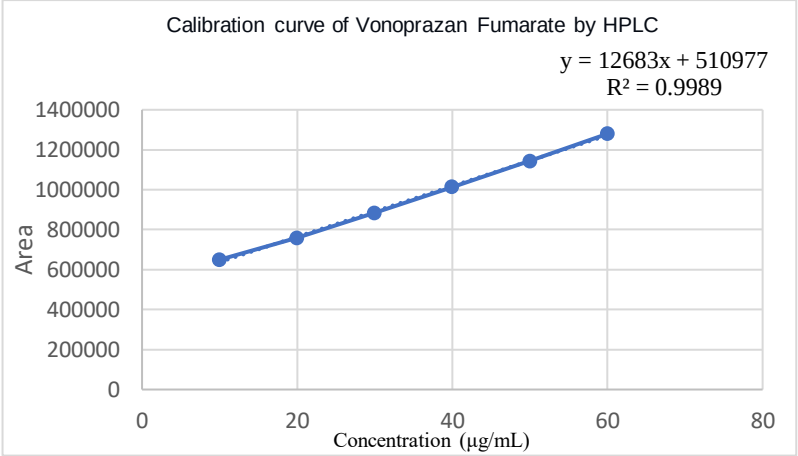

Figure 3: calibration curve of Vonoprazan Fumarate

Accuracy

In this method, the recovery of Vonoprazan Fumarate was evaluated by testing solutions at approximately 80%, 100%, and 120% of the target concentration. The percentage of recovery for each solution was determined, with results ranging from 98.0% to 102.0%, as summarized in Table No. 2.

Table No.2 Recovery Study Results for Drug Accuracy at Different Concentration Levels

Level (%)	Actual conc. (µg/mL)	calculated conc. (µg/mL)	Peak area	% recovery	mean concentration (µg/mL)	standard deviation	%RSD
80	32	32.3396	921140	100.70%	32.3902	0.09277	0.28642
		32.3338	921066				
		32.4973	923140				
100	40	39.4469	1011282	98.91%	39.6045	0.1365	0.34467
		39.6794	1014231				
		39.6871	1014328				
120	48	44.9447	1081011	101.83%	44.4621	0.34224	0.76973
		44.2525	1072231				
		44.1892	1071128				

Precision

To assess precision, the standard solution was injected six times. The results, expressed as the percentage relative standard deviation (%RSD) was found to be less than 2, are presented in Table No. 3 and 4.

Table No.3: Results of intraday precision

Sr no.	Sample name	Area	Theoretical plate	Tailing factor	Retention time
1	Test_1	881177	15650	1.4	3.92
2	Test_2	880361	15523	1.4	3.92
3	Test_3	879160	15389	1.4	3.92
4	Test_4	881703	15574	1.4	3.92
5	Test_5	879237	15680	1.4	3.92
6	Test_6	884021	15709	1.4	3.92
MEAN		880943.17	15587.50	1.40	3.92
SD		1818.50	119.14	0.0	0.00
%RSD		0.21	0.76	0.00	0.00

Table No 4: Results of interday precision.

Sr no.	Sample name	Area	Theoretical plate	Tailing factor	Retention time
1	Test_1	880358	15640	1.4	3.92
2	Test_2	880525	15413	1.4	3.92
3	Test_3	866546	15660	1.4	3.92
4	Test_4	883253	15438	1.4	3.9
5	Test_5	877224	15480	1.4	4.1
6	Test_6	866704	15632	1.4	3.92
MEAN		875768	15543.8	1.4	3.94
SD		7335.44	112.17	0.00	0.08
%RSD		0.84	0.72	0.00	1.91

Assay

Assay procedures are designed to quantify the amount of a specific substance present in a sample. In this study, the focus is on determining the concentration of the active ingredient in the drug substance. By comparing the concentration of the substance in the sample with the added known amount (spike), the % recovery can be calculated. This calculation provides an indication of the accuracy of the assay. The % recovery was found to be 98.90. Figure No.4 illustrates chromatogram of Vonoprazan Fumarate for Assay.

