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Method Development and Validation for Estimation of Vonoprazan By RP-HPLC Method In Bulk and Tablets Dosage Form

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Abstract

A rapid, simple, and sensitive reversed-phase HPLC-UV method of analysis for Vonoprazan in pharmaceutical formulation. Effective chromatographic separation was achieved using Agilent 5 HC C18 – (150X4.6mm, 5µm) with isocratic elution of the mobile phase consisting of Buffer (0.01M potassium dihydrogen phosphate (1.36 g/L) and 0.02M sodium dihydrogen phosphate (2.4 g/L) in 900ml water, adjust pH 6.8 by 5N sodium hydroxide solution than complete till 1000 ml by water): Acetonitrile (65: 35 v/v). The wavelength of detection was set to be 225 nm (UV detector), and a flow rate of 1.0 ml/min. was employed, 30 µl was used as injection volume and the column temperature was maintained at 35°C. Under these chromatographic conditions, the Peak of Vonoprazan was obtained at a retention time about 3.3 min. and run time of about 6.0 minutes. The developed method was validated according to ICH guidelines for the validation of analytical procedures and was successfully used.

Keywords: Vonoprazan; HPLC; Validation; Forced degradation.

1. Introduction

Vonoprazan as vonoprazan fumarate (1-(5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1 H-pyrrol-3-yl)-N-methyl methanamine mono fumarate) "Figure 1" which is a first-in-class potassium-competitive acid blocker, it was approved in the Japanese market in February 2015. Vonoprazan is used in the form of fumarate for the treatment of gastroduodenal ulcers (including some drug-induced peptic ulcers) and reflux esophagitis, and also can be combined with antibiotics for the eradication of Helicobacter pylori. The molecular formula is C21H20FN3O6S, and the molecular weight is equal to 461.4653 [1]–[3]. According to the safety data sheet for vonoprazan and toxicological information, it is irritant to skin and mucous membranes, it has an irritant effect on the eyes, and no sensitizing effects are known [4].

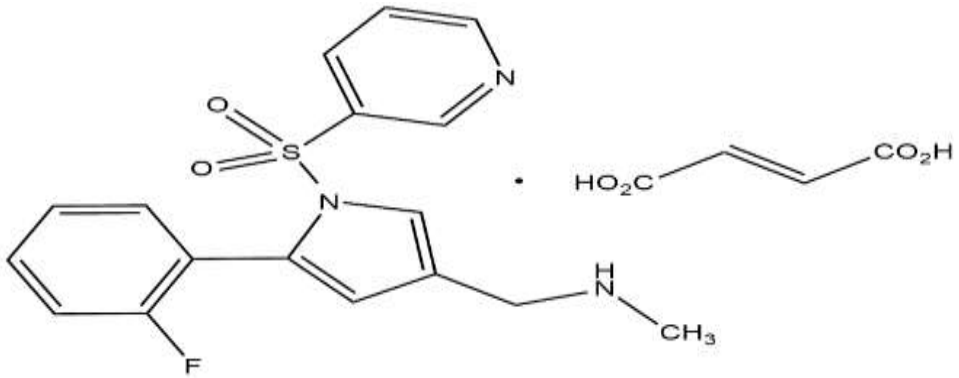

Figure 1. Chemical structure of vonoprazan fumarate.

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A tablet formulation containing 10 mg and/or 20mg of vonoprazan (as fumarate) has been introduced into clinical practice. A survey of literature revealed that few HPLC and spectrophotometric methods are reported for the determination of vonoprazan. A drug combination of vonoprazan and aspirin was determined by ultraviolet spectrophotometric [5] but spectroscopy is not a separation technique, so this technique cannot distinguish and separate the interferences, also other spectroscopic techniques; fluorimetric method for the estimation of vonoprazan in human plasma [6]. Liquid chromatography with mass spectrometry used to determine some impurities related to vonoprazan fumarate [7] this aspect makes use of an expensive technique such as LC-mass, but our objective is a simple and straightforward method that can be used to determine the percentage of the active substance and is suitable for use as a separation process in routine work and stability studies to separate interferences like unspecified impurities and prove that they are within the permissible limit, using simple, fast, effective and inexpensive methods, also there is other specific method for related by LC UV detector [8]. HPLC method for the determination of vonoprazan pyroglutamate and vonoprazan fumarate [9],and separation achieved by gradient elution but as for this method, it specializes in separating vonoprazan using isocratic elution, which makes it the simplest and most stable. The method of analysis that we are discussing is specific to the analysis of vonoprazan in particular in its dosage form, for which a special analysis monograph was not approved in any of the known pharmacopoeias (Table 1).

Analytical method objectives are often defined as method acceptance criteria for peak resolution, precision, limit of detection and quantitation, specificity, and sensitivity. The focus in reversed-phase liquid chromatography (RPLC), The separation is based on analytes partition coefficients between a polar mobile phase and a hydrophobic (nonpolar) stationary phase. The stationary phases were solid particles coated with nonpolar liquids. These were quickly replaced by more permanently bonding hydrophobic groups, such as octadecyl (C18) bonded groups, on a silica support [10], [11].

This work has been performed to develop and validate method for the quantification of vonoprazan and evaluate its stability. The proposed method was successfully applied to the assay of commercial tablets. This method has been successfully used in either the quality control testing or the stability testing under various conditions according to ICH guidelines [12] of the selected pharmaceutical formulations, brand product (Takecab 10mg film coated tablets) [13], [14], and pure raw material.

