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Analytical Method Development by HPLC for Quantification of Vonoprazan Fumarate in tablet Formulation

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Abstract: *A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Vonoprazan fumarate in tablet dosage formulations. Chromatographic separation was achieved using a C18 column with a suitable mobile phase consisting of buffer and organic solvent in optimized proportion under isocratic elution conditions. The flow rate was maintained at 1.0 mL/min, and detection was carried out using a UV detector at an appropriate wavelength. The retention time of Vonoprazan fumarate was found to be satisfactory with good peak symmetry and resolution.*

The developed method was validated according to International Council for Harmonisation (ICH) guidelines for parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). The calibration curve showed excellent linearity over the selected concentration range with a correlation coefficient greater than 0.999. The percentage recovery values indicated high accuracy of the method, while precision studies demonstrated low percentage relative standard deviation (%RSD), confirming reproducibility. The method was found to be robust against small deliberate variations in chromatographic conditions.

The proposed RP-HPLC method was successfully applied for routine quantitative analysis of Vonoprazan fumarate in tablet formulations and can be effectively utilized for quality control and pharmaceutical analysis due to its simplicity, reliability, and cost-effectiveness.

Keywords: Analytical Method Development by HPLC for Quantification of Vonoprazan fumarate in tablet Formulation

I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.1-6

HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20th century analytical chemistry found wide application in forensic, environmental, industrial and medical field.

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Importance of Analytical Chemistry:

- It finds numerous applications in various disciplines of chemistry.
- It finds wide applications in other fields of related sciences.

Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.
Qualitative analysis deals with the identification of elements, ions or compounds present in the sample.
Quantitative analysis
Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.1-6

METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.7

CONSIDERATIONS BEFORE METHOD DEVELOPMENT

Developing and validating new analytical methods is costly and time consuming. Before starting the arduous process, a thorough literature search should be conducted for existing methodologies of the intended analytes or similar compounds. This should include a computerized search of chemical abstracts and other relevant sources such as compendial monographs (USP, EP), journal articles, manufacturer literature, and the Internet. Although this search might not uncover a directly usable method, it often provides a starting point for method development or at least some useful references. 7
New analytical methods are needed for the following reasons:
Existing methods are not available (e.g., New Chemical Entity (NCE) for consideration as a new drug candidate).
Existing methods are not sufficiently reliable, sensitive, or cost effective.
New instrumentation or technique has better performance (ease of use, rapid turnaround, automation, higher sensitivity).
An alternate (orthogonal) method is required for regulatory compliance.

FACTORS AFFECTING THE CHOICE OF ANALYTICAL METHOD:

Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. An important task for the analyst is to select best procedure for a given determination this will require careful consideration of the following criteria:1-6
The type of analysis required: elemental or molecular, routine or occasional.
Problem arising from the nature of the material to be investigated, e.g. radio-active substance, corrosive substance, substances affected by water.
Possible interference from components of the material other than those of interes
The concentration range to be investigated.
The accuracy required.
The facilities available, particularly the instrument.
The time required to complete the analysis.
The number of analysis of similar type which have to be performed.

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Details of Pure drug:

Table No. 8: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Vonoprazan fumarate	Arrow Chem Mumbai.	10 g	99.9 % w/w

Marketed Preparation:

Table No. 9: Details of marketed Preparation

Brand Name	Mfd by	Content	Quantity
Vonity® tablet	General Pharmaceuticals	Vonoprazan fumarate 20 mg	10 tablets

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.

Methanol (HPLC grade).

Acetonitrile (HPLC grade)

Disodium hydrogen phosphate (AR grade).

Distilled Water (HPLC grade).

Triethylamine (HPLC grade).

Ortho Phosphoric Acid (HPLC grade).

Instruments:

Table No. 10: Instruments Used

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	L 50

Study of Functional Group by Using Infra Red Spectroscopy:

Vonoprazan fumarate API: - Accurately weighed 3 mg of Vonoprazan fumarate API was mixed properly with 300 mg of dried KBr, then carefully triturated in a mortar pestle. Keep this mixture in a die and IR spectrum was taken using the Diffused Attenuated reflectance mode.

