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DEVELOPMENT, VALIDATION AND STABILITY-INDICATING RP-HPLC METHOD FOR VONOPRAZAN: A RAPID APPROACH WITH SIMPLE MOBILE PHASE

G. LAVANYA[1], L. SIVA SANKER REDDY[2*], S. MUNNER[3], R. NAGESWARA RAO[3], L.A RAMA PRASAD[4]

[1]M. Pharmacy, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
[2]Department of Pharmaceutical Chemistry, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
[3]Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
[4]Associate Vice President, JSL Health Sciences Pvt.Ltd, A-17, Cooperative Industrial Estate, Balanagar, Hyderabad, Telangana 500037, India.

***Corresponding author**
L. SIVA SANKER REDDY
Professor and Head of the Department, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518501, Andhra Pradesh, India.
Email: shiva25shankerreddy@gmail.com

Abstract:

Background: Vonoprazan fumarate is a potassium-competitive acid blocker (P-CAB) that inhibits the $H^{+/}K^{+}$-ATPase enzyme to suppress gastric acid secreaction and treat acid-related disorders. **Materials and Methods:** Vonoprazan fumarate was used for the study. HPLC grade methanol, orthophosphoric acid (OPA), and HPLC water were used to prepare the mobile phase and standard solutions. The analysis was carried out using an RP-HPLC system with a UV-PDA detector. Separation was performed on a C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of methanol and 0.1% OPA in the ratio of 90:10 (v/v) at a flow rate of 1 ml/min, with detection at 254 nm. A new simple stability indicating method was developed and validate their parameters such system suitability, linearity, precision, accuracy, LOD, LOQ, robustness, assay and degradation studies by using RP-HPLC. **Results and Discussion:** The retention time of vonoprazan fumarate was found to be 2.6 minutes. The method showed good linearity in the concentration range of 10–50 µg/ml with a correlation coefficient ($R^2 = 0.9999$). The validation parameters and degradation studies were within acceptable limits according to ICH Q2(R1) guidelines. **Conclusion:** The developed method consumes nearly 20% less mobile phase as compared to the already reported methods, making it economical and environmentally friendly for routine analysis of vonoprazan fumarate in API and dosage forms.

Key words: Methanol, Ortho phosphoric acid, RP-HPLC, Vonoprazan fumarate.

1.INTRODUCTION:

Vonoprazan fumarate is the first-in-class drug belonging to the class of potassium-competitive acid blockers (P-CABs). Chemically, it is described as N-methylmethanamine fumarate of 1H-pyrrol-3-yl-(5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)). It is soluble in organic solvents such as dimethyl sulfoxide and methanol but practically insoluble in water. The molecular weight

of vonoprazan fumarate is 461.5 g/mol, and the empirical formula is $C_{21}H_{20}FN_3O_6S$.

Vonoprazan fumarate, a member of the potassium-inhibiting acid neutralizer (PIAN) class, exerts its pharmacological activity by selectively preventing the H^{+}/K^{+}-ATPase enzyme mechanism. At the secretory surface of the gastric parietal cells, vonoprazan inhibits both the basal and stimulated secretion of stomach acid. In healthy volunteers, the plasma protein binding of vonoprazan has been reported in the range of 85% to 88%. Majorly, vonoprazanis are discharged through urine (67%), and the remaining are excreted through faeces (31%). Pharmacokinetic evaluations have demonstrated that, following a single 20mg oral dose of vonoprazan, the elimiation half-life is approximately 7.1 hours. At steady state, the elimination half-life is slightly lowered to 6.8 hours. The apparent oral clearance has been reported as 97.3 L/h after a single dose, while at steady state it decreases to 81.3 L/h. [1,2]

