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DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR QUANTIFICATION OF VONOPRAZAN FUMARATE IN PHARMACEUTICAL DOSAGE FORMS AND HUMAN PLASMA

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ABSTRACT

Background: Vonoprazan Fumarate is a potassium-competitive acid blocker (P-CAB) that inhibits gastric H^+, K^+-ATPase, effectively reducing acid secretion and treating disorders such as Gastroesophageal Reflux Disease and Helicobacter pylori infection.

Objective: This study aimed to develop and validate a simple, sensitive, and cost-effective RP-HPLC method for quantifying Vonoprazan Fumarate in human plasma.

Materials & Methods: Chromatographic separation was achieved on an Agilent C18 column (150 × 4.6 mm, 5 µm) with a mobile phase of methanol and 0.1% acetic acid in water (50:50 v/v) at a flow rate of 0.7 mL/min. Detection was carried out at 225 nm. Plasma samples were prepared using acetonitrile-induced protein precipitation. Method validation was performed as per International Council for Harmonisation guidelines.

Results: The method showed a retention time of 10 min with good peak symmetry. Linearity was observed over 2–10 µg/mL ($r^2 \geq 0.999$). LOD and LOQ were 0.5140 µg/mL and 1.5577 µg/mL, respectively. The recovery was 98.66%, with %RSD <2%, confirming accuracy and precision.

Conclusion: The validated RP-HPLC method is reliable and suitable for routine plasma analysis and pharmacokinetic studies of Vonoprazan Fumarate.

KEYWORDS: Vonoprazan Fumarate, RP-HPLC, Bioanalytical Method Validation, Human Plasma Analysis, Pharmacokinetic Studies

1. INTRODUCTION

For millennia, plants have served as humanity's primary pharmacy, offering incredible solutions for disease

Bioanalysis plays a vital role in pharmaceutical research by enabling the quantitative determination of drugs and their metabolites in biological matrices such as plasma, serum, urine, and cerebrospinal fluid. These measurements are essential for understanding pharmacokinetic parameters including absorption, distribution, metabolism, and excretion, as well as for evaluating bioavailability, bioequivalence, and drug–drug interactions1. Due to the low concentration of analytes and the complexity of biological matrices, highly sensitive, selective, and reliable analytical methods are required. Bioanalytical method validation (BMV) is a systematic process that ensures an analytical method is accurate, precise, and reproducible for its intended application2. Regulatory agencies such as the International Council for Harmonisation (ICH) and the Food and Drug Administration (FDA) have established guidelines outlining critical validation parameters, including accuracy, precision, selectivity, sensitivity, linearity, and stability. Depending on the extent of method modification or application, validation may be classified as full, partial, or cross-validation. These validation procedures are essential for generating high-quality and reliable data during drug development and clinical studies. Various advanced analytical techniques such as liquid chromatography–mass spectrometry (LC–MS), gas chromatography–mass spectrometry (GC–MS), and capillary electrophoresis–mass spectrometry (CE–MS) are widely employed in bioanalysis. Among these, high-performance liquid chromatography (HPLC) remains one of the most preferred techniques due to its robustness, sensitivity, and reproducibility3, 4. In particular, reverse-phase HPLC (RP-HPLC) is extensively used in pharmaceutical analysis because of its efficiency in separating compounds based on hydrophobic interactions and its suitability for routine laboratory applications5. Vonoprazan Fumarate is a novel potassium-competitive acid blocker (P-CAB) used in the treatment of acid-related disorders such as gastroesophageal reflux disease (GERD), peptic ulcers, and Helicobacter pylori infection6, 7. Unlike conventional proton pump inhibitors, Vonoprazan exhibits rapid onset of action, sustained acid suppression, and improved stability under acidic conditions. Owing to its increasing clinical use, there is a growing need for accurate and reliable bioanalytical methods for its quantification in biological matrices to support pharmacokinetic studies, therapeutic monitoring, and bioequivalence evaluations8, 9. Method development in HPLC

involves careful selection and optimization of chromatographic parameters such as column type, mobile phase composition, flow rate, and detection wavelength to achieve optimal separation and sensitivity. System suitability testing further ensures consistent performance of the analytical system prior to analysis10. Therefore, the development and validation of a robust RP-HPLC method for the estimation of Vonoprazan Fumarate in biological matrices is of significant importance. A validated method not only ensures accuracy and reproducibility but also supports regulatory compliance and routine application in pharmaceutical analysis and clinical research11, 12.

MATERIALS & METHODS:

Chemicals and Reagents

The API sample of Vonoprazan Fumarate was obtained from Swapnroop Drug and Pharmaceutical, Shendraban, Maharashtra. Tablets of Vonoprazan Fumarate (Vacinti 5mg) were purchased from Local market. HPLC Grade methanol, Acetonitrile, Potassium Phosphate Buffer were procured from Merck Ltd., India. Human plasma samples were procured from RD Labs, Jalgaon.

Instrumentation

The analysis of Vonoprazan Fumarate was performed using an Agilent Technologies HPLC Gradient System equipped with an auto-injector, diode-array detector (DAD), and UV730D absorbance detector, operated via Chemstation 10.1 software. A reverse-phase C18 column (Agilent, 5 µm, 4.6 mm × 250 mm) was used for chromatographic studies. Additional instruments employed during method development included a Thermo C18 column (150 mm × 4.6 mm, 5 µm), a UV-spectrophotometer (Analytical Technology), a VSI pH meter (Model VSI 1-B), a precision balance from A&D Company, Japan, and a sonicator from ENERTECH Electronic Instruments.

