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UV Spectrophotometric Method Development and Validation of Vonoprazan Fumarate in Bulk and Pharmaceutical Dosage form; Green Profile Evaluation Via eco-scale and GAPI Tools

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Abstract

A novel, accurate, specific, precise, robust and first UV spectrophotometric technique for determining the recently approved FDA; vonoprazan fumarate, a first -in -class potassium -competitive acid blocker, in bulk and in tablet medicinal dosage form was developed and validated. Quantitative measurements were taken at 230 nm using methanol and phosphate buffer (50:50 v/v) as a solvent. According to ICH criteria, the parameters of linearity, precision, accuracy, stability, robustness, the limit of detection, and the limit of quantitation were investigated. The calibration graph was discovered to be linear over a concentration range of (2 -30µg/mL) with a correlation coefficient (r = 0.9998). The proposed method's accuracy was validated using a percentage recovery of 99.48±0.947%, which demonstrated that it was highly accurate. The detection limit was 0.24 µg/mL, and the quantification limit was 0.70 µg/mL. The results showed that the technique is accurate, precise with relative standard deviation lower than 2%, while also being easy and inexpensive. The proposed method was successfully used to determine the drug in a pharmaceutical formulation without interference from excipients. The research proved that the proposed method can be used in conventional analysis. The developed method scores excellent green profile on environment according to the novel green analytical procedure index (GAPI) and Analytical Eco - scale guidelines.

Keywords: Vonoprazan Fumarate; Helicobacter pylori; Gastric cancer; Proton pump inhibitors; potassium-competitive acid blockers; COVID-19

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1. Introduction

In recent years, it has been estimated that over four billion people worldwide are infected with Helicobacter pylori (H. pylori) which is categorized in (Group 1 - Definite carcinogen) by the World Health Organization (WHO) [1]. Infection with H. pylori causes a number of gastrointestinal diseases, including peptic ulcers and is the leading cause of stomach cancer [2 -4]. The first -line eradication therapy for its infection in the past depended on proton pump inhibitors (PPIs), which bind to the hydrogen - potassium ATPase pump irreversibly [5 - 10].

Unfortunately, due to PPIs mechanism of action with the proton pump, the class has been linked to a number of side effects and the US Food and Drug Administration (FDA) issued warnings against their prolonged use alone or in combination with other drugs as it is tightly linked to a number of negative outcomes such as achlorhydria which is the main reason for atrophic gastritis then gastric cancer. H. pylori also have become more resistant to PPI -based treatments and the treatment rate has recently dropped [11 -19].

It was also reported that the use of PPIs was linked to a higher risk of morbidity and death from coronavirus illness 2019 (COVID -19), with individuals who used PPIs having significantly higher chances of having positive COVID -19 testing

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with adverse effects when compared to those who did not use PPIs, as the SARS -CoV -2 working receptor is angiotensin -converting enzyme -2 (ACE -2) has been proven to be present in the gastrointestinal tract [20, 21]. As a result, alternate formulations for the classical prodrug class of PPIs have been developed to address these issues..A new era for treatment of acid related diseases is begun with Potassium competitive acid blockers (P -CABs) are a unique class of acid -suppressing drugs that have a greater eradication rate for Helicobacter pylori than PPIs with reversible manner of interaction with the pump with faster onset , long duration and safe profile more than PPIs [22, 23]. VPZ is a first -in -class of the new class (P -CABs) used to treat and prevent stomach ulcers and reflex esophagitis, with an inhibitory potency nearly 350 times that of standard proton pump inhibitor [24]. Vonoprazan Fumarate (VPZ); Nme -thylmethanam -ene[1 -[5 -(2 -fluorophenyl) - 1 -pyridin - 3 -ylsulfonylpyrrol - 3 -yl] (Figure.1) [25]. [25]. In May 04, 2022 , FDA authorized VPZ -based treatments for Helicobacter pylori infection as the first innovative acid suppressor from a new drug class licensed in the US in over 30 years [26]. VPZ was approved in Egypt and has been on the market as a tablet dosage form with a novel mechanism of action that competitively suppresses the binding of potassium ions to H+, K+ -ATPase (commonly known as the proton pump) in the final step of gastric acid secretion in parietal cells of the stomach, with a strong and prolonged inhibitory impact since June 1, 2022.