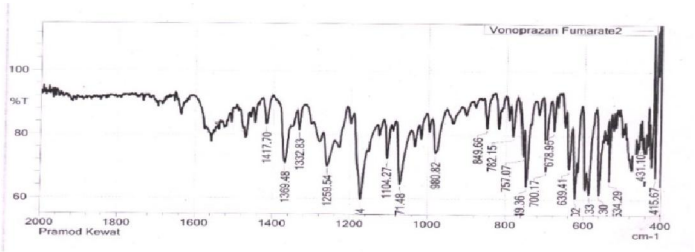

Fig. No. 8: IR Spectra of Vonoprazan fumarate

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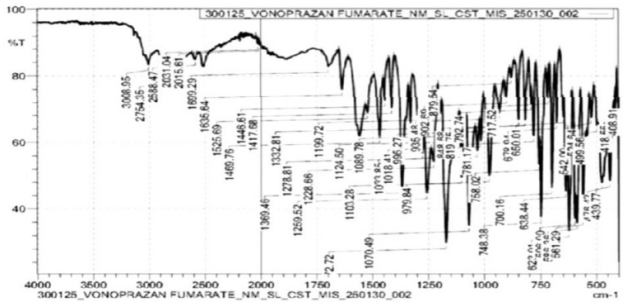


Fig. No. 9: Reference IR Spectra of Vonoprazan fumarate

Conclusion:

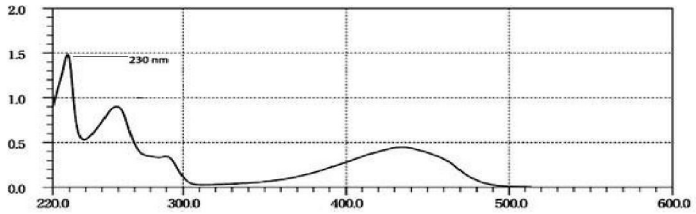
The IR spectra of the given test drugs is matches with the IR spectra of standard drugs.

Determination of wavelength maxima Vonoprazan fumarate standard stock solution:

An accurately weighed quantity of Vonoprazan fumarate (VNF) 5 mg was transferred to the 10 ml volumetric flask and dissolved in HPLC grade ACN. The volume was made up to the mark with the same to make (500 g/ml).□

The aliquot portions of stock standard solutions were diluted appropriately with HPLC grade g/ml of VNF. The solutions were scanned in the□ACN to obtain concentration 5 range of 400–200 nm in 1 cm cell against blank. The UV absorbance spectrum of VNF were recorded and found to be 230nm.

Fig. No. 10: Wavelength Maxima for Vonoprazan fumarate.



Development of HPLC method for estimation of Vonoprazan fumarate

Method Development Strategy:

Selection of Common Solvent (Diluents):

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in mobile phase. The selection was made after assessing the solubility of Vonoprazan fumarate in different solvents i.e Acetonitrile and water.

Preparation of standard stock solution:

Accurately weighted VNF 20 mg was dissolved in 100ml ACN. This solution was used as standard stock solution.

Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing VNF was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through Membrane filter (size 0.15µ). To achieve peaks with good symmetry

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various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Chromatographic Parameters:

Column:	C8 (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size
Flow Rate:	1.0ml/min
Wavelength:	230 nm
Injection volume:	20µl
Column oven Temperature:	Ambient (250C)
Run Time:	10 minutes
Mobile Phase:	10 mM phosphate buffer pH 3.5 modified with 0.1 % OPA and ACN (50:50)
Preparation of 0.1% OPA:	Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and makeup the volume upto the mark with HPLC water.