Chromatographic Conditions

Chromatographic separation was done by a C18 column using a binary gradient solvent system. Wavelength used for EGF detection was 225 nm. Ambient temperature conditions were used throughout the experiment. The degassing of the mobile was done for 15 minutes. The 20 µL injection volume with a flow rate of 0.7 mL/min and solvent, methanol: 0.1 % Acetic Acid 50:50 % v/v was used after optimizing conditions.

Standard Stock Solution

An accurately weighed 5 mg of Vonoprazan Fumarate was dissolved in 25 mL of methanol, followed by the addition of 1 mL of human plasma. The mixture was vortexed for 30 minutes and centrifuged at 5000 rpm for 1 hour. The supernatant was filtered through a membrane filter to obtain a clear solution. Appropriate aliquots (0.1–0.5 mL) of this stock solution were transferred into a 10 mL volumetric flask and diluted to volume with the mobile phase consisting of methanol and 0.1% acetic acid in water (5:5, v/v). The prepared solutions were transferred into HPLC vials and subjected to chromatographic analysis.

Preparation of Blank Plasma

For the preparation of blank plasma solution, 3 ml of extracted human plasma was taken and to this, 10 ml of solvent mixture (comprising 5 ml of methanol and 5 ml of water) was added. The separated plasma was then used for further HPLC analysis.

Preparation of Calibration curve standard:

From the previously prepared stock standard solution 0.1, 0.2, 0.3, 0.4, and 0.5 ml aliquots were pipetted into separate 10 ml volumetric flasks and diluted to the mark with mobile phase to obtained final concentrations of 2, 4, 6, 8, and 10 µg/ml of Vonoprazan Fumarate, respectively. These standard solutions were injected into the HPLC system under optimized chromatographic conditions, and the corresponding peaks were recorded at 225 nm.

Validation of Parameters

Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. From the working solution appropriate volumes were pipetted out and diluted with the mobile phase to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL. Area is plotted graphically as functions of analyte concentration

Precision and Accuracy

Precision of an analytical method refers to the closeness of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. It is usually expressed as Standard Deviation (SD) or Relative Standard Deviation (%RSD). The accuracy of the developed RP-HPLC method for Vonoprazan Fumarate was evaluated by spiking human plasma samples with known concentrations of the drug at three levels: 80% (1.6 mg), 100% (2.0 mg), and 120% (2.4 mg). Each level was analyzed in triplicate over three consecutive days. The percentage recovery was calculated to determine the method's accuracy.

LOD and LOQ

According to ICH Q2 (R1) guidelines, the Limit of Detection (LOD) and Limit of Quantification (LOQ) based on signal-to-noise (S/N) ratios, using an S/N ratio of 3:1 for LOD and 10:1 for LOQ.

Robustness:
Robustness was assessed by introducing deliberate, minor variations to the chromatographic conditions to evaluate the method's reliability under varied analytical settings. The mobile phase composition was changed in the proportion of methanol: 0.1 % Acetic water (49:51 & 51:49 % v/v). The change in detection wavelength and the effect of the results was examined.

Analysis of Marketed Formulation
The validated RP-HPLC method was applied for the quantification of Vonoprazan Fumarate in a marketed tablet formulation (label claim: 5 mg). Twenty tablets were weighed (total weight: 2.33 g) and finely powdered. A quantity of powder equivalent to 58.28 mg of Vonoprazan Fumarate was accurately weighed and extracted with 10 mL methanol, followed by sonication for 15 minutes to ensure complete extraction. An aliquot (0.4 mL) of the supernatant was diluted to 10 mL with the mobile phase. Prior to analysis, 1 mL of plasma was incorporated as per the bioanalytical procedure. The prepared sample was filtered and injected into the HPLC system, and the peak area was recorded. Quantification was performed using the calibration curve constructed from standard solutions. The concentration of Vonoprazan Fumarate in the sample was calculated using the regression equation, and the drug content per tablet was determined accordingly.

RESULTS
Melting point: The procured reference standard of Vonoprazan Fumarate was found to melt in the range of 194-196 °C. The λmax in the UV spectrum of a 10 µg/mL solution of Vonoprazan Fumarate was found to be 225 nm.

System Suitability Test
Trial run 7 was selected based on system suitability parameters including resolution, tailing factor, retention time, and the number of theoretical plates, all of which met the predefined acceptance criteria. These results confirmed the reproducibility and suitability of the chromatographic system for the analysis of Vonoprazan Fumarate.

Validation Parameters
Calibration Curve & Linearity
The data obtained from the calibration experiments, when subjected to linear regression analysis, showed a linear relationship between peak area and concentration in the range of 2310 µg/mL for Vonoprazan Fumarate (Table 3 and Figure 4) depicts the calibration data. The respective linear regression equation for Vonoprazan Fumarate was $y = 50.20x + 5.469$, where x is the concentration and y is the peak area. The correlation coefficient (r^2) was found to be 0.999, indicating excellent linearity. The calibration curve is shown in (Figure 3).

Accuracy
Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98- 102% (Figure 5, Table 4, 5).
Repeatability
Repeatability studies on RP-HPLC for Vonoprazan Fumarate was found to be 99.45% (Table 6). The % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded.

Precision
Intraday and Inter day Precision studies on RP-HPLC for Vonoprazan Fumarate, which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded. (Table 7, Figure 6)

Robustness
The changes were did, mobile phase composition (±1 ml/ min-1), and Wavelength (±1 ml/ min-1). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded (Table 8, Figure 7)

LOD and LOQ
The LOD and LOQ of Vonoprazan Fumarate were found to be 0.5140 (¼g/mL) and 1.5577 (¼g/mL)
Analysis of Tablet Formulation
Analysis of the marketed formulation of Vonoprazan Fumarate showed 98.41 % the label claim was 99-101% (Table 9 Figure 8).

