RESEARCH PAPER

“ A Novel Stability-Indicating Rp-Hplc Method For Vonoprazan Fumarate: Method Development, Validation And Forced Degradation Studies ”

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ABSTRACT:
A simple, rapid, precise, accurate, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Vonoprazan Fumarate in pharmaceutical dosage form. Chromatographic separation was achieved using a C18 column (150 mm × 4.6 mm) with a mobile phase consisting of 10 mM ammonium acetate and methanol in the ratio of 40:60 at a flow rate of 1 mL/min. Detection was carried out at 254 nm using a diode-array detector, and Vonoprazan Fumarate showed a retention time of approximately 3.7 min. The method was validated according to ICH guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated linearity over the concentration range of 10–60 µg/mL with a correlation coefficient of 0.999. Recovery values at 80%, 100%, and 120% levels were found to be 99.590%, 99.820%, and 100.099%, respectively. Intraday and interday precision showed %RSD values of 0.0844 and 0.0548, respectively. The LOD and LOQ were found to be 0.10 µg/mL and 0.30 µg/mL, respectively. Forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions demonstrated that the method could effectively assess the stability of Vonoprazan Fumarate. The developed RP-HPLC method was therefore found to be suitable for routine analysis and stability assessment of Vonoprazan Fumarate in pharmaceutical dosage forms.

KEYWORDS: Vonoprazan Fumarate; RP-HPLC; Stability-indicating method; Method validation; Forced degradation; ICH guidelines; Pharmaceutical dosage form; Diode-array detector.
How to cite this article: Samruddhi Sonawane Archana Gorle RupePingale. “ A Novel Stability-Indicating Rp-Hplc Method For Vonoprazan Fumarate: Method Development, Validation And Forced Degradation Studies ”. Int J Drug Deliv Technol. 2026;16(79s):466-472. DOI: 10.25258/ijddt.16.79s.49.

INTRODUCTION:

Acid-related disorders remain a major public health concern because they are common, chronic, and often associated with substantial impairment in quality of life, recurrent symptoms, mucosal injury, and the need for long-term pharmacotherapy. Gastroesophageal reflux disease (GERD), peptic ulcer disease (PUD), NSAID-associated ulceration, and Helicobacter pylori infection continue to account for a large proportion of upper gastrointestinal morbidity and healthcare use. In clinical practice, acid suppression is central to symptom control, mucosal healing, ulcer prevention, and eradication therapy, which makes the search for more effective and more predictable antisecretory agents highly relevant.(1–3)

Proton pump inhibitors (PPIs) have been the standard therapy for decades, but their limitations have become increasingly apparent. They require acid activation before they can irreversibly inhibit the gastric H+/K+-ATPase, and their effect depends on timing with meals and on intragastric pH, which contributes to delayed onset and variable acid control. Even with appropriate use, some patients continue to experience nocturnal acid breakthrough, incomplete symptom relief, delayed mucosal healing, or recurrence of disease, especially in severe reflux esophagitis and in settings that require stronger acid suppression. Interindividual variability, drug interactions, and limited nighttime control have further encouraged the development of alternatives with more rapid, sustained, and predictable action.(2,4)

These unmet needs led to the development of newer acid suppressants, especially potassium-competitive acid blockers (P-CABs), among which vonoprazan fumarate is the first-in-class agent. Vonoprazan inhibits gastric acid secretion by reversibly and competitively blocking the potassium-binding site of the H+, K+-ATPase, resulting in rapid onset of action and more prolonged acid suppression than conventional PPIs. Its pharmacologic profile has made

it an important therapeutic option for reflux esophagitis, peptic ulcer disease, NSAID-associated ulcers, and H. pylori eradication regimens. Because it can achieve stronger and more consistent intragastric acid control, vonoprazan has attracted considerable attention as a candidate to overcome several limitations of PPI-based therapy.(2–4)

The purpose of this review is to summarize recent advances in the development and therapeutic use of vonoprazan fumarate, with emphasis on its chemical and pharmacological characteristics, clinical applications, and comparative performance with PPIs. In addition, the review highlights current analytical methods used for its quantification and quality assessment, as these are important for formulation development, bioanalysis, and regulatory evaluation. Byintegrating the available evidence, this review aims to provide a concise and clinically relevant overview of vonoprazan fumarate for researchers, clinicians, and pharmaceutical scientists.