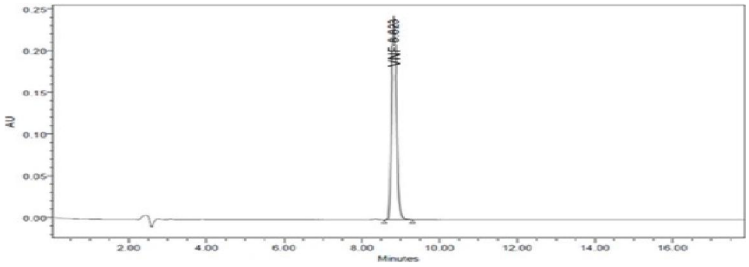

Fig. No. 11: Separation of VNF in selected mobile phase showing retention time at 8.823 min.

System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions.

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of VNF was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

B) Procedure:

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was L std. drug solution was injected which was made in five□obtained. A 20 replicates and the system suitability parameters were recorded.

Table No. 11: Result of System suitability test

Sr. No	Peak area VNF	Retention Time VNF	Symmetry VNF	No. of theoretical Plates VNF
1	650269	8.90	1.20	9526
2	648952	8.92	1.30	9588
3	645896	9.01	1.10	9645
4	652698	8.98	1.35	9600
5	647895	8.99	1.30	9550
Mean	649142	8.96	1.25	9582
S.D	1551.61	0.057	0.1	46.07

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%R.S.D.	0.34	0.47	1.5	0.48

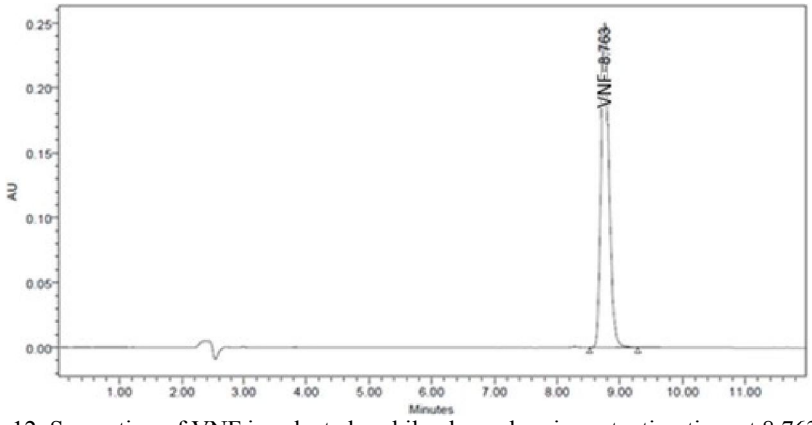

Fig. No 12: Separation of VNF in selected mobile phase showing retention time at 8.763 min.

Application of proposed method for estimation of VNF in tablet formulation: Standard stock solution:

a) Preparation of standard solutions:

Vonoprazan fumarate standard stock solution: Accurately weighed quantity of 20 mg VNF was dissolved in ACN and volume was made up to 100 ml mark by same to obtain 200 µg/ml stock solution.

Vonoprazan fumarate standard working solution: Pipette out 1 ml from standard stock solution and dilute it with 10 ml ACN to obtain 20µg/ml of VNF.

Sample solution preparation:

Entire content of Vonity® tablet (20 mg) was transferred to a 100 ml volumetric flask, the volume was made upto the mark with methanol, the resultant concentration was 200 µg/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 µ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with methanol, the concentration of working sample solution was 20 µg/ml.

Procedure:

Equal volume L)□(20 of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response

i.e. peak area of major peaks were measured. The content of VNF was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using following formula-

$$\% \text{ Estimation} = \frac{At \times Ds \times Ws \times 100}{As \quad Dt \quad Wt}$$

Where,

At=Area count for sample solution. As= Area count for standard solution. Ds= Dilution factor for standard.

Dt= Dilution factor for sample. Ws =Weight of standard (mg) Wt =Weight of sample (mg)

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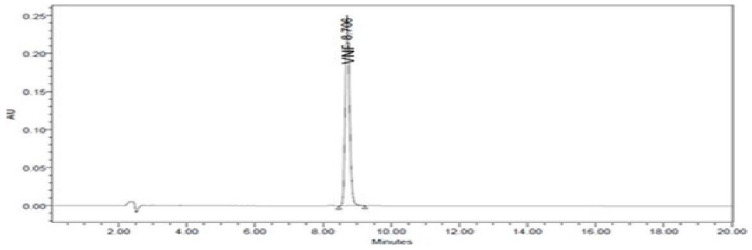
Fig. No.13: Chromatogram of VNF marketed formulation showing retention time 8.706 min.