DISCUSSION
The present study successfully developed and validated a simple, rapid, and precise RP-HPLC method for the quantification of Vonoprazan Fumarate in human plasma. Drug extraction was performed using a simple protein precipitation technique. The method was validated as per ICH guidelines and demonstrated to be accurate, precise, specific, and sensitive. Parameters such as selectivity, specificity, calibration curve linearity, accuracy, precision, sensitivity were within acceptable limits. Chromatographic separation was achieved on an Agilent C18 column (150 mm × 4.6 mm, 5 µm) using a mobile phase of methanol: 0.1% acetic acid (50:50, v/v, pH 3), delivered at 0.7 mL/min and 25°C. The retention time was found to be 3.8 minutes. The calibration curve showed good linearity, and the % nominal concentration ranged between 98 -102%.Overall, the method is rapid, selective, and requires minimal sample preparation.

Due to its lower solvent consumption and robustness, it is suitable for routine analysis of Vonoprazan Fumarate in plasma and can be applied to pharmacokinetic, safety, and efficacy studies.

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method was successfully developed and validated for the quantification of Vonoprazan Fumarate in API, tablet dosage forms, and human plasma. The method exhibited excellent sensitivity, with low limits of detection and quantification, enabling reliable trace-level analysis. Efficient sample preparation using protein precipitation provided satisfactory recovery and reproducibility. The absence of interference from excipients and biological matrix components, along with peak purity values close to unity, confirmed the specificity and selectivity of the method. Validation results complied with ICH guidelines, demonstrating the method's accuracy, precision, robustness, and reliability. Overall, the developed method is suitable for routine quality control analysis and can be effectively applied in pharmacokinetic studies, bioavailability and bioequivalence assessments, as well as therapeutic drug monitoring and toxicological evaluations of Vonoprazan Fumarate.

ACKNOWLEDGEMENT:

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CONFLICT OF INTEREST:

The authors declare that they have no conflicts of interest related to this study.

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Table 1: Chromatographic Behaviour of Vonoprazan Fumarate Using Mobile Phases of Various Compositions

Sr. No.	Mobile Phase	Remark
1	Methanol : 0.1% Acetic acid (90:10 % *v/v*) 225 nm, flow 0.7mL/min	No sharp peak (Peak splitting)
2	Methanol : 0.1% Formic acid (80:20% *v/v*) pH 3, 225 nm flow 0.7 mL/min	No Sharp peak
3	Methanol : 0.1% Formic acid (75:25% *v/v*) pH 3,225 nm, flow 0.7 mL/min	No Sharp peak
4	Methanol : 0.1% formic acid (70 : 30% *v/v*) pH3 225 nm, 0.7 mL/min	No Sharp peak
5	Methanol: 0.1% Formic acid (65:35% *v/v*) pH 3 225 nm, 0.7 mL/min	No Sharp Peak obtain

6	Methanol: 0.1% Acetic Acid (55:45% *v/v*) pH 3 225 nm,0.8 mL/min	Sharp Peak was not obtain
7	**Methanol: 0.1% Acetic Acid (50:50% *v/v*) pH 3 225 nm, 0.7 mL/min**	**Sharp Peak Obtain**

Table 2: Details of Chromatogram of System suitability No- 1

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.935	289.45615	6127	0.75	-

Table 3: Linearity data for Vonoprazan Fumarate

Method	Conc. µg/mL	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	2	91.11	91.14	91.1250	0.02	0.02
	4	197.05	194.14	195.5950	2.06	1.05
	6	302.25	302.02	302.1350	0.16	0.05
	8	399.81	396.07	397.9400	2.64	0.66
	10	492.89	491.07	491.9800	1.29	0.26
	Equation		y =50.20x-5.469			
	R^2		0.9992			

Table 4: Result of Recovery data for Vonoprazan Fumarate

Drug	Level (%)	Amt. taken (µg/mL)	Amt. Added (µg/mL)	Amt. found * ± S.D.	Amt. recoverd Mean *±S.D.	%Recovery Mean *± S.D.
VPZ	80%	2	1.6	3.60±0.010	1.60±0.010	99.89±0.61
	100%	2	2	4.00±0.014	2.00±0.014	100.23±0.70
	120%	2	2.4	4.37±0.012	2.37±0.012	98.66±0.49

Table 5: Statistical Validation of Recovery Studies Vonoprazan Fumarate

Method	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
RP-HPLC Method	80%	VPZ	99.89	0.61	0.61
	100%	VPZ	100.23	0.70	0.70
	120%	VPZ	98.66	0.49	0.49

Table 6: Repeatability studies on RP-HPLC for Vonoprazan Fumarate

Method	Concentration of Vonoprazan Fumarate (mg/Ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for VPZ	6	299.45	6.07	101.11
	6	298.72		
		Mean	**6.07**	**99.45**
		SD	**0.52**	**0.55**
		%RSD	**0.17**	**0.17**

Table 7: Result of Intraday and Inter day Precision studies on RP-HPLC for Vonoprazan Fumarate

Drug	Conc. (µg/mL)	Intraday Precision		Interday Precision	
		Mean± SD	%Amt Found	Mean± SD	%Amt Found

VPZ	4	195.75±2.90	100.21	196.11±0.74	100.39
	6	300.63±2.41	101.62	298.34±1.44	100.87
	8	398.46±1.68	100.58	391.39±1.22	98.82