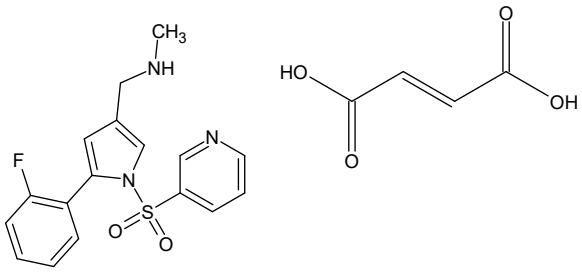

Figure1: Chemical structure of Vonoprazan fumate

In the Journal of Separation Science [3], Na Cao, and his co-workers detailed the process-related impurity detection, characterisation, and HPLC-quantification of vonoprazan fumarate. As a mobile phase, they employed B-Trisodium phosphate orthophosphate buffer (35:65) (pH 6.0) and A-Trisodium phosphate buffer & methanol (95%, 5v/v). C_{18} (250 mm x 4.6 mm x 5µm) column was put to use to evaluate the drug, and the column temperature was kept at 30°C. Nader Mohamed Alzaghal et al. [4] developed and validated an RP-HPLC method for the estimation of vonoprazan in bulk and tablet dosage forms. The mobile phase consisted of buffer (0.01 M potassium dihydrogen phosphate and 0.02 M sodium phosphate in 900 mL water) and acetonitrile (65:35 v/v) adjusted to pH 6.8, with a flow rate of 1.0 mL/min. The reported retention time was 3.3 min, in this system they used Agilent 150 x 4.6 mm, 5 µm, 5HC C_{18} column at 35 °C column temperature. Kanaga P, outlined an Analytical method development & validation of RP- HPLC [5]. Acetonitrile water (65:35 v/ v) (6.5 pH) was utilized as a mobile phase for the simultaneous estimation of vonoprazon and dopamine in pharmaceutical dosage form. The elution time was 7.185 minutes at flow rate of 1.0 mL/min. Mona M. Abdel-Moneim et al. [6] developed a chromatographic assay for the recently approved co-formulation of vonoprazan fumarate with low-dose aspirin (AGREE). The study was published in the BMC Chemistry journal, where a C18 column was used for the analysis. Under optimized chromatographic conditions, vonoprazan fumarate showed a retention time of 6.59 minutes.

Yang Liu, Aoxue Liu, et al. [7] reported the development of a stability-indicating HPLC method for the simultaneous determination of ten related substances in vonoprazan fumarate drug substance. The study was published in the Journal of Pharmaceutical and Biomedical Analysis. The mobile phase consisted of A: 0.03 M Na phosphate buffer: methanol: acetonitrile

(72:25:3 v/v/v) and B: 0.03 M Na phosphate buffer: acetonitrile (30:70 v/v) at pH 6.5. A Phenomenex Kinetex EVO C18 column was maintained at a column temperature of 30 °C, and the drug was eluted at 5.5 min with a flow rate of 1.0 mL/min. Similar findings were reported in the literature for the quantification of vonoprazan. The aim of the present study was to develop and validate a chromatographic method for the quantification of vonoprazan fumarate using various validation parameters.

2. MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used in this work included Vonoprazan fumarate (JSL Laboratories Pvt Ltd), Methanol-HPLC grade (Merck), Water (HPLC grade, Merck), ortho phosphoric acid (s d fine-chem limited).

Instrumentation

Chromatographic analysis was carried out using a Shimadzu LC-20AD high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA) detector. UV–Visible spectroscopic measurements were performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Sample weighing was conducted using an ATY224 analytical balance (Shimadzu, Kyoto, Japan). Sonication was performed using a SONICA 2200MH ultrasonicator (Soltec, Milan, Italy). A hot air oven (Vision Lab Equipment, Hyderabad, India) and a UV cabinet (Monoquartz, Chennai, India) were used for stress degradation studies.

RP-HPLC Chromatographic conditions

Chromatographic Conditions

RP-HPLC Chromatographic conditions: Chromatographic analysis was performed using a Shimadzu HPLC system equipped with a PDA detector. Separation was achieved on a C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of methanol and 0.1% ortho-phosphoric acid (90:10, v/v), filtered (0.45 µm) and degassed prior to use. The flow rate was maintained at 1.0 mL/min with an injection volume of 20 µL. Detection was carried out at 254 nm, and the column temperature was set at 30°C. A stock solution (1000 µg/mL) was prepared by dissolving 10 mg of vonoprazan fumarate in 10 mL methanol, and further dilutions were made using the mobile phase. The analysis was performed under isocratic conditions with a run time of 5 min, and the retention time was approximately 2.6 min.

Preparation of Mobile phase:

Medication was dissolved in the stock solution using only methanol. To create the following dilutions, 90 milliliters of methanol and 10 milliliters of 0.1% orthophosphoric acid were combined to create a 90:10v/vv/v ratio.