This method is distinguished in that it is the most straightforward in terms of using a straightforward mobile phase composition (isocratic elution), as well as using the appropriate organic solvent to sharpen the peak and its compatibility with the type of stationary phase, choosing the right flow rate and mobile phase proportions to provide quick separation and to prevent negative peaks, select the proper wavelength without sacrificing chromatographic quality. Also, a wide range of variables that may occur was tested and studied to ensure the quality of the presented analysis method and to cover this fully in the section on robustness and ruggedness.

This work aims to achieve a suitable analytical method used to estimate the percentage of vonoprazan which is a novel active pharmaceutical ingredient in the pharmaceutical preparations that it contains, with the separation of any of the unknown impurities resulting from the degradation processes for exposure to different conditions, and to evaluate its ratio to the percentage of the active substance in order to see its suitability to the permissible limit, taking into account the daily doses of the drug. Forced degradation as part of the validation of the analytical method, sensitivity, and selectivity and take advantage of this to achieve the aforementioned desired goal.

Table 1. Comparison with the previous methods.

Previous methods	Title	Reference number	Improvement in the proposed method
A drug combination of vonoprazan and aspirin was determined by ultraviolet spectrophotometric.	Spectrophotometric Quantitative Analysis of Aspirin and Vonoprazan Fumarate in Recently Approved Fixed-Dose Combination Tablets Using Ratio Spectra Manipulating Tools.	[5]	Spectroscopy is not a separation technique, so this technique cannot distinguish and separate the interferences.
Fluorimetric method for the estimation of vonoprazan in human plasma.	Ultra-Sensitive Fluorimetric Method for the First Estimation of Vonoprazan in Real Human Plasma and Content Uniformity Test.	[6]	It is also other spectroscopic not a separation technique and specific for plasma.
Liquid chromatography with mass spectrometry used to determine some impurities related to vonoprazan fumarate.	Identification, characterization, and high-performance liquid chromatography quantification of process-related impurities in vonoprazan fumarate.	[7]	This aspect makes use of an expensive technique such as LC-mass, but our objective is a simple fast, effective, inexpensive, and straightforward method.
	Development of a stability – indicating HPLC method for simultaneous determination of ten related substances in vonoprazan fumarate drug substance.	[8]	
HPLC method.	Determination of vonoprazan pyroglutamate and vonoprazan fumarate by HPLC.	[9]	Separation achieved by gradient elution but as for this method, it specializes in separating vonoprazan using isocratic elution, which makes it the simplest and most stable.

2. Experimental.

2.1. Equipment.

The chromatographic technique performed on Agilent HPLC 1260 infinity II with Chemstation software, reversed phase column (Agilent 5 HC C18–150X4.6mm, 5µm) as stationary phase, Mettler Toledo Analytical Balance, Mettler Toledo pH meter, Ultrasonic (Elmasonic S 60H). Vacum micro filtration unit with 0.45µ membrane filter (Rocker 801), and Centrifuge (HERMILE Z 300).

2.2. Materials and Reagents.

Potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Acetonitrile (HPLC grade), Sodium hydroxide, and Hydrochloric acid from Scharlau. and 30% Hydrogen peroxide from Merck KGaA, and Ultrapure water from Laboratory Water Purification Systems (ELGA- 18.2 MΩ.cm ultrapure water). The working standard of Vonoprazan fumarate was provided by Alandalous Pharmaceutical Company.

2.3. Pharmaceutical formulation.

Vondalous 10 mg Tablets are white to off-white round film coated tablet (with average weight, 118.5 mg), Vondalous 20 mg tablets are Light pink to pink round film coated tablets (with average weight, 237.0 mg) were Produced by Alandalous Pharmaceutical Company and Takecab 10 mg FC Tablets Produced by Otsuka Pharmaceutical Co., Ltd - Japan.

2.4. Method development.

2.4.1. Preparation of mobile phase.

The mobile phase consisting of Buffer (0.01M potassium dihydrogen phosphate (1.36 g/L) and 0.02M sodium dihydrogen phosphate (2.4 g/L) in 900ml water, adjust pH 6.8 by 5N sodium hydroxide solution then complete till 1000 ml by water): Acetonitrile (65: 35 v/v).
Diluent (Solvent): consisting of (Water: Acetonitrile 1:1).

2.4.2. Chromatographic conditions.

Chromatographic separation was achieved using Agilent 5 HC C18 – (150X4.6mm, 5µm) with isocratic elution of the mobile phase. The wavelength of detection was set to be 225 nm (Uv detector), and a flow rate of 1.0 ml/min. was employed, 30 µl was used as injection volume and the column temperature was maintained at 35°C. Under these chromatographic conditions, Peak of Vonoprazan was obtained at Retention time about 3.3 min. (**Figure 1**) and Run time of about 6.0 minutes.

2.4.3. Preparation of standard solution.

An accurately weight 26.7 mg Vonoprazan fumarate working standard (equivalent to 20 mg Vonoprazan) into 100 ml volumetric flask and dissolve in 80 ml diluent, sonicate 3 min., then complete to volume with the same solvent (Standard stock 1). In 50 ml volumetric flask, transfer 5 ml from (Standard stock 1) and then complete to volume with diluent (Standard solution).

2.4.4. Preparation of sample solution.

A total of 10 tablets were weighed and finely powdered or from bulk, an accurately weighed quantity of powdered equivalent to Weight of one tablet (about 118.5mg from conc.10mg or 237.0 mg from conc. 20mg) transferred into 100 ml volumetric flask and dissolve in 80 ml diluent, sonicate 20 min, cool and complete to volume with the same solvent (sample stock 1). For Conc. 10mg/tablet: In 50 ml volumetric flask, transfer 10 ml from (Sample stock 1) and then complete to volume with diluent (Sample test solution). For Conc. 20mg/tablet: In 50 ml volumetric flask, transfer 5 ml from (Sample stock 1) and then complete to volume with diluent (Sample test solution). Filter all solutions through a 0.45 µ Nylon filter, discard the first 5 ml of the filtrate.