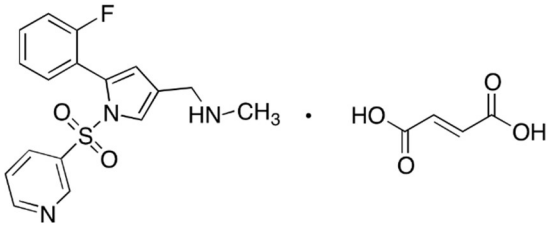


Fig No. 1 Structure of Vonoprazan Fumarate

Materials and Methods:

Active Pharmaceutical Ingredient (API), Vonoprazan Fumarate was procured from Dhamtec Pharma and Consultants.The other chemicals such as HPLC Grade Methanol and Water, AR grade Dimethyl Sulfoxide (DMSO) were from Loba Chemie,PVT, LTD.

Instruments:

Development and validation of the method were performed on Agilent’s High Performance Liquid Chromatography (RP-HPLC) with Diode-Array Detector

Preparation of Standard stock solution:

Weighed accurately about 13.3 mg of Vonoprazan Fumarate standard and transferred it into a 10 ml volumetric flask. 2 mL of diluent (Methanol) was added and sonicated for 10 minutes. Volume made up to mark with diluent and mix well.

Pipette out 1 ml from the stock solution and make up the volume to 10 ml mark with diluent to give 100 µg/ml. from this pipette out 1ml and transferred into a 10 ml volumetric flask and make up volume with diluent to give 10 µg/ml.

Preparation of Sample stock Solution:

Triturate 10 tablets of drug Vonoprazan Fumarate , weigh quantity of tablet equivalent to 10 mg of drug in to 10 ml volumetric flask.Add 2 ml of diluent disperse the tablet completely and sonicate for 10 min with intermittent swirling cool the flask, cool and dilute up to mark with diluent. Pipette out 1 ml in 10 ml of volumetric flask and make up volume with diluent to give 100 µg/ml. from this pipette out Iml and transferred into a 10 ml volumetric flask and make up volume with diluent to give 10 µg/ml.

Preparation of Mobile Phase:

10mM Ammonium Acetate: Methanol

Mobile Phase was prepared by mixing 0.77gm of Ammonium Acetate in Water and Methanol in ratio of 40:60.

Method Validation:

Validation was done according to ICH guidelines for the determination of the developed RP-HPLC method for Vonoprazan Fumarate in transdermal dosage form. Validation was carried out for the

Specificity

To evaluate for excipient interference, diluent, and sample solution were injected to determine specificity.

Linearity:

Accurately weighed 13.3 mg of Metformin HCL and transferred to 10ml volumetric flask. Diluted up to the mark with solvent to get concentration of 1000 µg/ml. From the above solution 1 ml was pipette out and transfer to 10 ml volumetric flask and diluted up to the mark with solvent to get concentration of 100 µg/ml. From the above solution suitable solutions were made to obtain concentration in the range of 10-60 µg/ml. Linearity response was assessed over the range of 10-60 µg/ml. Linear relationship should be evaluated should be evaluated across concentration and peak area. Each linearity preparation was injected six times. Solvent was used as blank. Calibration curve was obtained by plotting peak area Vs concentration.

Accuracy:

Accuracy of the method was determined by spiking known amount of standard in sample solution at 3 different levels (i.e. 80%, 100%, 120%) in duplicates as per the method of analysis and % recovery was calculated.

Precision:

System Precision:

Six different standard preparations 20µg/ml Vonoprazan Fumarate injection samples were prepared the same as specified earlier procedure. The % RSD was calculated.

Method precision:

Six different sample preparations 20µg/ml Vonoprazan Fumarate injection samples were prepared the same as specified earlier procedure. The % Assay was calculated for each preparation.