Table 8: Result of Robustness Study of Vonoprazan Fumarate

Parameters	Conc.(µg/ mL)	Amount of detected (mean ±SD)	%RSD
Chromatogram of comp change 49 ml Methanol + 51ml buffer	8	391.18±1.99	0.51
Chromatogram of comp change 51 ml Methanol + 49 ml buffer	8	389.86±1.89	0.48
Chromatogram of wavelength change buffer 224nm	8	417.8±4.12	0.99
Chromatogram of wavelength change buffer 226nm	8	371.11±2.22	0.60

Table 9: Analysis of marketed formulation

Drug	Conc.	Amt. Found	% Label Claim	SD	%RSD
VPZ	8	7.8726	98.41	0.018	0.224
VPZ	8	7.8477	98.10	0.018	0.224

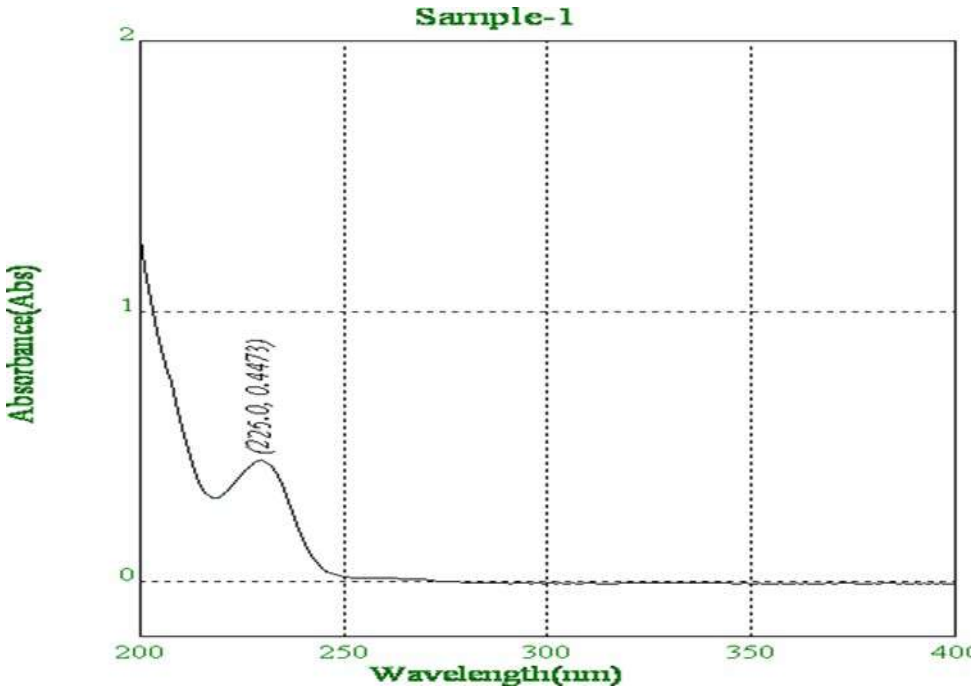
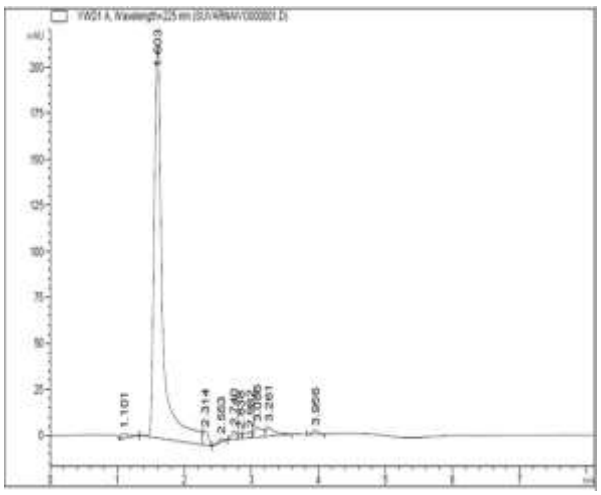