2.5. Organic impurities.

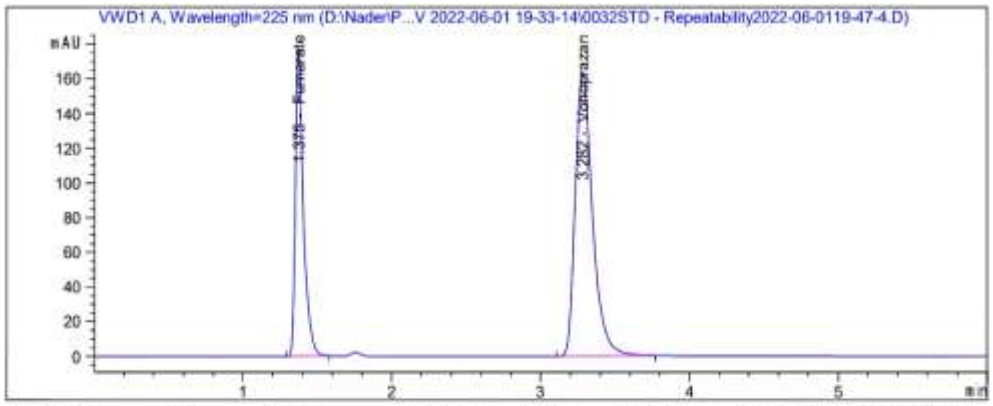
To determine any percentages of unknown impurities resulting from some degradations of the active substance in pharmaceutical preparations and compare their ratio to the ratio of the principal substance apply the method of assay regarding: chromatographic conditions, mobile phase, and diluent, except run time is 20 minutes.

2.5.1. Preparation of sample.

Weigh and transfer accurately the powdered equivalent to 20 mg Vonoprazan into 20 ml volumetric flask, then add 15 ml of diluent, sonicate for 20 mins with occasional shaking. Cool at a room temperature and complete to the volume with diluent. Centrifuge at 5000 RPM for 5 minutes and use the supernatant.

2.5.2. Preparation of standard solution.

Transfer 5 ml of sample solution (supernatant) into 100 ml volumetric flask, and complete to the volume with diluent, then Dilute 5 ml into 50 ml volumetric flask, and complete to the volume with diluent. Inject diluent as blank.



RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.375	-	666.67084	176.40973	0.60	0.0534	3670	-	-
3.282	-	1314.93201	163.19492	0.65	0.1207	4098	12.87	2.39

Figure 1. Chromatogram of vonoprazan peak.

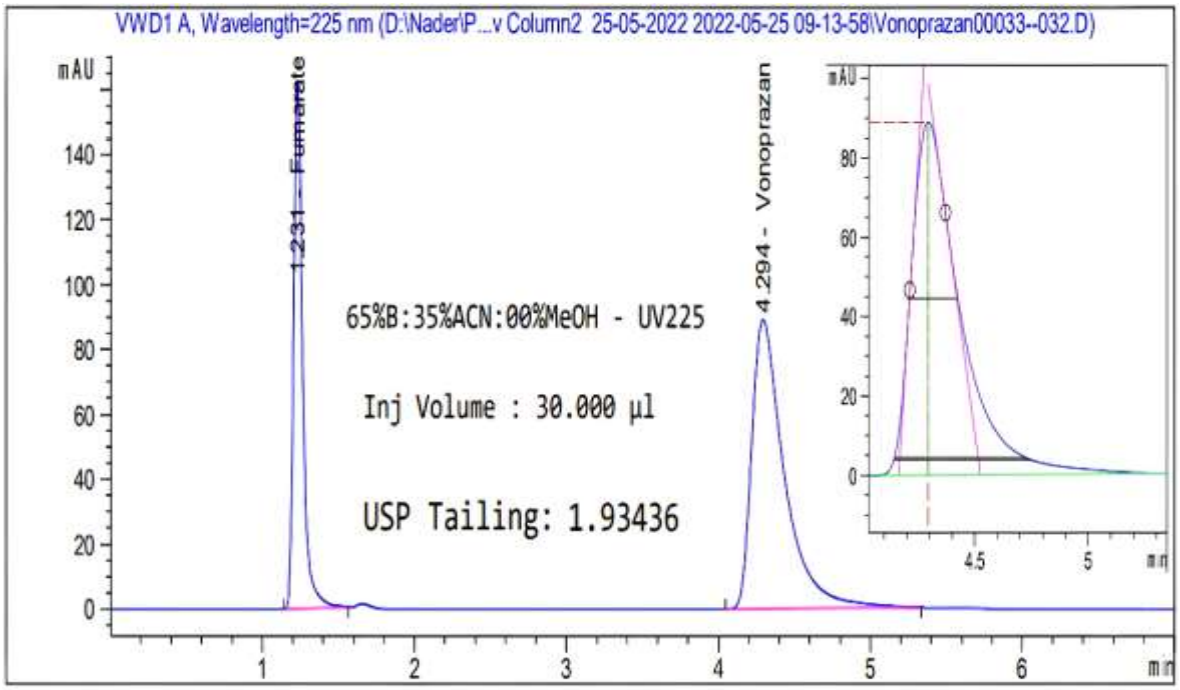
3. Results and discussion.

The aim during development of any method that achieve good resolution between analytes and peaks with acceptable peak symmetry, sharp peak, and in a reasonable analysis time.