Robustness:

Robustness of an analytical procedure is it’s ability to remain unaffected by small, but deliberate variations. Parameters like flow rate, temperature, wavelength

were changed one by one in triplicate and %RSD calculated.

FORCE DEGRADATION STUDIES

To evaluate the stability indicating nature of the developed RP-HPLC method, the samples were subjected to various stress conditions including acidic oxidative thermal and photolytic degradation as per ICH guidelines. Following exposure to each stress condition the samples were analyzed using the developed RP-HPLC method. The percentage degradation of Metformin was calculated for each condition to assess the method's capability to separate the drug from its degradation products thereby confirming the specificity and stability indicating property of the method.

Acid Degradation:

Pipette out 1 mL of the sample solution and add 1mL of 0.1 N HCl. Transfer the mixture into a 10 mL volumetric flask and allow it to heat for 2 hours at 60 °C. After 2 hours, add 1 mL of 0.1 N NaOH to neutralize the solution. The final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45 µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Alkaline Degradation:

Pipette out 1ml from sample solution and transfer in 10 ml of volumetric flask and add 1ml of 0.1N NaOH. Transfer the mixture into a 10 mL volumetric flask and allow it to heat for 2 hours at 60 °C. After 2 hours, add 1 mL of 0.1 N HCL to neutralize the solution. The final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45 µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Oxidation degradation:

Pipette out 1ml from sample solution and transfer in 10 ml of volumetric flask and add 1ml of 3% H2O2. Above solution was kept for 2 hrs. After 2 hrs, the final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Thermal degradation:

Weigh 50mg of Vonoprazan Fumarate and , it was placed in a Petri dish. It was exposed to thermal stress by keeping it in a hot air oven at 80 °C for 24 hours. After 24 hours, the sample was allowed to cool to room temperature. Then took 10 mg of degraded sample and prepared 100 µg/ml. The solution was filtered through a 0.45 µm syringe filter and analyzed using a validated HPLC method. The chromatogram was recorded, and the percentage degradation was calculated based on the peak area.

Result and Discussion:

Table No.1: Optimized Chromatographic Conditions

Column	C18-150mm*4.6mm
Flow Rate	1 ml/min
Wavelength	254 nm
Detector	Diode Array Detector (DAD)
Mobile Phase	10mM Ammonium Acetate : Methanol (40;60)
Column Temperature	25°C
Injection Volume	10 µL
Retention Time	3.7 min

Assay Method:

Six replicates of Standard and Sample solutions were prepared and % Assay was calculated.

Fig No. 2:Chromatogram of Standard Vonoprazan for Assay

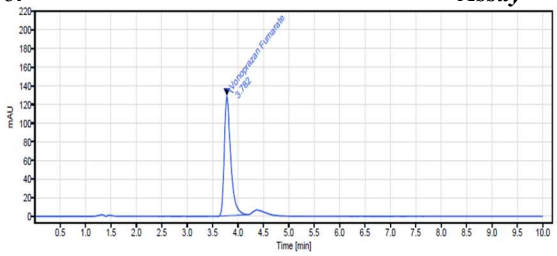
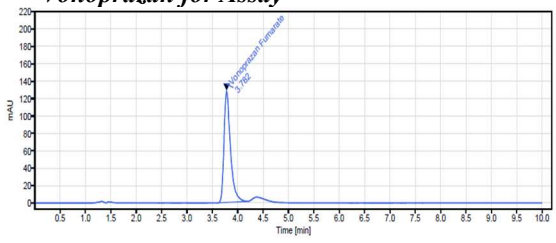


Fig No. 3: Chromatogram of Sample Vonoprazan for Assay



Observation: Vonoprazan Fumarate present in analyze dosage form was found to be 98.29 Acceptance criteria: Analyzed dosage form should be 98 % and 102 % pure respectively.