Figure 1: UV Spectrum of Vonoprazan Fumarate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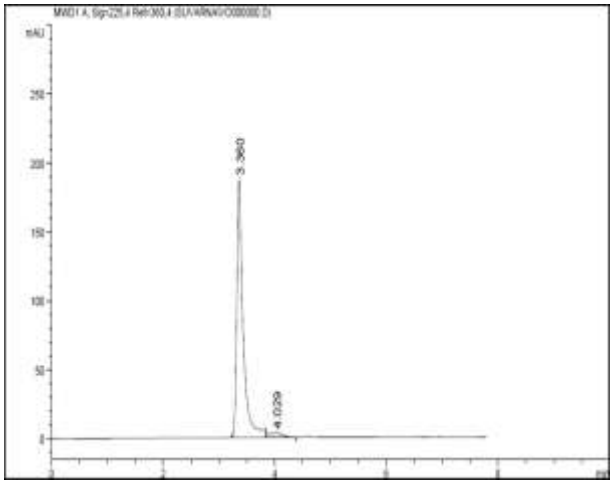
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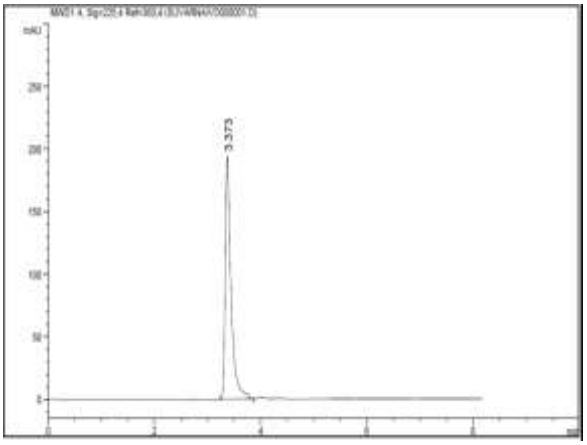
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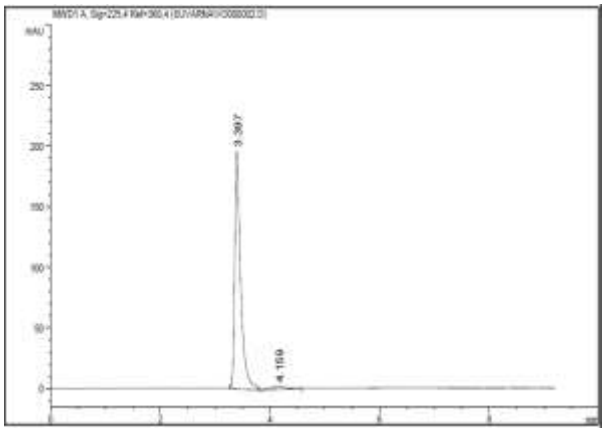
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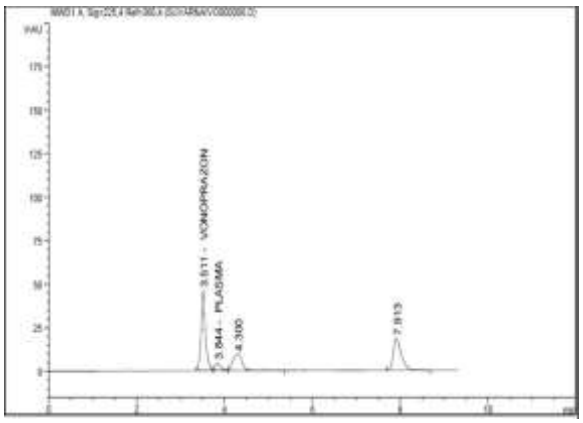
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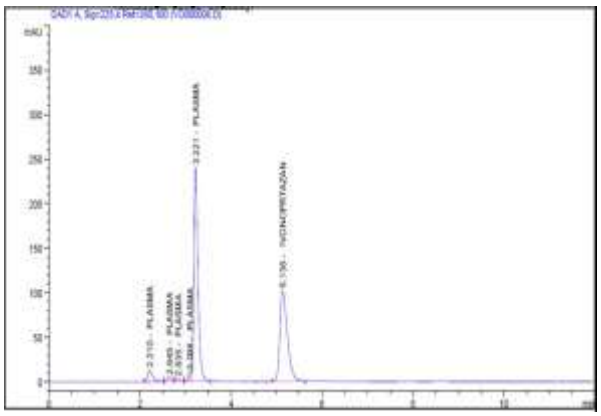
3.944 - PLASMA
4.300
7.913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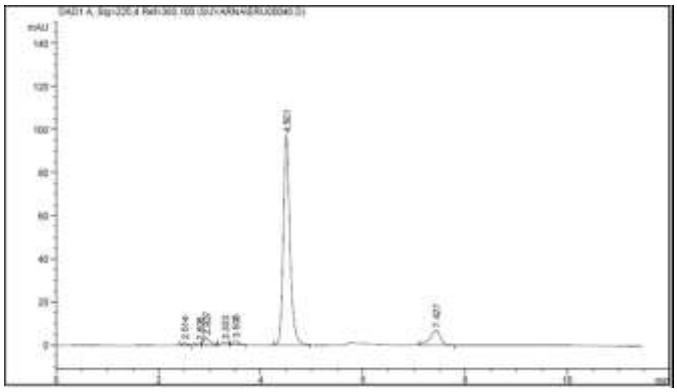
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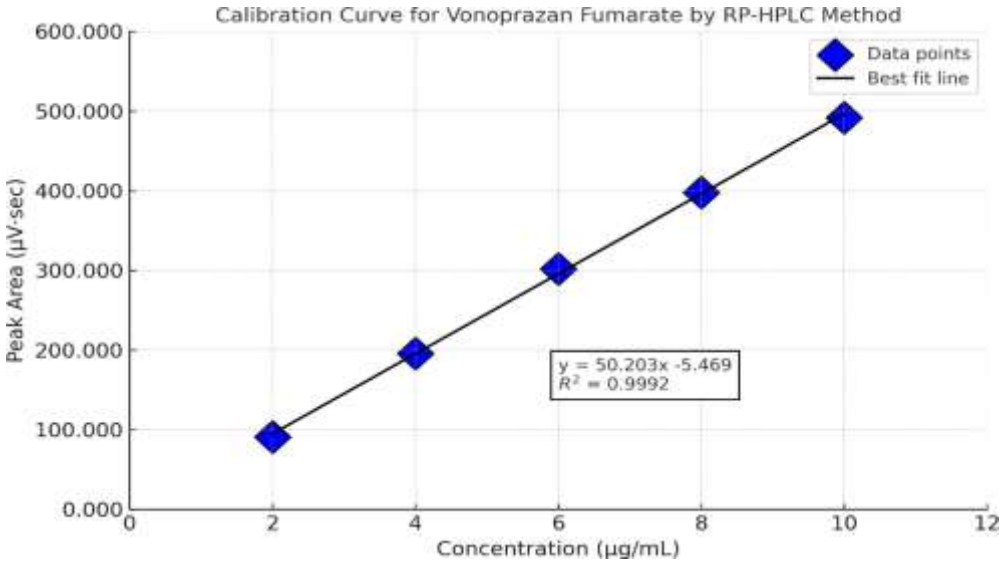