3.1. Stationary phase.

For optimization of the stationary phase, several C18 columns from different brands were tried (**Figure 2**).

The best of them was found to be Agilent 5 HC C18 – (150X4.6mm, 5µm) Peak separation was symmetrical concerning Vonoprazan peak. In addition, acceptable theoretical plates (about 4098.00) and tailing factor not more than 2.0 (about 1.42) and resolution not less than 5.0 (about 12.87).



RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.231	-	669.24982	162.60019	0.70	0.0594	2383	-	-
4.294	-	1338.97546	89.10868	0.48	0.2125	2264	13.24	3.49

Figure 2. Chromatogram of vonoprazan peak with other HPLC columns (Luna C18).

3.2. Mobile phase.

To increase column lifetime and to make the method more suitable for frequent analysis, 0.01M potassium dihydrogen phosphate and 0.02M sodium dihydrogen phosphate in water, pH 6.8 was used as a buffer for a mobile phase, organic content of the mobile phase was optimized. The ratio of acetonitrile and buffer was found to be the best ratio that gives good peak separation and Suitable retention time.

3.3. Wavelength of detection.

Wavelengths from 210 nm to 240 nm were tried. The response of the analyte was found to be low at a wavelength higher than 240 nm and found to be too high at 210 nm till 230 nm (UV spectrum of Vonoprazan obtain in **Figure 3**), but the best peak response and signal to noise ratio was obtained when using 225 nm as a wavelength for detection.

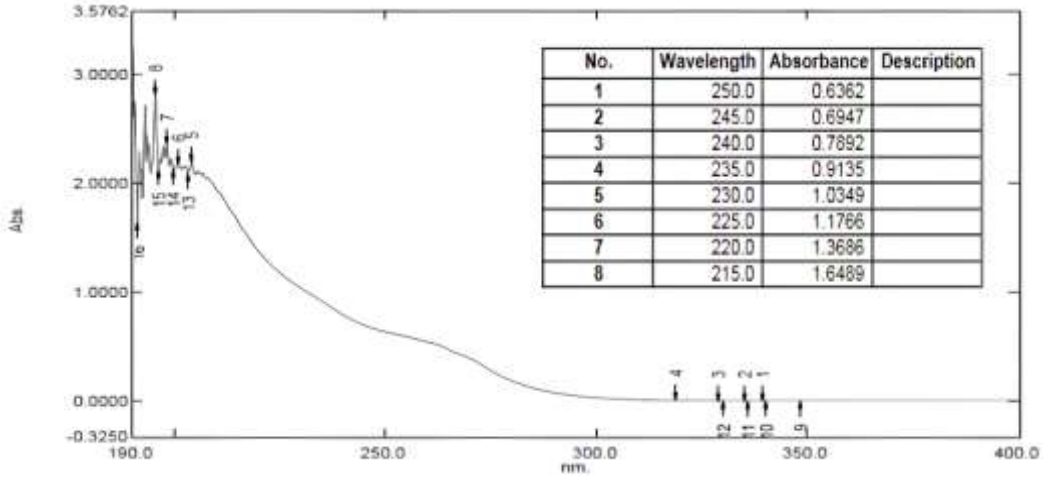


Figure 3. UV spectrum of vonoprazan.

3.4. Concentration of standard and test solutions.

An appropriate concentration of the standard substance, about 0.02, was used to give an appropriate response and samples were prepared by weighing a certain amount of the sample to equal the same concentration so that there would be an equal response in the same amount in the standard substance and samples.

4. Validation of the proposed method.

The proposed HPLC-UV method was validated as per the International Conference on Harmonization (ICH) guidelines on validation. Validation of analytical procedures: text and methodology [15], [16]. Also, there are many other guidelines and references that talk about the validation of analytical methods [17]–[20].

4.1. Linearity and concentration ranges.

The ability of analytical procedure, within a given range, to obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity was performed by preparing a minimum 5 different concentrations, and then making 3 replicates of each concentration.

The linearity study was evaluated based on the following criteria: Correlation coefficient (r) and r-squared: should have values higher than 0.998 (**Figure 4**), Significance of intercept: the 95 % confidence interval of intercept should include the zero value. This indicates statistically insignificant intercept. ANOVA test (analysis of variances) of linearity data: This indicates the probability that the regression was not obtained by chance. A small significance of F confirms the validity of the regression output (**Table 2)**.

Eight percentages of different concentrations were prepared from Vonoprazan standard (20%, 40%, 60%, 80%, 100%, 120%, 160% & 200%).

Table 2. Data of linearity for study analysis of vonoprazan.

Level (%)	Conc. (µg/ml)	Peak area	*SD*	*%RSD*
20	4.0	262.04879	0.28	0.11
40	8.0	529.14811	0.73	0.14
60	12.0	798.64868	0.65	0.08
80	16.0	1062.94726	0.20	0.02

100	20.0	1313.59562	0.81	0.06
120	24.0	1589.67513	7.08	0.45
160	32.0	2122.90877	0.66	0.03
200	40.0	2650.55241	0.79	0.03
Slope	66.3062			
Intercept	-1.7811			
R^2	0.99996			
Data from ANOVA and Statistical analysis for linearity				
Standard Error	5.217524881	**P - Value**	0.643524771	
Observations	8	**SD intercept**	3.65721877	
F	167639.8	**Significance F**	1.43262E-14	