HPLC Validation Parameters:

Specificity:

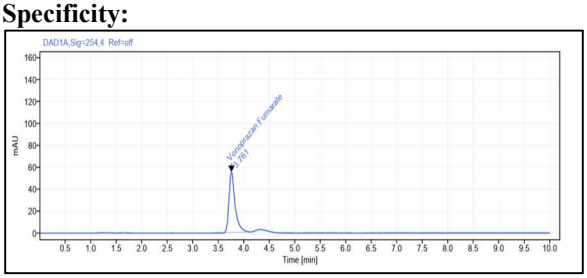


Fig No. 4 Standard Chromatogram for Specificity

Observation: No major interference of mobile phase, solvents, retention time, and impurities were observed, hence, the method was found to be specific**.**

Linearity: The linearity of Vonoprazan was found to be in the range of 10-60 µg/ml

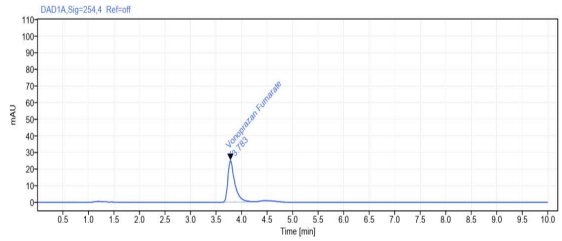


Fig No. 5 Chromatogram of Vonoprazan for Linearity 10 ppm

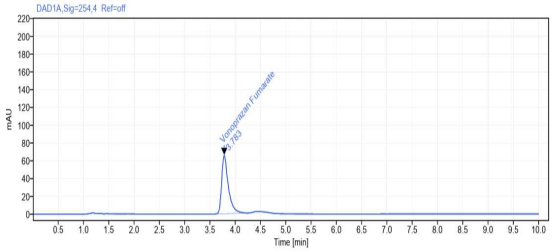


Fig No. 6 Chromatogram of Vonoprazan for Linearity 20 ppm

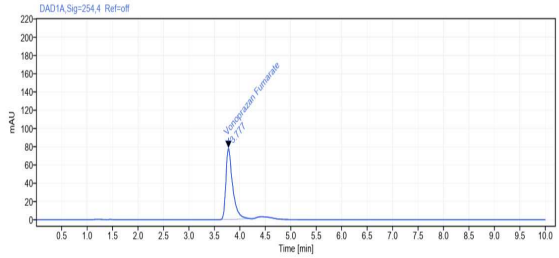


Fig No. 7 Chromatogram of Vonoprazan for Linearity 30 ppm

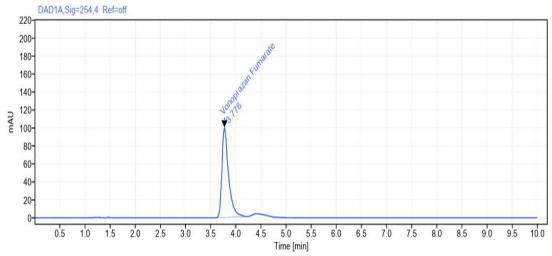


Fig No. 8 Chromatogram of Vonoprazan for Linearity 40 ppm

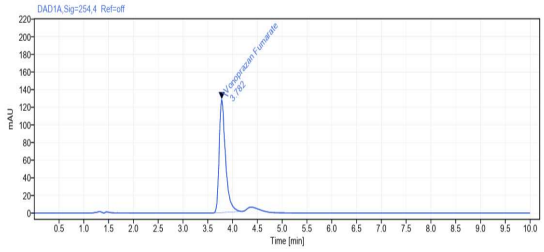


Fig No. 9 Chromatogram of Vonoprazan for Linearity 50 ppm

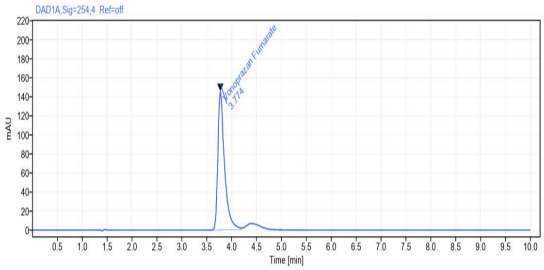
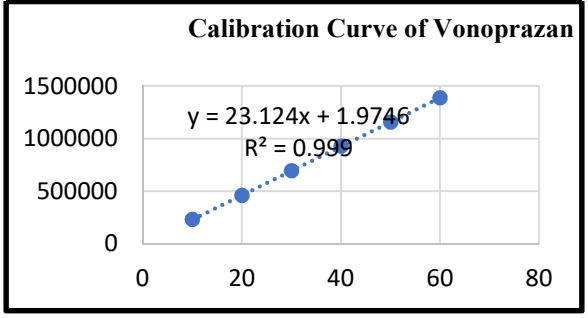