Preparation of Standard solution:

The standard solution contains 1000 µg/ml. First, 10 mg of vonoprazan fumarate API was precisely weighed on an analytical electronic balance. The drug was then transferred into a 10 ml volumetric flask, and a few ml of methanol was added as a solvent. The drug was then shaken

for a few minutes to dissolve it, and finally, the solvent was made up to the mark and thoroughly mixed.

The mobile phase (methanol: OPA0.1% 90:10v/v) is used to generate different concentrations from the aforementioned standard stock solution. It is well mixed, filtered through a 0.45µ- nylon membrane filter, and then injected into an HPLC. The peak responses are clearly documented.

HPLC METHOD VALIDATION

System Suitability

Standard solutions were prepared and injected into the chromatographic system. System suitability studies were performed to evaluate the performance of the system. Chromatograms were obtained under optimized conditions at a concentration of 30 µg/mL.

Linearity

Linearity was established over the concentration range of 10–50 µg/mL for vonoprazan fumarate. A calibration curve was constructed by plotting peak area versus concentration of the drug solution.

Precision

1. Intraday precision
2. Interday precision

Intra-day Precision

Intra-day precision was evaluated by analyzing six replicate injections of a 30 µg/mL solution at three different time points (9:00 AM, 12:00 PM, and 4:00 PM) on the same day. The peak areas were recorded, and %RSD was calculated to assess method precision.

Inter-day Precision

Inter-day precision was assessed by analyzing six replicate injections of a 30 µg/mL solution over three consecutive days. The peak areas were recorded, and %RSD was calculated to evaluate the reproducibility of the method.

Accuracy

Accuracy was assessed by preparing solutions at 50%, 100%, and 150% levels of the target concentration using tablet powder equivalent to vonoprazan fumarate. These solutions were injected into the chromatographic system, and the results were evaluated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Detection limit:

LOD= 3 × standard deviation/ slope

Quantification limit:

LOQ= 10 × standard deviation/ slope

Robustness

Robustness of the method was evaluated by introducing deliberate variations in chromatographic conditions. Standard solutions at a concentration of 30 µg/mL were prepared from the stock solution.

The flow rate was varied at 0.8, 1.0, and 1.2 mL/min; the detection wavelength was adjusted to 252 nm, 254 nm, and 256 nm; and the column temperature was altered to 25°C, 30°C, and 35°C. The resulting chromatographic responses were recorded.

During robustness testing, the flow rate was varied by ±0.2 mL/min, the wavelength by ±2 nm, and the column oven temperature by ±5°C from the optimized conditions.

Assay

Twenty tablets were weighed to determine the average weight. Tablet powder equivalent to 10 mg of vonoprazan fumarate (115 mg) was dissolved in methanol, sonicated, and diluted to obtain a stock solution (~1000 µg/mL). This was further diluted with mobile phase (methanol: 0.1% orthophosphoric acid, 90:10 v/v) to 30 µg/mL, filtered (0.45 µm), and injected (n = 6) into the HPLC system.

Degradation Studies

1. Acid Degradation

An aliquot of 0.3 mL of the sample solution was transferred into a 10 mL volumetric flask. To this, 1 mL of 0.1 N HCl was added, and the solution was heated at 50°C for 15 minutes, then cooled to room temperature. The solution was neutralized with 1 mL of 0.1 N NaOH and diluted to volume with the mobile phase. The prepared solution was injected into the HPLC system, and the chromatographic responses were recorded.

2. Base Degradation

An aliquot of 0.3 mL of the sample solution was transferred into a 10 mL volumetric flask. To this, 1 mL of 0.1 N NaOH was added, and the solution was heated at 50°C for 15 minutes, then cooled. The solution was neutralized with 1 mL of 0.1 N HCl and diluted to volume with the mobile phase. The solution was injected into the HPLC system, and the chromatographic responses were recorded.

3. Peroxide Degradation

An aliquot of 0.3 mL of the sample solution was transferred into a 10 mL volumetric flask. To this, 0.1 mL of 3% hydrogen peroxide (H_2O_2) was added, and the volume was made up with the mobile phase. The solution was kept at room temperature for 12 hours. After incubation, the solution was injected into the HPLC system, and the chromatographic responses were recorded.