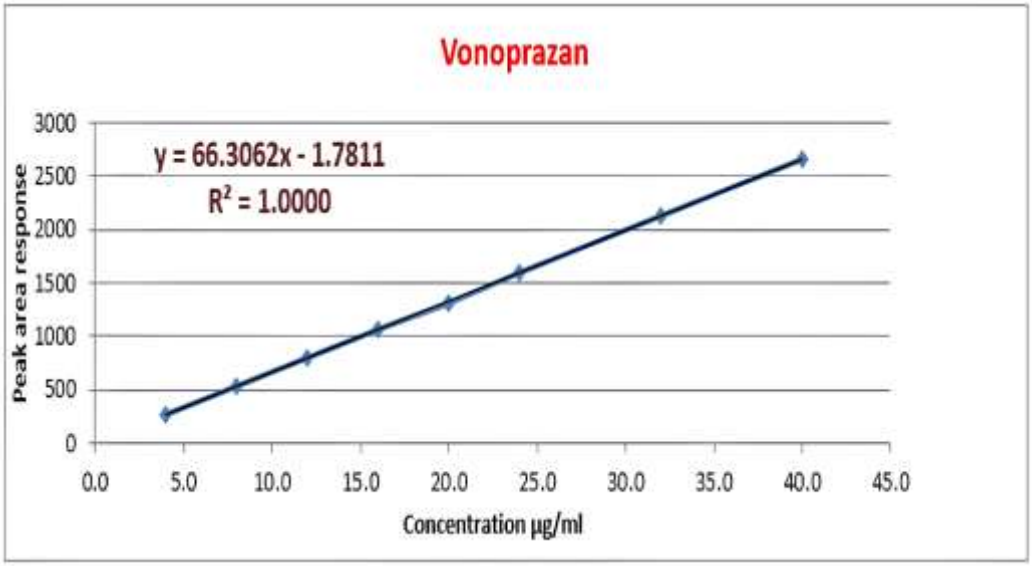


Figure 4. Linearity plot of vonoprazan.

4.2. Sensitivity.

4.2.1. Limit of Detection (LOD): It is the concentration of analyte that can be detected but not necessarily quantified.

LOD= 3.3 x SD intercept / Slope, SD mean standard deviation. Calculated from linearity and result is equal to 0.182 µg/ml.

4.2.2. Limit of Quantitation (LOQ): It is the concentration at which the peak of analyte quantified.

LOQ= 10 x SD intercept / Slope, calculated from linearity and result is equal to **0.552** µg/ml.

4.3. Precision &Repeatability.

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Repeatability expresses the precision under the same operating conditions over a short interval of time (**Table 3**). Repeatability is also termed intra-assay precision. A minimum of 6 determinations of the same homogenous sample at 100% of the test concentration (**Table 4**).

Table 3. Data of standard repeatability for vonoprazan.

Standard Repeatability	
Replicate number	**Standard response (PA)**
1	1314.93201
2	1313.96851

3	1314.37781
4	1313.20227
5	1313.36548
6	1313.23657
Average	**1313.84711**
Standard deviation (STDEV)	**0.706**
% RSD	**0.054**

Table 4. Data of test repeatability for analysis of vonoprazan.

Sample test Repeatability		
Test No.	**Peak area**	**% Assay**
1	1250.68964	97.71
2	1258.97333	98.97
3	1263.47504	97.46
4	1233.65857	97.46
5	1246.43909	98.39
6	1243.34772	97.26
Average		**97.87**
Standard deviation (STDEV)		**0.666**
% RSD		**0.680**
Acceptance criteria: RSD ≤ 2 % for Assay		

4.4. System suitability.

System suitability testing was assessed by analyzing replicate injections of standard solution [21]. The system suitability solution was analyzed using the HPLC-UV method at the previously mentioned settings. The acceptance criteria set for system suitability parameters: Theoretical plate number (N) should be more than 2000 (Found to be 4098), the tailing factor should be less than 2.0 (Found to be 1.42), the resolution should be not less than 2.0 (Found to be 12.87), and % RSD of analyte peak in replicate injections should be less than 2.0% (Found to be 0.054 %).

4.5. Accuracy and Recovery.

The accuracy of the method was determined by calculating the recoveries of Vonoprazan by analyzing solutions containing approximately 80%, 100% and 120% of the working strength of Vonoprazan. The percentage recovery results obtained are listed in **Table 5** . Recovery is between 98.0%-102.0 %.

Table 5. Data of accuracy& recovery for analysis of vonoprazan.

%Conc.	**Av. three Peak area**	**Standard deviation**	**%RSD**	**Actual conc. (µg/ml)**	**Theoretical conc. (µg/ml)**	**Recovery %**
80	**1011.77930**	1.331	0.132	**15.96**	**16.16**	**98.80**
100	**1255.94137**	2.351	0.187	**19.82**	**20.20**	**98.12**
120	**1511.18929**	0.911	0.060	**23.85**	**24.24**	**98.38**
Average Recovery						**98.44**

4.6. Robustness.

Determined by observing how a method stands up to slight variations in normal operating parameters. The Robustness of analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedure parameters listed in the procedure documentation and provides an indication of its suitability during normal usage. For instance,

slightly variation in normal operating parameters could be applied (Flow rate, Wavelength, pH, and/or Temperature, etc.).
The robustness of the analytical procedure was examined by studying the effect of changes in flow rate, wavelength, temperature, and pH value of the buffer on the suitability of the analytical method where: changing the flow rate by ± 5%, changing the wavelength by ± 3nm, changing the temperature by ± 5°C, changing the pH value of buffer by ± (0.3).

4.7. Ruggedness (Intermediate precision).

Expresses within-laboratories variations: different days and different analysts, a minimum of 6 determinations of sample at 100% of the test concentration done for each change i.e.: for analysts, two analysts will perform six determinations of samples. RSD for each set should be reported.
Acceptance criteria for Robustness and Ruggedness:
Pooled RSD for the results ≤ 3 %, On 5 % level of significance, Apply ANOVA statistics, P value should be more than 0.05 indicating the resemblance of results of the sets see

Table 6.
System suitability parameters were tested for each change. Results obtained showed that these variations did not have a significant effect on the measured system suitability parameters for the proposed.