Fig No. 10 Chromatogram of Vonoprazan for Linearity 60 ppm

Sr. No.	Concentration (µg/ml)	Peak Area
1.	10	234068
2.	20	462369
3.	30	695704
4.	40	929613
5.	50	1156959
6.	60	1389231
7.	Y-Intercept	y = 23.124x + 1.9746

8.	Correlation Coefficient	0.999



Observation:

The correlation coefficient for the linear curve obtained between concentrations vs. area for standard preparations of Vonoprazan was found to be 0.999. The relationship between the concentrations of Vonoprazan was linear in the range examined since all the points lie in a straight line, and the correlation coefficient was well within limits.

Accuracy: The recovery studies was carried out 3 times, chromatogram was recorded and %Recovery and Mean %Recovery was calculated

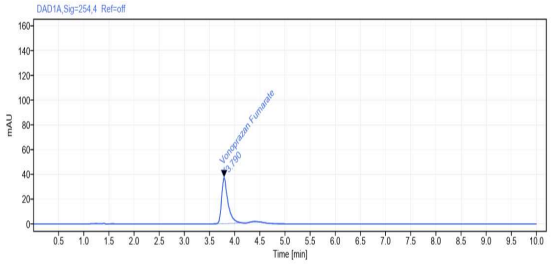


Fig No. 11 Chromatogram of Vonoprazan for Accuracy (80%)

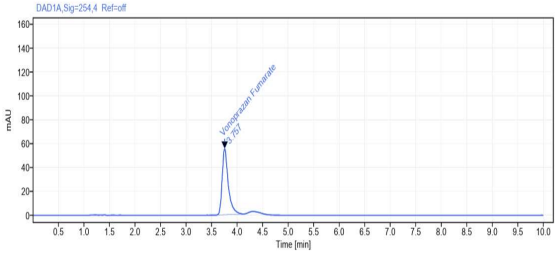


Fig No. 12 Chromatogram of Vonoprazan for Accuracy (100%)

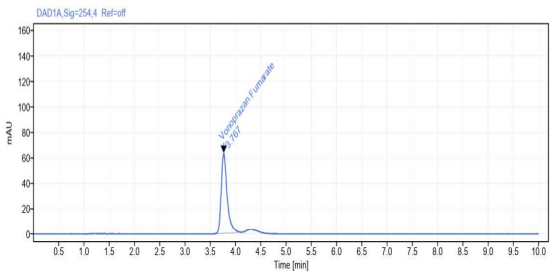


Fig No. 13 Chromatogram of Vonoprazan for Accuracy (120%)

Observation Table:

Level	Actual Concentration	Calculated Concentration	Peak Area	% Recovery	Mean Concentration	SD	%RSD
-	16	15.940	370.002	-	--	-	-
80%	16	15.929	369.739	99.590	15.934	0.00055	0.0346
-	16	15.934	369.863	-	-	--	-
-	20	19.989	463.705	-	-	-	-

100%	**20**	**19.984**	**463.590**	**99.820**	**19.964**	**0.03090**	**0.19556**
--	**20**	**19.919**	**463.752**	**-**	**-**	**-**	**-**
-	**24**	**24.010**	**556.775**	**-**	**-**	**-**	**--**
120%	**24**		**556.678**	**100.099**	**24.024**	**0.06626**	**0.26607**
-	**24**	**23.969**	**555.829**	**-**	**-**	**-**	**-**

Table No. 2 Accuracy data of Vonoprazan by HPLC Method

Observation: Recovery was found to be 99.590, 99.820 and 100.099% for the levels of 80, 100 and 120% respectively.

Precision: Six replicate of the standard solution were prepared, and %RSD was calculated.