Figure 3: Calibration Curve of Vonoprazan Fumarate

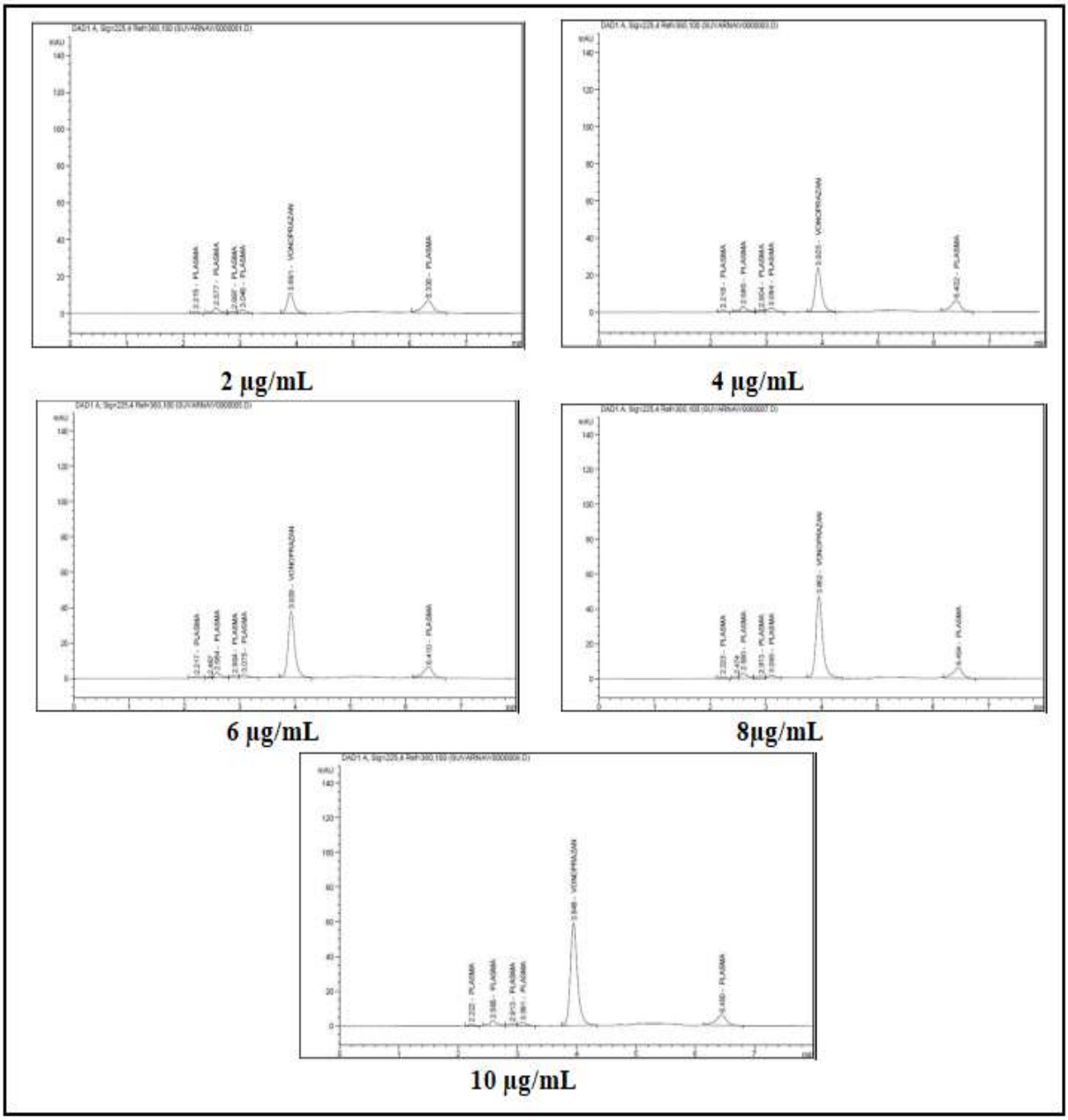

Figure 4: Linearity Chromatograms

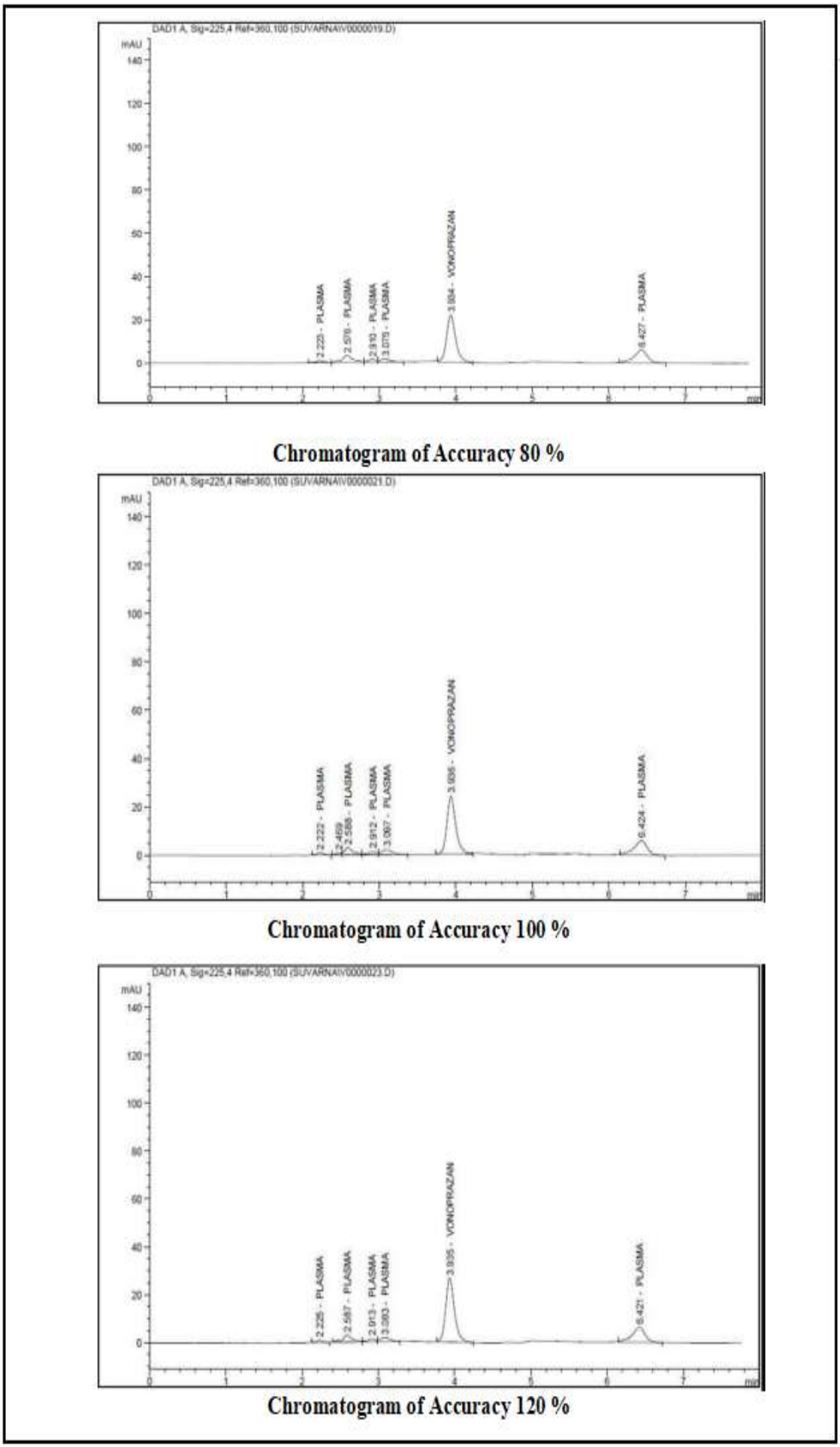