Table 6. Data and results ANOVA statistical analysis for robustness& ruggedness.

Effect of changes	Pooled RSD	F	P-value
Flow rate	1.023	0.13752	0.87419
Wavelength	1.666	0.00039	0.99961
Temperature	2.170	0.04479	0.95651
pH of buffer	2.642	0.00310	0.99690
Ruggedness	0.615	1.94698	0.19312

4.8. Selectivity and Specificity.

Forced degradation studies were performed to provide an indication of the stability indicating properties, selectivity, and specificity of the procedure. Accelerated degradation was attempted using acid and base hydrolysis, effect of heat, light and oxidation **Table 7** explain results of effecting. As it showed relatively no effect on the samples in the case of acid, heat, white light, and the oxidation process, while it was more affected by sunlight, and a strong and significant effect occurred in the case of alkaline or base.

Table 7. Results of forced degradation.

Conditions	% Remaining of API (raw material)	% Remaining of API (sample product)
Acid	98.34	101.32
Base	25.46	26.85
Heat	101.98	103.78
Sun Light	70.10	72.18
Lab white light	98.34	101.38
Oxidation	99.26	98.37

4.9. Acid hydrolysis.

By using 0.1N hydrochloric acid for 60 minutes in a water bath at 70°C, chromatogram as shown in **Figure 5**.

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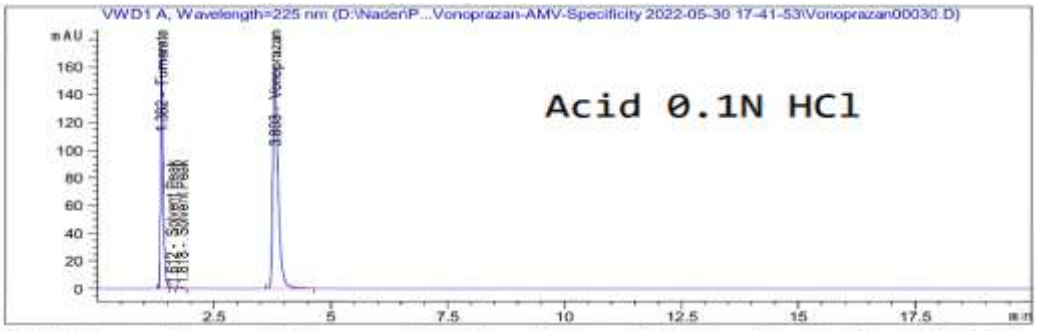
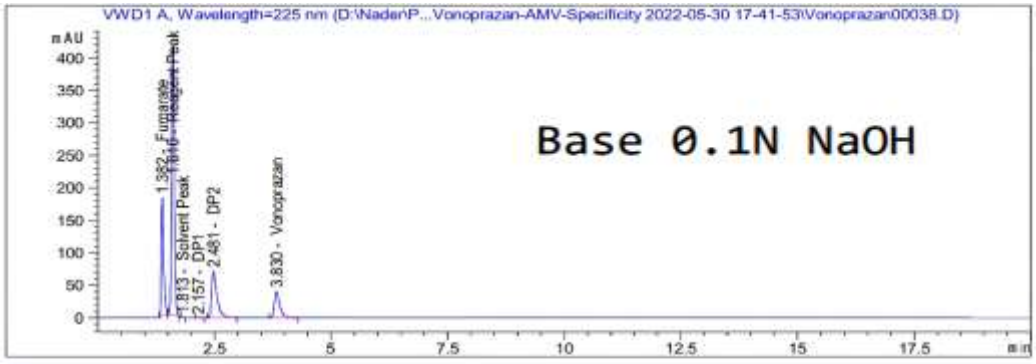


RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.382	-	640.07574	178.20367	0.64	0.0513	4032	-	-
1.612	-	1.38252	4.18510e-1	0.56	0.0542	4900	2.56	1.17
1.818	-	4.10985	9.70412e-1	0.84	0.0647	4372	2.03	1.13
3.803	-	1248.33154	159.74063	0.55	0.1128	6293	13.14	2.09

Figure 5. Acid-forced degradation chromatogram for vonoprazan.

4.10. Base hydrolysis.

By using 0.1N Sodium hydroxide for 60 minutes in a water bath at 70°C, chromatogram as shown in **Figure 6**.

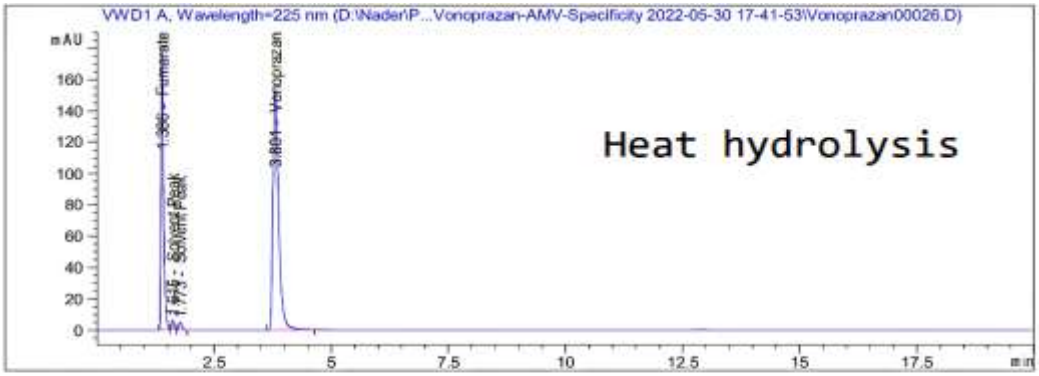


RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.382	-	641.93646	182.67696	0.66	0.0511	4056	-	-
1.610	-	1615.58984	416.65671	0.82	0.0593	4077	2.42	1.16
1.813	-	1.58485	4.92152e-1	0.46	0.0552	5968	2.09	1.13
2.157	-	3.31491e-1	5.07503e-2	0.65	0.1033	2413	2.55	1.19
2.481	-	572.24524	71.37611	0.48	0.1142	2614	1.75	1.15
3.830	-	324.79868	40.36320	0.58	0.1182	5817	6.83	1.54