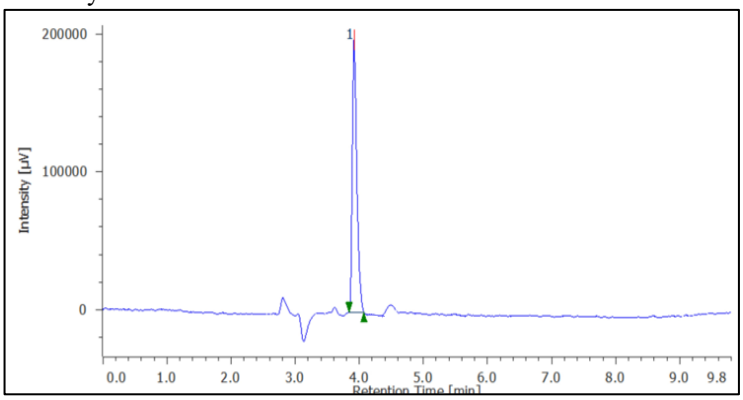

Figure 4. Assay of Vonoprazan Fumarate

Robustness

The robustness was determined by assessing the impact of small, yet significant, variations in specific analytical parameters that could influence selectivity or quantitative results. The following parameters have been changed one by one and their effect on system suitability test. Change in flowrate ±0.2 ml/min, change in temperature ±5°C and change in wavelength ±2nm. Percentage relative standard deviation (%RSD) was found to be less than 2. shown in Table No.5.

Table No.5. Robustness data of Vonoprazan Fumarate

SR.NO -			1	2	3	MEAN	SD	%RSD
FLOW RATE	1ml/min	AREA	661608	640608	649250	650489	10554.65	1.11
		RT	3.393	3.393	3.357	3	0.02	
		NTP	9554	9353	9504	9470	104.64	
	0.6ml/min	AREA	1193311	1156048	1196785	1182048	22583.56	1.21
		RT	5.637	5.66	5.633	6	0.01	
		NTP	18106	18213	17715	18011	262.15	
TEMPERATURE	20°C	AREA	1042290	1048560	1048560	1046470	3619.99	0.67
		RT	3.973	3.96	3.96	4	0.01	
		NTP	13511	13861	13861	13744	202.07	
	30°C	AREA	1081689	1076857	1081689	1080078	2789.76	1.19
		RT	3.883	3.9	3.8	4	0.05	
		NTP	6249	6013	6148	6137	118.41	
WAVELENTH	111nm	AREA	850018	853132	842709	848620	5350.35	

		RT	4.277	4.28	4.28	4	0.00	0.68
		NTP	12266	12601	12501	12456	171.97	
	115nm	AREA	852004	851990	866801	856932	8547.10	0.58
		RT	4.267	4.267	4.263	4	0.00	
		NTP	13035	12999	12861	12965	91.85	

FORCED DEGRADATION STUDY

The drug was subjected to stress factors including acidic, basic, thermal, photolytic, and oxidative conditions (9). For the forced degradation studies, 1 mL of the filtered stock solution was transferred to a 10 mL volumetric flask, and diluent was added to bring the volume to 10 mL. The drug exhibited increased degradation under alkaline conditions. For each stress condition, sample solutions were prepared, each with a concentration of 30 µg/mL, and subsequently exposed to the respective stress conditions. Table No.6 explains results of effecting. As it showed relatively no effect on the samples in the case of acid, heat, sunlight, and the oxidation process, while it was more affected by UV light, and a strong and significant effect occurred in the case of alkaline or base. In Figure No.5 – (A), (B), (C), (D) and (E) indicate photolytic, oxidative, alkaline, thermal, and acidic degradation, respectively.