4. Thermal Degradation

An aliquot of 0.3 mL of the sample solution was transferred into a 10 mL volumetric flask. To this, 1 mL of mobile phase was added, and the solution was heated at 60°C for 10 minutes, then cooled. The volume was adjusted with the mobile phase, and the solution was injected into the HPLC system. The chromatographic responses were recorded.

3. RESULTS AND DISCUSSION

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Table 1: System suitability parameters for the suggested HPLC technique.

S. No.	Peak area	Ret Time(min)	Theoritical plate count	Tailing Factor
1	1266257	2.614	2264	1.298
2	1294931	2.617	2245	1.254
3	1284722	2.616	2242	1.293
4	1246663	2.616	2256	1.294
5	1249022	2.614	2242	1.262
6	1259700	2.598	2319	1.278
Average	1266882.50	2.61	2261.33	1.26
STDEV	19415.31	0.01	29.58	0.02
%RSD	1.53	0.28	1.31	1.46
Limits	-	-	>2000	<2.0
%RSD	<2.0	<2.0	<2.0	<2.0

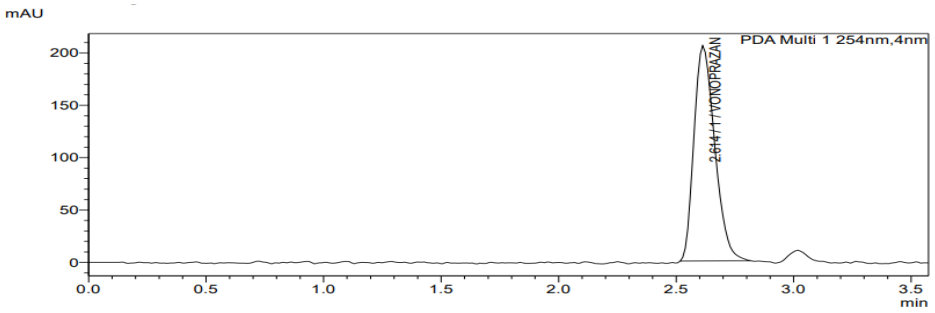

Figure 2: Chromatogram of Vonoprazan fumarate under optimized conditions.

Remark: As shown in Table 1, all system suitability parameters were found to be within the acceptable limits specified by ICH Q2(R1). The representative chromatogram is presented in Figure 2.

Linearity

Table 2: Vonoprazan fumarate Linearity

S. No	Linearity level	Conc. (µg/ml)	Peak area
1	50%	10	638010
2	75%	20	947298
3	100%	30	1275504
4	125%	40	1585816
5	150%	50	1918373

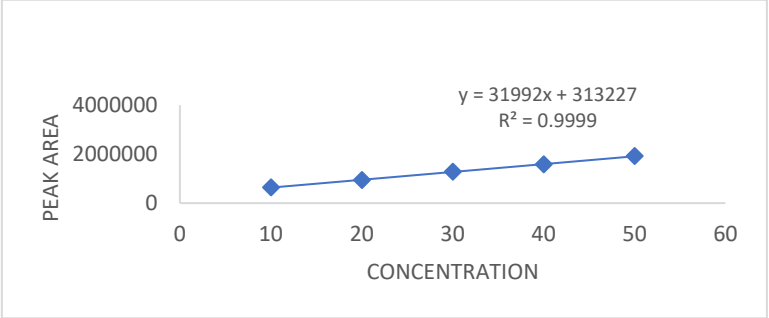

Figure:3 Linearity graph of vonoprazan fumarate

Remark: Five concentrations (10, 20, 30, 40, and 50 µg/mL) were evaluated, yielding a correlation coefficient of 0.9999. The corresponding peak area values are presented in the calibration curve shown in Figure 3.