Figure 6. Base-forced degradation chromatogram for vonoprazan.

4.11. Heat hydrolysis.

Samples solutions were heated in water bath at 70°C for 4 hours, chromatogram as shown in **Figure 7** .

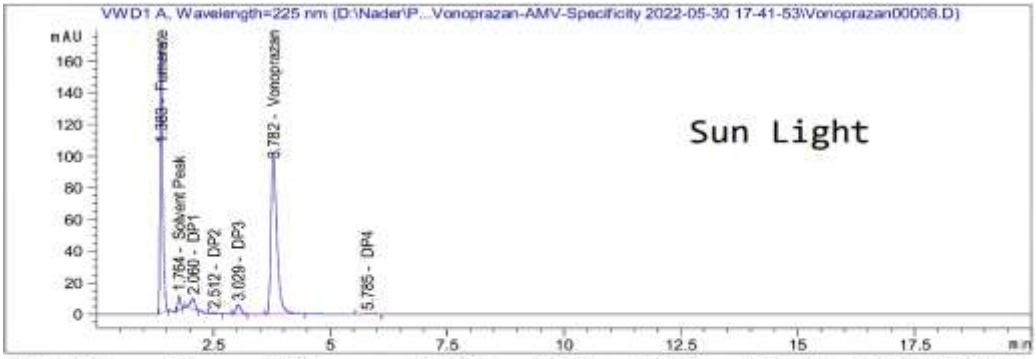


RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.386	-	663.80426	181.43475	0.63	0.0522	3911	-	-
1.615	-	18.73392	5.49180	0.76	0.0533	5073	2.55	1.17
1.773	-	21.79446	4.64158	0.65	0.0733	3242	1.46	1.10
3.801	-	1297.81775	152.81940	0.58	0.1245	5166	12.05	2.14

Figure 7. Heat-forced degradation chromatogram for vonoprazan.

4.12. Sun light hydrolysis.

Samples solutions were exposed to direct sun light for 1 hour, chromatogram as shown in **Figure 8**.

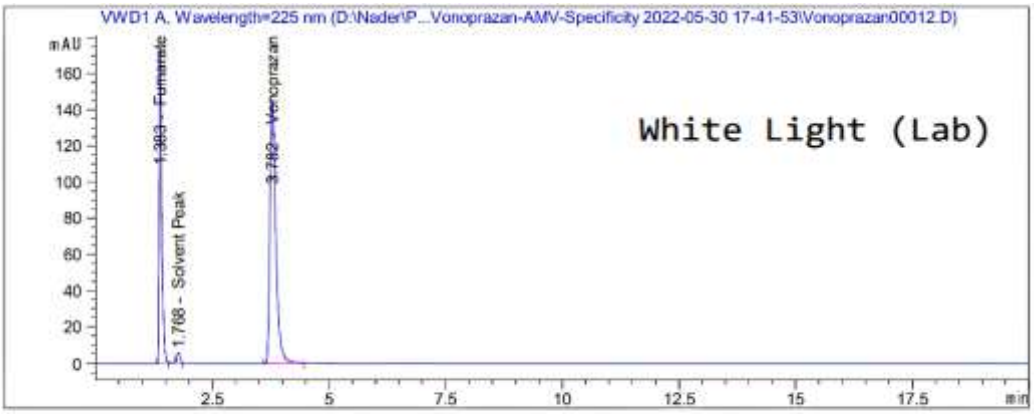


RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.383	-	639.41266	170.54042	0.61	0.0540	3628	-	-
1.764	-	37.12489	9.22181	0.82	0.0678	3758	3.67	1.28
2.060	-	47.81178	6.74252	1.32	0.1144	1798	1.91	1.17
2.512	-	6.13972	7.67979e-1	0.81	0.1300	2068	2.17	1.22
3.029	-	43.01704	5.88554	0.82	0.1156	3806	2.47	1.21
3.782	-	894.23596	101.71210	0.59	0.1294	4729	3.61	1.25
5.785	-	6.74247	3.66540e-1	0.52	0.3100	1929	5.36	1.53

Figure 8. Sun light forced degradation chromatogram for vonoprazan.

4.13. Lab white light hydrolysis.

Samples solutions were exposed to Lab white light without any protection for 2 hours, chromatogram as shown in **Figure 9**.