Brand name : Vonity® tablet (20 mg)

Sr.No.	Assay (mg)	% Purity
1	19.98	99.99
2	19.95	99.75
3	19.98	99.99
4	19.96	99.80
5	19.98	99.99
Average	19.97	99.90
SD	0.014	0.11
% RSD	0.07	0.11

Table No. 12: Results and statistical data for estimation of VNF in marketed formulation

Validation parameters:

Accuracy
Precision
Ruggedness
Robustness
Linearity and range
Specificity
Placebo Interference study

Accuracy:

The accuracy samples were prepared by spiking the standard into the pre-analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

The % Recovery was then calculated by using formula

$$\% \text{ Recovery} = A\text{-}B \times 100 / C$$

Where- A = Total amount of drug estimated.
B = Amount of drug found on pre analyzed basis.
C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

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	VNF		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	16	20	24
	16	20	24
	16	20	24
Amt taken (µg/ml)	16	20	24
	16	20	24
	16	20	24
Amt recovered (µg/ml)	15.98	19.98	23.98
	15.98	19.98	23.99
	15.99	19.99	23.98
% Recovery	99.88	99.90	99.91
	99.88	99.90	99.95
	99.93	99.99	99.91
Mean % recovery	99.89	99.93	99.92
% RSD	0.48	0.39	0.56

Table No 13: Accuracy studies by standard addition method Acceptance criteria:

The % RSD for the triplicate at each spike level shall be NMT 2.0.

The overall % RSD for % recovery for all spike levels shall be NMT 2.0.

The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

Precision:

System precision

Prepared the standard solution as per test method and inject into the HPLC system in three replicates. Calculate the % RSD for the area responses and record the observations into the following table

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.217	NMT 2.0
2	Theoretical plates	9557.53	NLT 2000
3	Tailing factor	1.278	NMT 2.0

Table No. 14: Results for System Precision showing system suitability

Where, NMT - Not More Than NLT – Not Less Than

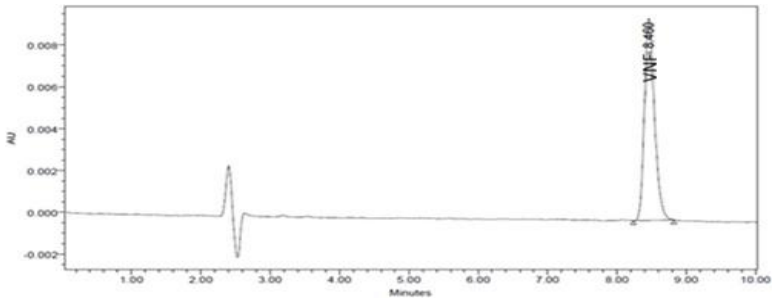

Fig.no 14: Chromatogram System precision Showing Repeatability

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Injection No.	Area Response
	VNF
1	650298
2	651600
3	649500
Average	650466
SD	11060
% RSD	0.70

Table No. 15: System precision Showing Repeatability Acceptance criteria:

% RSD for replicate injections shall be NMT 2.0

Method precision:

Prepared three sample solutions as per the test method and injected into the HPLC system by following the conditions prescribed in the Test method.

Procedure:

Sample solution was prepared and injected into the HPLC system, the chromatograms were recorded for peak area response for the VNF. The assay and % label claim for VNF was calculated.