Figure 5: Chromatogram of Accuracy

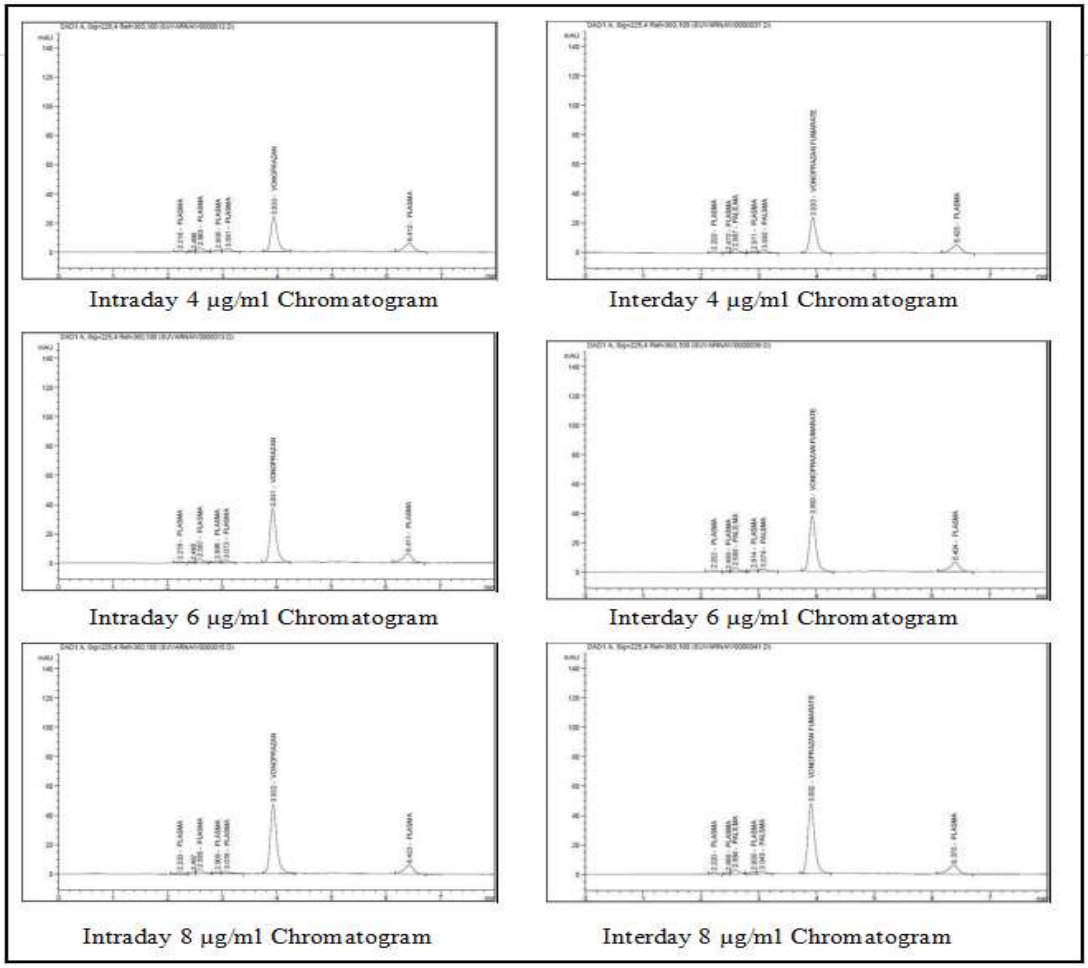

Figure 6: Chromatogram of Intraday & Interday