Precision

Table 3: Intra- day Precision

S. No	9:00 AM	1:00 PM	5:00 PM
1	1219126	1221118	1242635
2	1215822	1223368	1258930
3	1240973	1223083	1260867
4	1204113	1211651	1244644
5	1261107	1267202	1258600
6	1248209	1252105	1217717
Average	1231558.33	1233087.83	1247232.17
STDEV	21883.54	21553.73	16437.48
% RSD	1.78	1.75	1.32
Limits	**% RSD <2.0**	**% RSD <2.0**	**% RSD <2.0**

Inter-day Precision

Table 4: The suggested HPLC method's precision

S. No	Day 1	Day 2	Day 3
1	1266257	1219126	1250383
2	1294931	1215822	1231909
3	1284722	1240973	1264460
4	1246663	1204113	1278203
5	1249022	1261107	1272192
6	1259700	1248209	1245785
Average	1266882.52	1231558.33	1257155.33
STDEV	19415.31	21883.54	17521.36
% RSD	1.53	1.78	1.39
Limits	**% RSD: <2.0**	**% RSD: <2.0**	**% RSD: <2.0**

Remark: The precision parameter also met the required limits of %RSD which is must be less than 2.

Accuracy

Table 5: Accuracy displaying recovery percentage

S. No.	Levels	Conc taken (µg/ml)	Conc added (µg/ml)	Peak Area	Conc recovered (µg/ml)	% Recovery	Average Recovery
1.	**50%**	30	15	1743319	14.7016	98.0103	**100.72**
2.		30	15	1774375	15.6723	104.4820	
3.		30	15	1751853	14.9683	99.7887	

4.		30	30	2210261	29.2971	97.6571	
5.	**100%**	30	30	2209879	29.2852	97.6173	**98.85**
6.		30	30	2244981	30.3824	101.2747	
7.		30	45	2714500	45.0585	100.1301	
8.	**150%**	30	45	2713143	45.0161	100.0358	**100.10**
9.		**30**	**45**	**2714358**	**45.0541**	**100.1202**	

Figure 4.1

Accuracy (50%)

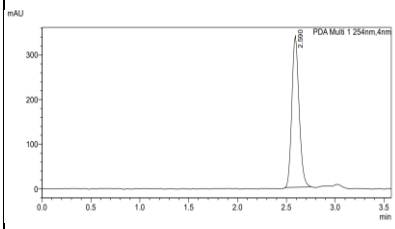

Figure 4.2

Accuracy (100%)

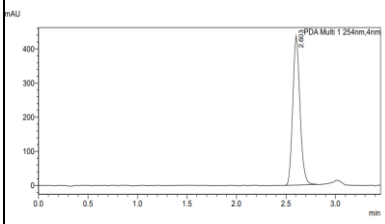

Figure 4.3

Accuracy (150%)

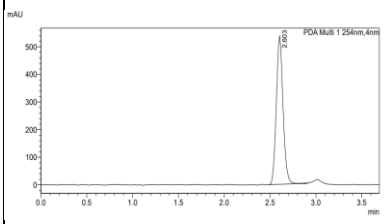

Figure:4 Accuracy Chromatogram of vonoprazan fumarate

Remark: The accuracy test was performed at 50 %, 100%, 150% and the respective % average values are 100.72, 98.85 and 100.10.

Table 6: HPLC Method LOD and LOQ

Parameters	**Slope from Linearity**	**SD of peak from system suitability**
	31992	19415.31
LOD = 3.3 x SD/Slope	2.003µg/ml	
LOQ = 10 x SD/Slope	6.069 µg/ml	

Robustness

Table 7: HPLC method Robustness

Parameter	**Condition**	**Condition**	**RT**	**Peak area**	**Theoretical plates**	**% Assay**
Flow (ml/min) min±0.2 ml	Less flow	0.8ml/min	3.602	1250383	3923	101.40
	Optimized	1.0 ml/min	3.599	1241909	3991	100.71
	More flow	1.2 ml/min	2.174	1210303	3876	98.15
Temp (°C) min±5°C	Less temp	25°C	2.602	1216907	4123	98.68
	Optimized	30°C	3.599	1241909	3991	100.71
	More temp	35°C	2.585	1226850	4213	102.73
Wave length (nm) min±2 nm	Low WL	252 nm	2.598	1239141	4448	100.49
	Optimized	254 nm	3.599	1241909	3991	100.71
	High WL	256 nm	2.596	1270018	4056	102.99

Remark: The change in flow rate, temperature, wave length to check the robustness of the method was successful for all the above mentioned parameters. The observed values are within the acceptance limits.