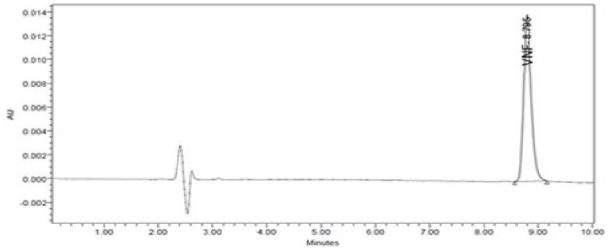

Fig.no 15: Chromatogram of Method precision

Sr.no.	VNF	
	Assay (mg)	Assay % of LC
1	19.99	99.99
2	19.99	99.99
3	19.98	99.95
Average	19.95	99.96
SD	0.70	0.45
% RSD	0.47	0.45

Table No.16: Method Precision Studies Set – I

Acceptance criteria: The% RSD for the three determinations shall be NMT 2.0

Ruggedness: Intermediate precision

Prepared three sample solutions as per the test method. Injected into the different HPLC system (preferably with different manufacturer or same manufacturer with different configuration) by using the different column and by the different analyst at different date.

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Sr.No.	VNF	
	Assay (mg)	Assay % of LC
1	19.98	99.95
2	19.97	99.90
3	19.98	99.95
Average	19.98	99.87
SD	0.012	0.824
% RSD	0.83	0.825

Table No. 17: Intermediate precision Studies (Ruggedness) Set – II Acceptance criteria: The % RSD for the three determinations shall be NMT 2.0

Data analysis between method precision and Intermediate precision:

Compared the data obtained in this section verses the data obtained in method precision and evaluate the overall average, overall SD and overall % RSD and recorded the observation into the following table

Sr.no.	% Assay of LC	
	VNF	
	Set – I	Set - II
1	99.99	99.95
2	99.99	99.90
3	99.95	99.95
Average	99.94	
SD	0.036	
% RSD	0.036	

Table No. 18: Intermediate precision (Ruggedness) evaluation of data SET – I: Method Precision data , SET – II: Intermediate Precision data

Acceptance criteria: The overall % RSD for the twelve determinations shall be NMT 2.0

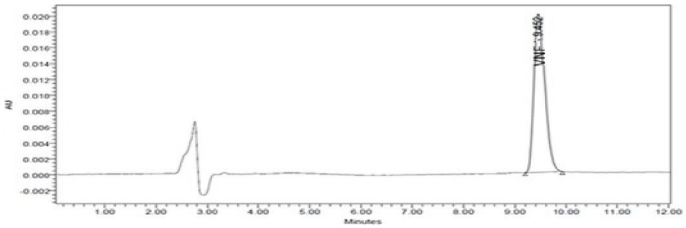
Robustness:

Effect of Variation in flow rate of mobile phase by ± 10%:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at –10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min) when compared with the Test method flow rate.

Procedure: Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

0.9 ml/min



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1.1 ml/min

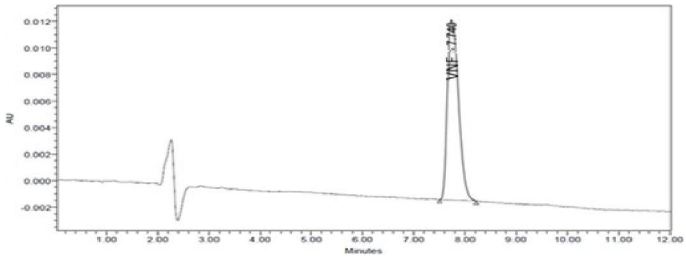


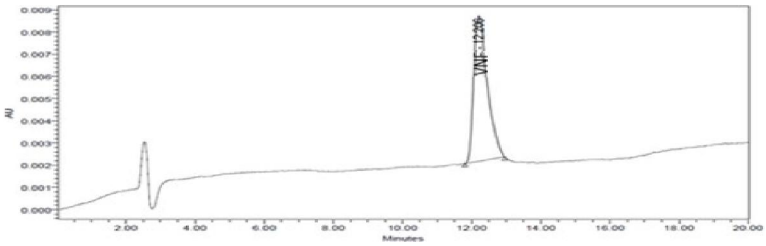
Fig. No. 16: Chromatograms of Change in Flow Rate

System suitability parameters were recorded and the results are presented in the table below.