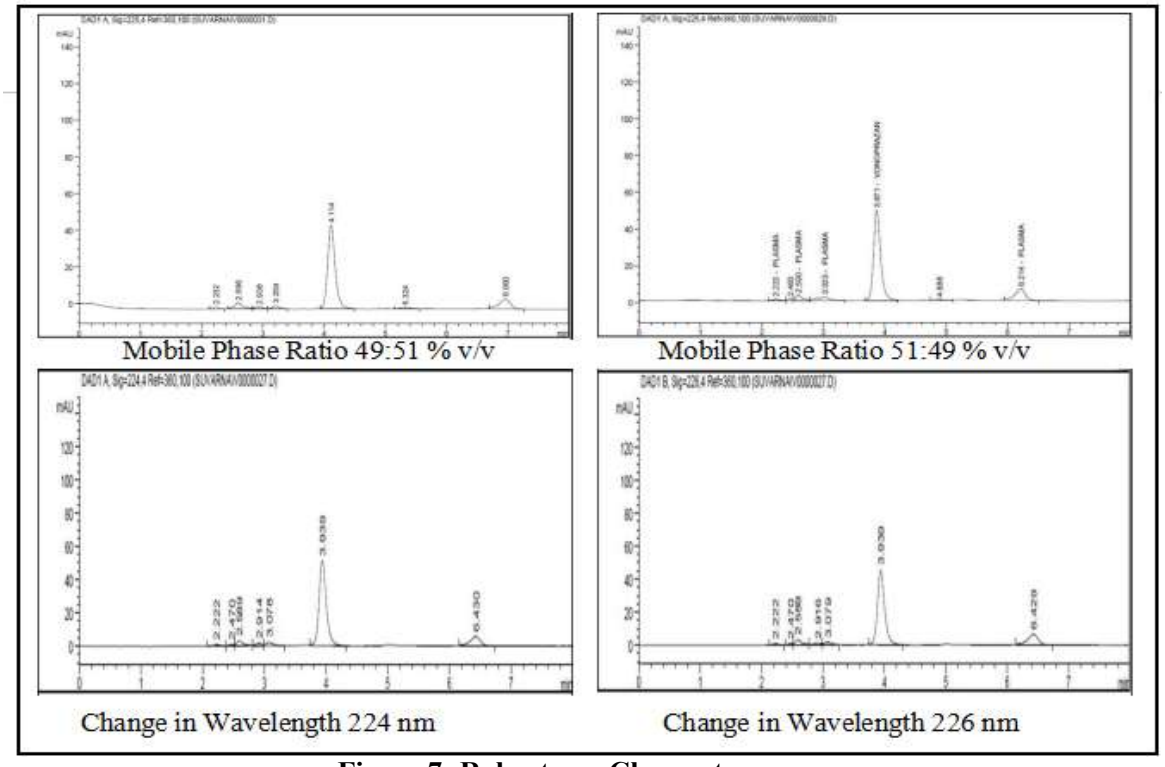

Figure 7: Robustness Chromatogram

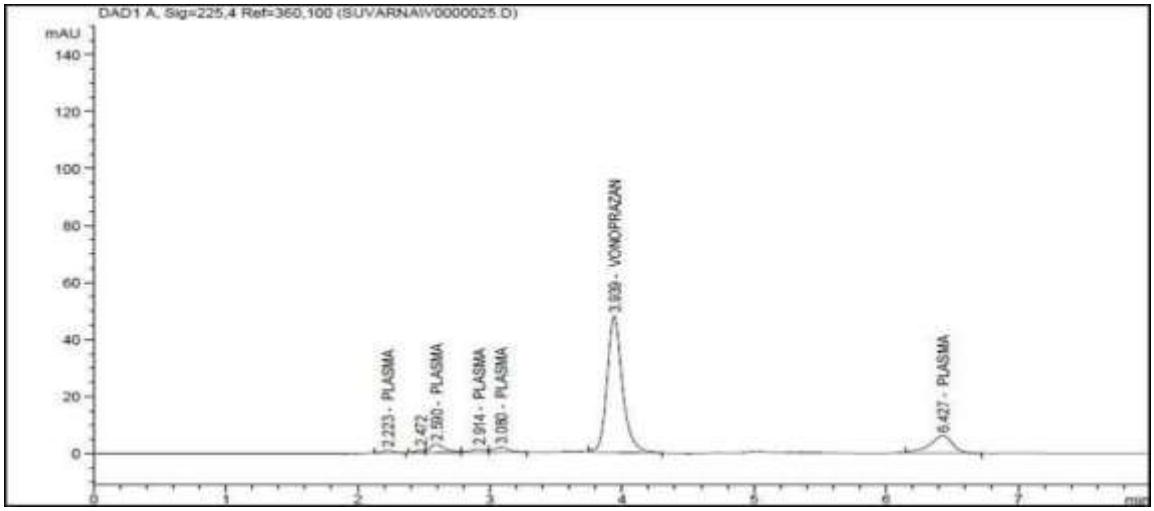

Figure 8: Chromatogram for Marketed Formulation