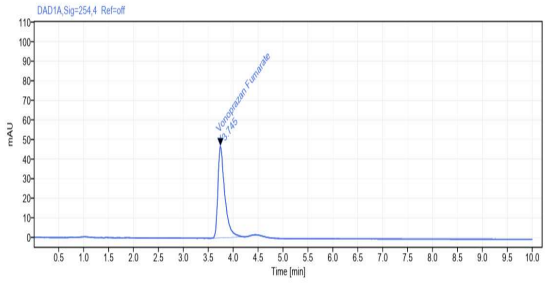


Fig No. 13 Chromatogram of Intraday Precision for Vonoprazan Fumarate

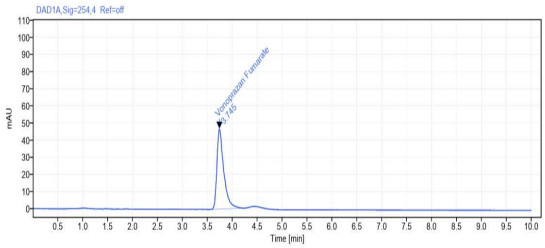


Fig No. 14 Chromatogram of Intraday Precision for Vonoprazan Fumarate

Sr. No.	**Concentration**	**Peak Area of Intraday**	**Peak Area of Interday**
1.	20	463.208	463.454
2.	20	463.934	463.879
3.	20	463.340	463.137
4.	20	463.025	463.671
5.	20	463.731	463.452
6.	20	463.939	463.654
7.	**Mean**	463.530	463.541
8.	**SD**	0.391	0.254
9.	**%RSD**	0.0844	0.0548

Observation: The %RSD for intraday and interday precision was found to be 0.0844 and 0.0548 respectively.

Robustness: The mobile phase and wavelength of the three replicates of the standard were varied, and the effect was evaluated for system suitability.

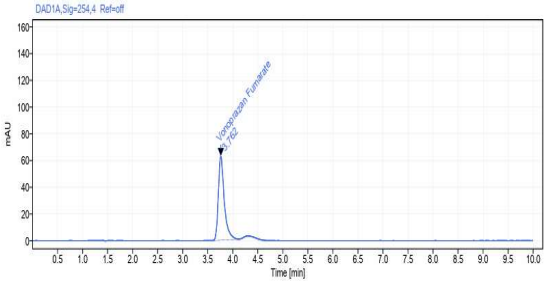
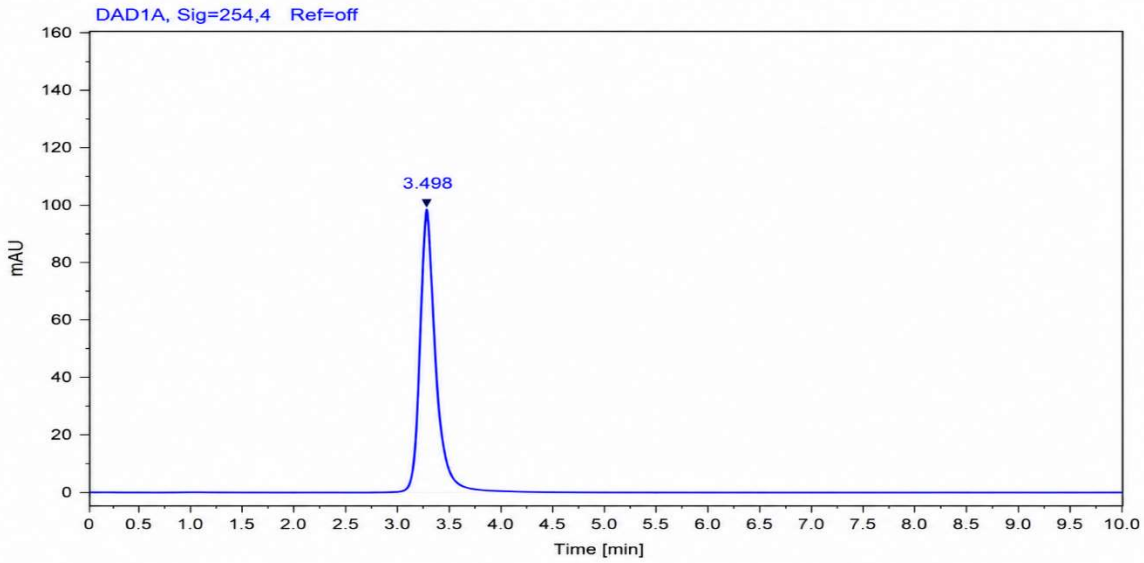