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	VNF	1.12	0.82	0.95	NMT 2.0
2	Theoretical plates	VNF	9197.53	8938.7	8657.9	NLT 2000
3	Tailing factor	VNF	1.28	1.91	1.10	NMT 2.0
4	Retention Time (Min)	VNF	8.895	9.54	7.74	

Table No. 19: System suitability of change in Flow Rate

Observation: The allowable variation in flow rate of the method is from 0.9ml/min to
1.1 ml/min
Acceptance criteria: All the system suitability parameters shall pass
Change in organic composition of mobile phase + 10% (10mM buffer: ACN)
System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.
Procedure: Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table.
-10% ACN: (10mM buffer: ACN 55:45)



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+10% ACN: (10mM buffer: ACN 45:55)

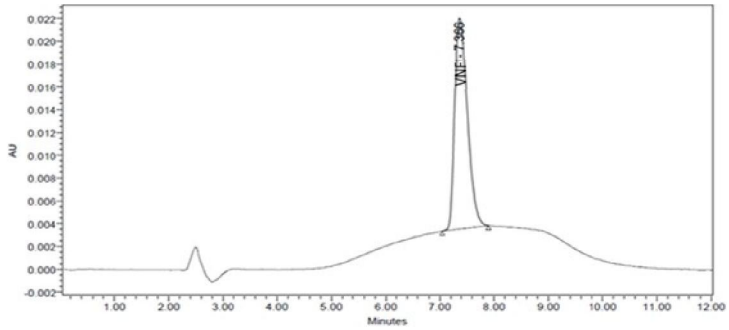


Fig. No. 17: Chromatograms of Change Organic Composition of mobile Phase

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	VNF	1.027	0.855	0.246	NMT 2.0
2	Theoretical plates	VNF	9197.53	8996	8947.6	NLT 2000
3	Tailing factor	VNF	1.28	1.166	1.08	NMT 2.0
4	Retention Time (Min)	VNF	8.98	12.206	7.360	

Table No. 20: System suitability of change in Organic Composition Observation: The allowable variation in ACN composition of method is from 90% to
110%. Acceptance criteria: 1. All the system suitability parameters shall pass.

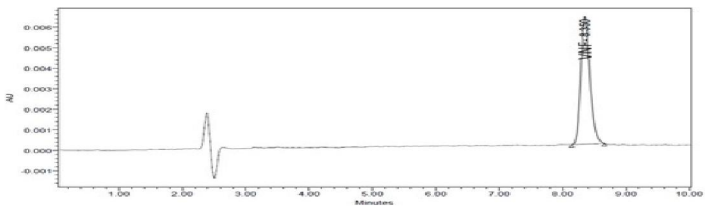
Effect of Variation in Wavelength by ± 2 units:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

Procedure:

Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks.

At 228nm wavelength



At 232 nm wavelength

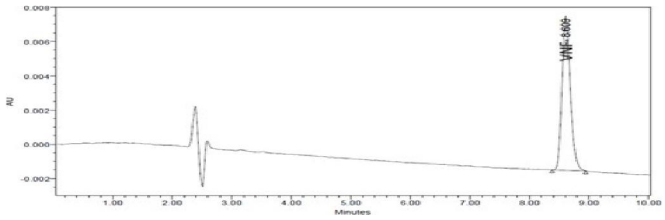


Fig. No. 18: Chromatograms of Change in wavelength.

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Sr. No.	System Suitability parameter	Observations for wavelength				Limits
		Unchanged	228nm	232nm		
1	The % RSD of peak area response for five replicate injections	VNF	1.21	0.263	0.241	NMT 2.0
2	Theoretical plates	VNF	8957.53	8887.9	8878.3	NLT 2000
3	Tailing factor	VNF	1.06	1.00	0.94	NMT 2.0
4	Retention Time (Min)	VNF	8.80	8.35	8.60	

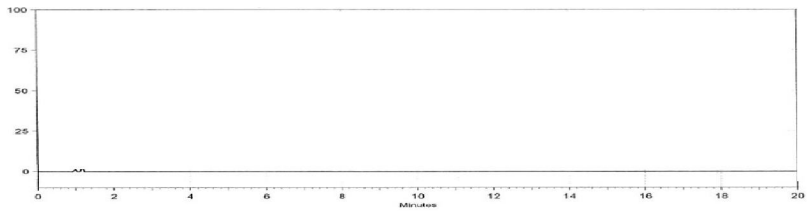
Table No.21: System suitability of change in wavelength