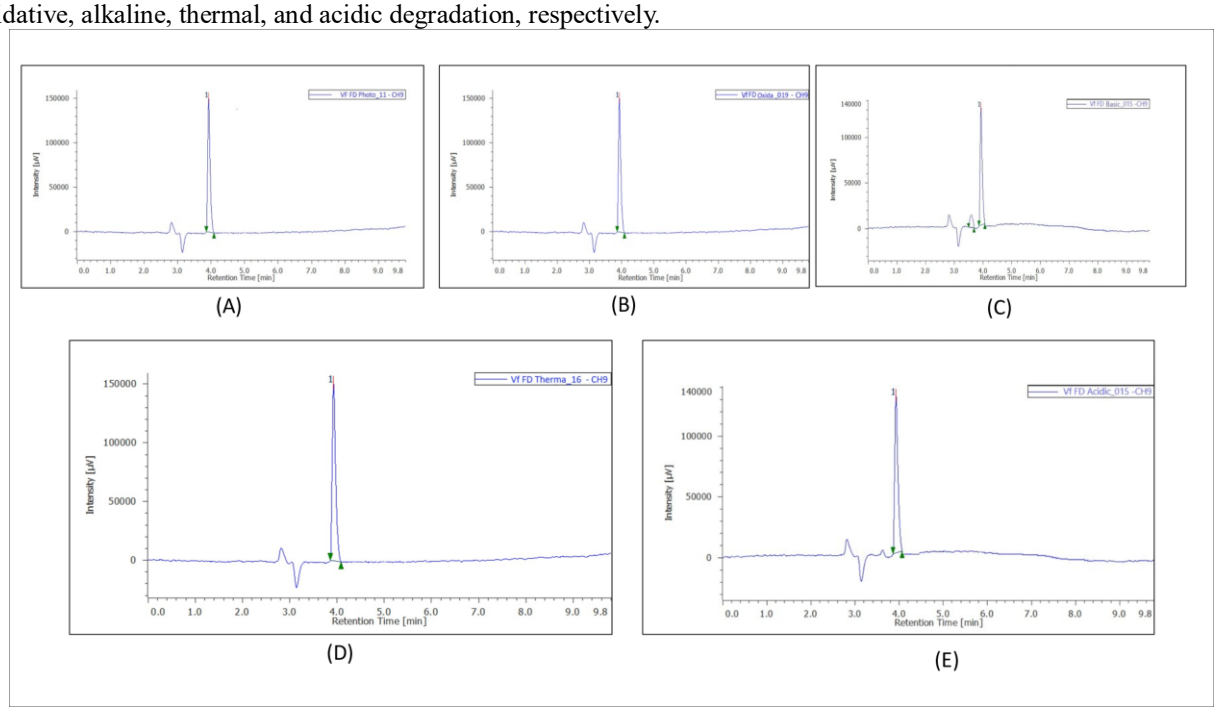

Figure 5. Forced degradation study of Vonoprazan Fumarate

Table No 6. Results of forced degradation.

Conditions	%Recovery	%Degradation
Acid (0.1N HCL)	90.20	9.8
Alkaline (0.1N NaOH)	70.25	29.75
Oxidative (30% H_2O_2)	98.36	1.64
Thermal (60ºC)	99.55	0.45
Photolytic (UV Chamber)	89.50	10.5

The method demonstrated moderate greenness with an AGREE score of 0.86, an AGREEprep score of 0.77, and a BAGI score of 75, indicating a balance between efficiency, sustainability, and practicality for pharmaceutical quality control.
AGREE: A flexible tool for evaluating analytical method sustainability based on the 12 principles of green analytical chemistry. It assigns a 0–1 scale score, integrating multiple criteria like reagent toxicity, waste, and energy use. Results are visualized via a pictogram for intuitive assessment**.** The HPLC method reviewed herein obtained an AGREE score of 0.86, hence indicating moderate level of greenness with strong adherence to green analytical chemistry principles. This score suggests that the method effectively minimizes environmental impact by optimizing solvent usage, energy consumption, and waste production while maintaining analytical efficiency (11) Shown in figure no.6

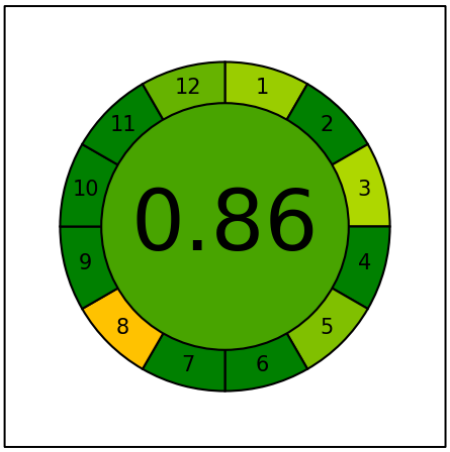

Figure 6. Pictogram of AGREE

BAGI: A practical index assessing analytical method applicability using ten criteria. A BAGI score of 75 highlights the strong industrial feasibility of the RP-HPLC method, confirming its suitability for large-scale pharmaceutical analysis. The predominance of blue color in the evaluation reflects key advantages, such as efficiency, reliability, and environmental consideration, making the method a practical choice for routine use. While some white areas suggest opportunities for further optimization, the overall score demonstrates a well-balanced approach that successfully integrates sustainability with industrial applicability.(12)