Fig No. 15 Chromatogram of Vonoprazan Fumarate



wavelength at 260 nm

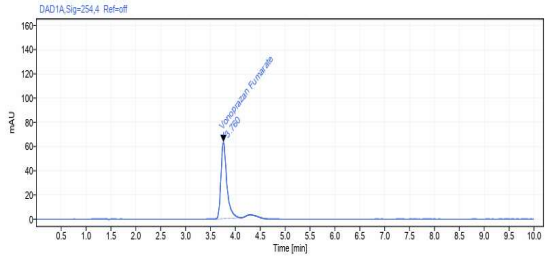


Fig No. 16 Chromatogram of Vonoprazan Fumarate wavelength at 280 nm

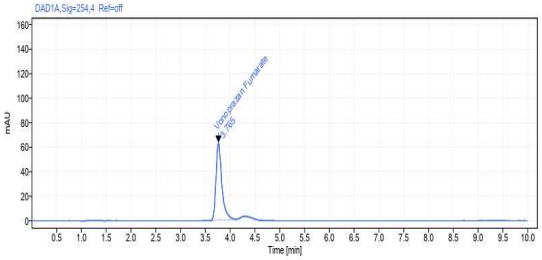


Fig No. 17 Chromatogram of Robustness with Flowrate of 0.8ml/min

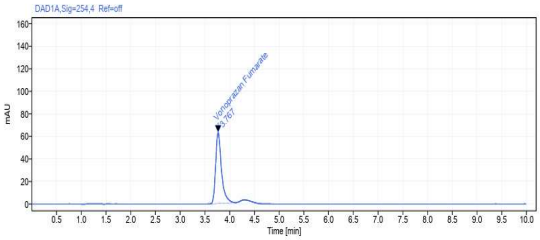


Fig No. 18 Chromatogram of Robustness with Flowrate of 1.2ml/min

Observation:

Table No. 4 Robustness data of Vonoprazan Fumarate by HPLC Method

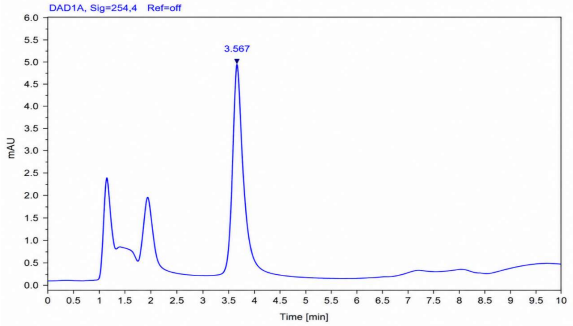
Parameters	Changed Conditions	Mean Area	SD	%RSD
Wavelength	260 nm 280nm	515.162 515.657	0.3500	0.068
Flow Rate	0.8ml/min 1.2ml/min	516.247 516.941	0.4907	0.095

Observation: Robustness parameters were found to be within the limit at different wavelengths and flow rates.

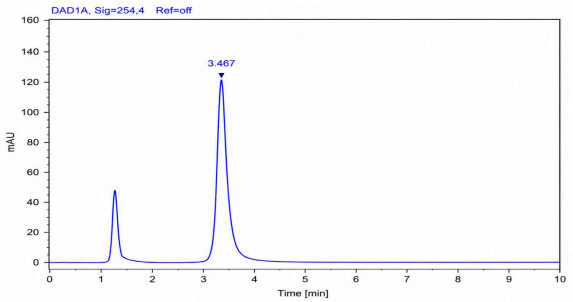
LOD and LOQ: LOD and LOQ were found to be 0.10 µg/ml and 0.30 µg/ml.