Assay

Table 8: Accuracy displaying recovery percentage

S. No	Peak area	% Assay
1	1264103	98.134
2	1276514	99.098
3	1289123	100.076
4	1268694	98.490
5	1289568	100.111
6	1286579	99.879
Average	1279096.83	99.30
STDEV	11007.79	0.85
% RSD	0.86	0.86
Limits	**Average Assay: 98 – 102%** **%RSD : < 2.0**	

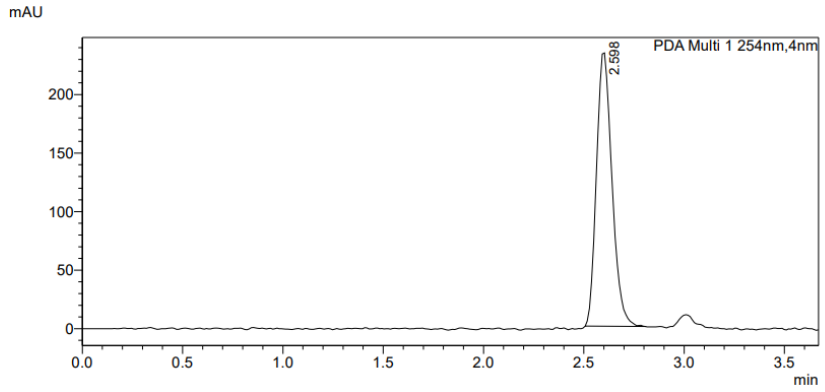

Figure:5 Assay of chromatogram

Remark: The mean assay was found to be 100.35 and this method is validated for assay.

DEGRADATION STUDIES

Table 9: Degradation studies

S. No	Condition	Peak Area	% Assay	% Degradation
1	Acid	1110829	90.081	9.919
2	Base	1102622	89.415	10.585
3	Peroxide	1118866	90.733	9.267
4	Thermal degradation	1125987	91.310	8.690

Remark: % degradation was within the limits for acid, base, peroxide, thermal degradation.

Summary table of the parameters for the method development, validation, and degradation studies

Table 10: Summary

S. No	Parameters	Results
1	Mobile phase	Methanol:0.1%OPA (90:10)
2	Wave length detection	254nm
3	Calibration range (µg/ml)	10-50µg/ml
4	Regression equation	y = 31992x+ 313227
5	Slope (m)	31992
7	Correlation coefficient (r^2)	0.9999
8	Retention time	2.6min
9	Systems Suitability	1.53(%RSD)
10	LOD (µg/ml)	2.003µg/ml
11	LOQ (µg/ml)	6.069µg/ml
12	Accuracy	98.85-100.72%
13	Inter-day Precision	1.39-1.78(%RSD)
14	Intraday Precision	1.32-1.78 (%RSD)
15	Robustness Flow rate	98-101%
16	Robustness Temperature	98-102%
17	Robustness Wave length	100-102%
18	Acid degradation	90%
19	Base degradation	89.4%
21	H_2O_2 degradation	90.7%
22	Thermal degradation	91.3%

4. Conclusion

A method for the estimation of vonoprazan fumarate in API and its tablet dosage form was developed using RP-HPLC. The method was successfully validated following ICH Q2R1 guidelines and for stability studies ICH Q1A(R2). The linearity was in the range of 10µg/ml to 50µg/ml, and the theoretical plates were found well beyond 2000. The %RSD for accuracy, precision, robustness were found <2 which indicates that the parameters are within the limits of the guidelines. The LOD & LOQ were found to be 2.003µg/ml and 6.069µg/ml respectively. The degradation studies were also performed to the tablet dosage form and we have taken care that nearly 10% of the drug degraded to show that our method was still applicable for the assay in the presence of the unknown degraded products.

This method utilizes nearly 40-50% less mobile phase compared to the reported methods and it only consumes methanol and water containing 0.1% OPA (no pH adjustment is required here) which are easy to prepare and considerably cheaper and environment friendly compared to the solvents used in the already existing methods. We could achieve the retention time of 2.6 min, which is much less when compared to existing methods.

Thus, we can consider that this method as sensitive, economical, reproducible and considerably rapid in the assay of a vonoprazan fumarate in API and Dosage form. This method can be used without any hesitation and with confidence for the routine analysis of vonoprazon fumarate.

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Author Contributions

All authors contributed to the study design, data acquisition, and data interpretation. All authors agreed to submit the manuscript to the current journal and approved the final version of the manuscript.

Ethical Approval

This study did not involve any human participants or experimental animals.

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