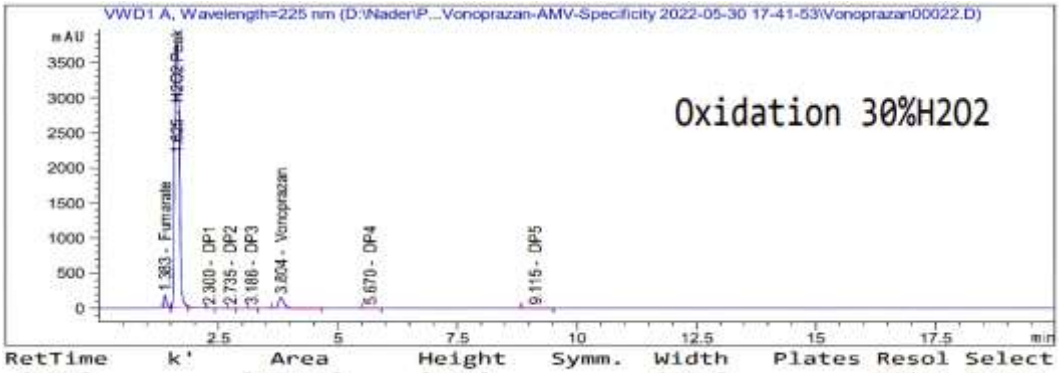


RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.383	-	649.26569	172.43628	0.61	0.0537	3682	-	-
1.768	-	23.21771	5.18024	0.76	0.0733	3218	3.56	1.28
3.782	-	1254.48376	144.57927	0.58	0.1277	4856	11.77	2.14

Figure 9. Lab white light forced degradation chromatogram for vonoprazan.

4.14. Oxidation hydrolysis.

By using 30% hydrogen peroxide and stood for 1 hour, chromatogram as shown in **Figure 10**.



RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.383	-	646.64581	179.81329	0.63	0.0522	3883	-	-
1.625	-	2.70665e4	3773.75269	0.72	0.1160	1084	1.69	1.17
2.300	-	3.36131	5.46807e-1	0.33	0.0889	3707	3.87	1.42
2.735	-	1.05481	2.20044e-1	0.47	0.0783	6758	3.06	1.19
3.186	-	1.38230	2.64580e-1	0.64	0.0808	8606	3.33	1.16
3.804	-	1265.87012	151.93021	0.58	0.1221	5370	3.58	1.19
5.670	-	3.26851	3.70150e-1	0.91	0.1400	9085	8.37	1.49
9.115	-	4.13715	3.04816e-1	0.91	0.2100	10439	11.57	1.61

Figure 10. Oxidation-forced degradation chromatogram for vonoprazan.

Several degradations products were observed with forced degradation for vonoprazan shown in

where explain also relative retention time (RRT) for about five unspecified degradation products (DP) with acceptable resolution with the principle peak, which in turn was used to apply to various samples in different conditions and proved the ability of the method to determine the presence of it and determine its ratio to the percentage of the active substance, which was proven not to exceed the minimum limit stipulated by comparing the daily doses of the pharmaceutical preparation as shown in the part on the practical application of the analysis method, the guidance on the content and qualification

of impurities ICH Q3A [22].

Table 8. Degradation's product that observed with forced degradation

DP	RRT	Acid	Base	Heat	Sun light	White light	Oxidation
DP1	**0.57**	-	exists	-	exists	-	exists
Dp2	**0.68**	-	exists	-	exists	-	exists
DP3	**0.82**	-	-	-	exists	-	exists
DP4	**1.51**	-	-	-	exists	-	exists
DP5	**2.40**	-	-	-		-	exists

4.15. Stability of solution.

The solution containing the drug substance is stored under conditions that ensure stability. The stability of this solution is analyzed over specified period, using a freshly prepared solution at each time interval for comparison. Acceptance criteria: between 98.0% and 102.0% of the expected final concentration.

% Stability of solution equal to **101.29%**, so the stability of standard solution was examined over 24 hours at room temperature and the solutions remained unchanged with no sign of degradation during the day of the preparation.

4.16. Practical Application.

This part represents a practical application on different samples that contain the active substance (Vonoprazan - API) in pure form and pharmaceutical preparations with different concentrations and different manufacturing sites, using the valid method of analysis for vonoprazan. This method has been successfully used in either the quality control testing or the stability testing of the selected pharmaceutical formulations, brand product, and pure raw material. **Table 9** represents all data and results about the samples used in the analysis of vonoprazan.

Table 9. Raw data and results for vonoprazan analysis.

No.	Sample name and Conc.	B.No.	Conditions	Av.wt. mg/tablet	%Assay	%Large impurities (NMT 0.2%)	%Total impurities (NMT 1.0%)
1	Vonoprazan fumarate (Raw material)	MIR-P/20003 (Metrochem API Private limited)	Ambient temperature	Powder	99.7	0.06	0.10
2	Vondalous 10mg FCT	210278	Ambient temperature	117.89	97.8	0.13	0.22
3	Takecab 10mg FCT	510976	Ambient temperature	115.29	99.8	0.08	0.12
4	Vondalous 20mg FCT	210295	06m Accelerated stability (40°C,75% RH)	239.18	95.4	0.12	0.23
5	Vondalous 20mg FCT	210295	06m Lon term stability (30°C,65% RH)	239.69	98.1	0.12	0.22

5. Conclusion.

A newly developed method for the estimation of vonoprazan had a wide linear concentration range, and was found to be rapid, simple, LOD and LOQ sensitivities, accurate. precise, and high resolution. Shorter retention time makes this method more acceptable and cost-effective, and it can be effectively applied for routine analysis in research institutions, stability studies and quality control department and other testing laboratories. Furthermore, in the case of forced degradation, its by-products could be separated in the majority of types used in the process of degradation for the active substance with recognised resolution between the peaks. This demonstrated the effectiveness of the technique used to demonstrate the by-products of

degradation or decomposition during stability testing in a range of temperature and humidity settings.
The method was validated according to ICH guidelines for the validation of analytical methods. The proposed method was successfully applied.

6. Conflicts of interest.

The authors declare no conflict of interest.

7. Acknowledgement.

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