Forced Degradation Studies:

1. **Acid Degradation:**
2. **Alkaline Degradation:**

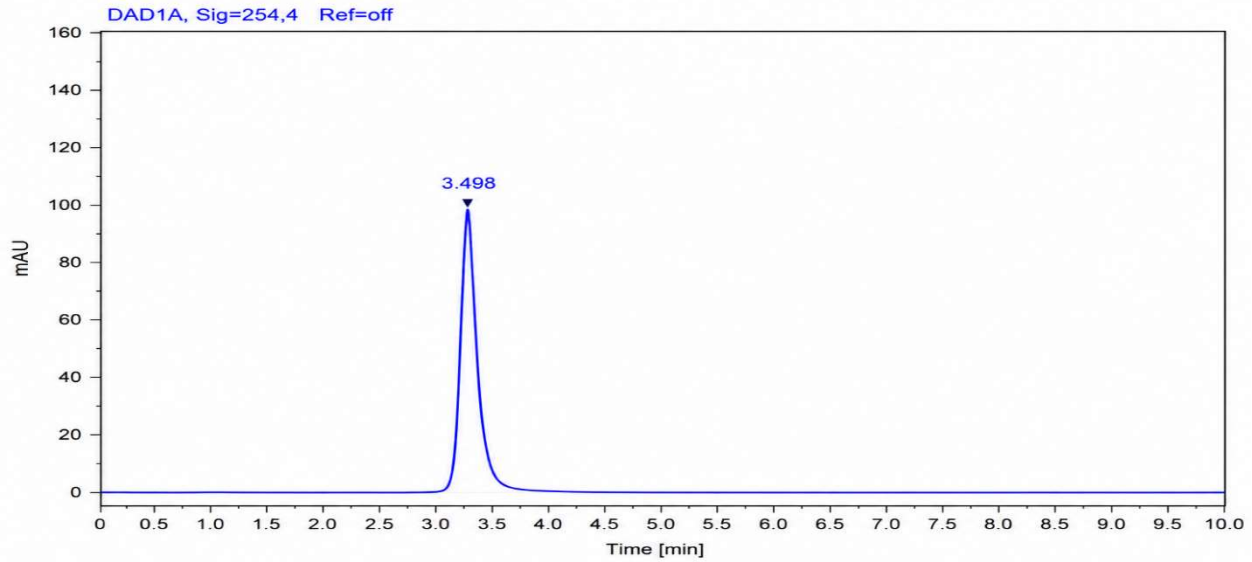


3. **Oxidative Degradation:**



1.	Acid(0.1 N HCL)	2hrs	2.5	97.5
2.	Alkaline(0.1 N NaOH)	2hrs	13.4	86.6
3.	Oxidation(3% H2O2)	2hrs	8.4	91.6
4.	Thermal(80 °C)	24hrs	0.5	99.5

4. Thermal Degradation:



Sr. No	Degradation Condition	Time	%Degradation	% Recovery

CONCLUSION:

A simple, rapid, accurate, precise, and stability-indicating RP-HPLC method was successfully developed for the estimation of Vonoprazan Fumarate in pharmaceutical dosage form. The optimized chromatographic conditions provided satisfactory separation with a retention time of approximately 3.7 min. The developed method exhibited good linearity over the concentration range of 10–60 µg/mL, with a correlation coefficient of 0.999.

The method demonstrated satisfactory accuracy, with recovery values ranging from 99.590% to 100.099%, and excellent precision, as indicated by low intraday and interday %RSD values. The method was also found to be robust against small variations in wavelength and flow rate. The low LOD and LOQ values demonstrated the sensitivity of the developed method.

Forced degradation studies showed that Vonoprazan Fumarate undergoes degradation under acidic, alkaline, oxidative, and thermal stress conditions, with the highest degradation observed under alkaline conditions. The developed method was capable of evaluating the drug under these stress conditions, supporting its stability-indicating nature.

Therefore, the proposed validated RP-HPLC method is suitable for the routine quantitative analysis and stability assessment of Vonoprazan Fumarate in pharmaceutical dosage forms and may be useful for quality-control applications.

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