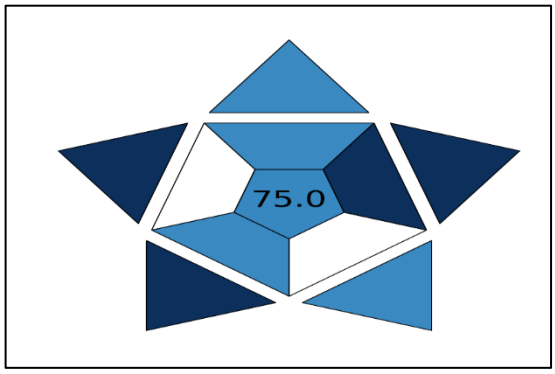

Figure 7. Pictogram of BAGI

AGREEprep: A metric prioritizing sample preparation sustainability, evaluating ten impact categories on a 0–1 scale. It assesses solvents, waste, energy, and sample size, generating a pictogram to highlight environmental concerns. Validated through case studies, it aids in green analytical method optimization (13). Score obtained is 0.77 which shows developed method is maintaining process efficacy and economic viability. The dark green to light green shades in the evaluation indicate strong compliance with sustainability goals, such as reduced solvent usage, minimal waste generation, and efficient resource management. These colours reflect the method's eco-friendliness and cost-effectiveness, making it a practical choice for routine analysis.

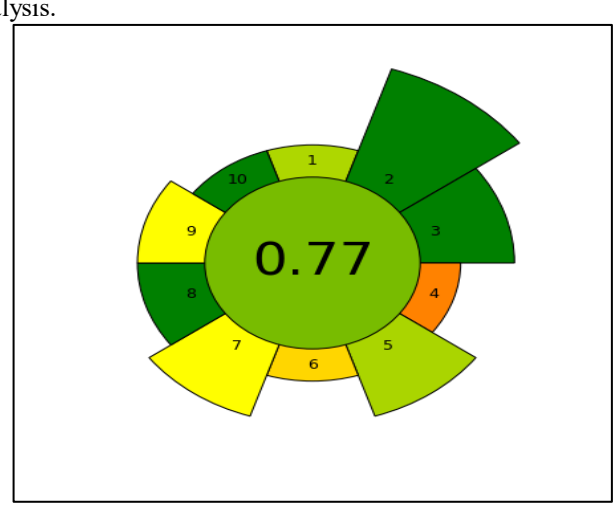

Figure No.8.Pictogram of AGREEprep

CONCLUSION

A new approach to estimating Vonoprazan Fumarate offers a broad range of concentrations and is quick, straightforward, and sensitive enough for low detection and quantification limits. Its accuracy, precision, and high resolution make it stand out. Additionally, with shorter retention time, it is a more convenient and cost-effective method, making it suitable for regular use in research labs, stability tests, quality control, and other testing facilities. The correlation coefficient (r^2) was determined to be 0.9989. The results of Accuracy showing the % recovery fall between 98.0% and 102.0%. Result of precision and Robustness: % RSD was less than 2. Additionally, when it comes to forced degradation, this method can effectively separate byproducts in most degradation processes involving the active substance, clearly distinguishing between the peaks. Drug shows 29.75% degradation in alkaline condition. This shows how well the technique works in identifying degradation or decomposition byproducts during stability tests under various temperature and humidity conditions. The method meets ICH standards for validating analytical procedures and has been successfully applied. The greenness evaluation confirmed the method's minimal environmental impact, reinforcing its suitability for routine laboratory use. These assessments establish the proposed method as a superior alternative, surpassing existing methods across multiple green analytical metrics. It effectively reduces environmental footprint, minimizes waste, and promotes sustainable resource utilization.

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ABBREVIATION

RP-HPLC: Reversed Phase High-performance liquid chromatography,
API: Active pharmaceutical ingredient,
GERD: Gastroesophageal Reflux Disease
LOQ: Limit of quantitation;
LOD: Limit of detection,
RSD: Relative standard deviation,
PDA: Photo Diode Array,
r^2: Coefficient of correlation,
ICH: International Council for Harmonisation,

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