Specificity:

Placebo Interference study:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

Sample matrix



Placebo Preparation

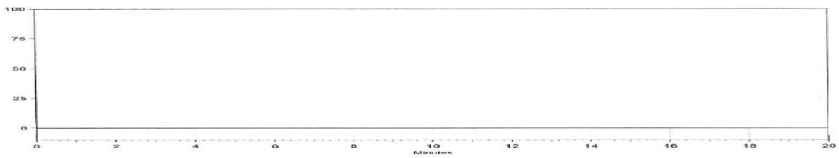


Fig. No. 19: Chromatograms of placebo interference study

Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

Table No. 22: Placebo Interference Acceptance criteria:

No interference should observe from placebo at the retention time of VNF.

Linearity and range:

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of VNF. Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

Procedure:

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for VNF peaks.

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The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for VNF, the correlation coefficient and equation of regression were recorded.

Sr. No.	% Level	VNF	
		Conc. (µg/ml)	Mean peak area
1	80	16	501000
2	100	20	649850
3	120	24	800100
4	160	32	1125000
5	180	36	1255600

Table No.23:- Observations of Linearity and range study for VNF Acceptance criteria: The correlation coefficient shall be NLT 0.99

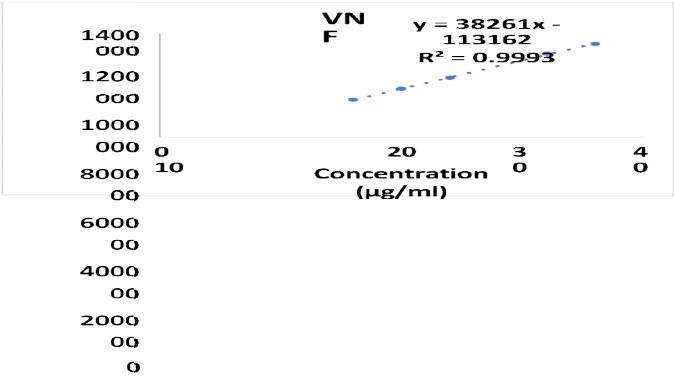

Fig. No. 20: Plot of linearity and range study for VNF

II. RESULT AND DISCUSSION

High Performance Liquid Chromatography which is a highly sophisticated technique, it is used for the determination of active molecules from their formulations. In the present study a HPLC method was developed for analysis of VNF from its tablet formulation.

Recently a tablet formulation containing VNF have been introduced in market for treating hyperacidity conditions.

Very few methods are so far reported for estimation of VNF. In the present investigation an attempt has been made to develop a simple HPLC method for estimation of VNF from its formulation. Pure standards of VNF were procured from the Arrow chem Mumbai. Percent purity of above-mentioned drug was reported by Supplier Company as follows-

Table No. 24: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Vonoprazan fumarate	Arrow Chem Mumbai.	10 g	99.9 % w/w

These were not analyzed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs

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from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system. This technique was employed in the present investigation for estimation of VNF in tablet formulation. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

HPLC Column Selected:

HPLC Waters 600 system with C18 (Thermo Hypersil gold) /4.6 x 250mm, 5µ particle size column and PDA detector were used for the study. The standard and
sample solution of VNF were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

Mobile phase composed of 10mM phosphate buffer pH 3.5 modified with 0.1% OPA and ACN (50:50 % v/v). An isocratic program was developed contributing a total run time of 20 min. The wavelength 230 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor(<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Table No. 25: Chromatographic Parameters:

Column	C18 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	230 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	20 minutes
Mobile Phase	10mM phosphate buffer pH 3.5 modified with 0.1% OPA and ACN (50:50 % v/v)

Mobile phase-preparation

Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and make up the volume upto the mark with HPLC water.

Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation ofstock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

	Peak area	Retention Time	Symmetry	No. of theoretical Plates
Sr.No	VNF	VNF	VNF	VNF
1	650269	8.90	1.20	9526
2	648952	8.92	1.30	9588
3	645896	9.01	1.10	9645
4	652698	8.98	1.35	9600
5	647895	8.99	1.30	9550
Mean	649142	8.96	1.25	9582
S.D	1551.61	0.057	0.1	46.07
%R.S.D.	0.34	0.47	1.5	0.48

Table No. 26: Summary of system suitability of Test results

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After establishing the chromatographic conditions, Mix standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Amount of drug in tablet was calculated using following formula:

$$\text{Assay (mg/ml)} = \frac{At \times Ds \times Ws \times p \times Wt \text{ mg/ml of test sample}}{As \quad Dt \quad Wt \quad 100}$$

$$\% \text{ Label claim} = \frac{\text{Assay (mg/ml)} \times 100}{\text{Label claim in mg/ml}}$$

Where,

At =Area count for sample solution. As = Area count for standard solution. Ds = Dilution factor for standard.
Dt = Dilution factor for sample. P = Potency of drug

VALIDATION

Validation of these methods was performed as per the USP guidelines for these following parameters:

Precision:

System Precision

Prepared the standard solution as per test method and injected into the HPLC system in three replicates. It was found that all system suitability parameters are well within the limits.

Method Precision

Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed R.S.D. less than 2.

Table No. 27: Data showing system Precision

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.217	NMT 2.0
2	Theoretical plates	9557.53	NLT 2000
3	Tailing factor	1.278	NMT 2.0

Table No.28: Method Precision Studies Set – I

Sr.no.	VNF	
	Assay (mg)	Assay (mg)
1	19.99	19.99
2	19.99	19.99
3	19.98	19.98
Average	19.95	1.499
SD	0.70	0.70
% RSD	0.47	0.47

Linearity & Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 80% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y-axis. The R2value was found to be 0.999 for VNF. The result show that an excellent correlation exists between concentration and mean peak areas within the concentration range. Thus the method developed is accurate, precise, specific, & linear. Hence it can be said that, RP-HPLC is the most accurate, precise and reproducible among all methods.

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Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results werewell within the range 99-101%. Thus the method was found to be accurate.

Table No. 29: Result of Accuracy Studies

	VNF		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	16	20	24
	16	20	24
	16	20	24
Amt taken (µg/ml)	16	20	24
	16	20	24
	16	20	24
Amt recovered (µg/ml)	15.98	19.98	23.98
	15.98	19.98	23.99
	15.99	19.99	23.98
% Recovery	99.88	99.90	99.91
	99.88	99.90	99.95
	99.93	99.99	99.91
Mean % recovery	99.89	99.93	99.92
% RSD	0.48	0.39	0.56

Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.
Effect of variation in flow rate of mobile phase by ± 10%
Organic phase composition (± 10%)
Change in Wavelength by ± 2 units
The results suggested all the system suitability parameters were within limits.

Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of VNF. Thus, no interference was foundat the Retention time of VNF.

III. SUMMARY AND CONCLUSION

SUMMARY

Tablet formulation containing VNF is recently introduced in market to treat hyperacidity conditions. Literature survey revealed very few methods for the estimation of VNF.

The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for estimation of VNF in the oral tablet formulation.

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In the developed RP-HPLC method the analyte were resolved using Mobile phase composed of 10 mM phosphate buffer pH 3.5 modified with 0.1 % OPA and ACN (50:50). A isocratic program was developed contributing a total run time of 20 min. using HPLC auto-sampler system having PDA detector with EMPOWER Software and C18 (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size column, the detection wavelength was 230 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.

CONCLUSION

From the results of the study it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the VNF in the tablet formulation.

The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of tablet formulation of VNF was done by the developed and validated RP-HPLC method.

The RP-HPLC method was also simple, accurate, precise, reproducible and economical too. It may be adopted for routine control analysis of VNF alone or in combined dosage form.

No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

Suitability of these methods on biological samples needs to be studied.

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