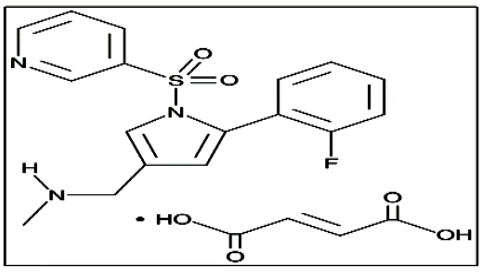

Fig.1. Chemical structure of Vonoprazan Fumarate (VPZ)

A review of the literature revealed that only a few methods for evaluating VPZ alone or in combination with other medicines were described. These approaches include electrochemical [27], fluorometric [28], and HPLC [29, 30] with gradient system for separation and high negative impacts on environment. As far as we know, no spectrophotometric technique for determining VPZ alone or in its pharmaceutical dosage form has been published, as it is not yet official in any of the pharmacopeia for applications. Under those conditions, there is an urgent need to develop a first, simple and rapid spectrophotometric method of determination because UV -spectrophotometry is likely to be the most convenient analytical method for routine analysis due to its inherent simplicity, low cost, and widespread availability in quality control laboratories. The goal of this work was to provide the first UV spectrophotometric method with excellent green profile according to the novel guidelines of the green analytical procedure index (GAPI) and Analytical Eco -scale for determining VPZ in pure and pharmaceutical formulations.

2. Experimental

2.1. Instrumentation

Shimadzu (Kyoto, Japan) UV -1601 PC, UV -visible double -beam Spectrophotometer with matched 1 cm path length quartz cells was used for the spectrophotometric analysis. For pH modifications, a Jenway pH meter, model 3510 (Jenway, Staffordshire, UK.

2.2. Materials

Hikma Pharma Co., 6th of October City, Egypt, kindly supplied standard Vonoprazan Fumarate (VPZ). The purity was certified to be 99.70%. (According to the official certificate provided by the manufacturer).

2.3. Pharmaceutical Dosage form

Hikma Pharma Co., 6th of October City, Egypt, manufactured and supplied Topoprzan®, which was labelled with 26.72 mg Vonoprazan Fumarate.

2.4. Chemicals

Methanol (MeOH), 1 -propanol, ethyl acetate, and ethanol were HPLC -grade organic solvents acquired from Fisher Chemical (Loughborough, UK). All analytical grades of sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, phosphoric acid, trisodium citrate dehydrate, citric acid monohydrate, and boric acid were acquired from El -Nasr Pharmaceutical Chemicals in Cairo, Egypt. Phosphate Buffer was freshly made in accordance with USP [31]. Millipore water filtration system produces de -ionized water in -house (Millipore, Milford, MA, USA).

2.5. Preparation of the diluent

To create the solvent, 50 ml of (Phosphate buffer 20 mM, pH= 6.8) were combined with 50 ml of methanol as a diluent in all preparations throughout the experiment at a (50:50 v/v) ratio.

2.6. VPZ stock standard solution

By dissolving 0.01 gm of VPZ in the diluent (previously mentioned under 2.5.), a standard solution of (100.00 µg/mL) of VPZ was created .

2.7. Procedure

2.7.1. Spectral characteristics of VPZ

An accurate portion of 1 mL of VPZ standard stock solution (100.00 µg/mL) was individually and precisely put into a 10 mL volumetric flask. The volume was
finished to the mark with the diluent and well mixed. The absorption spectrum was measured against the same diluent that was used as a blank.

2.7.2 Method validation

The method validation was carried out in accordance with the ICH recommendations [32].

2. 7 .2.1. Linearity

Various aliquots corresponding to 20 -300 g of VPZ were accurately transferred from the standard stock solution (100 µg/mL) into a succession of 10 -mL volumetric flasks before being completed to volume with the diluent. Using the same solvent as a blank, the spectra of the produced standard solutions were scanned from 200 to 400 nm. The VPZ calibration curve was created by graphing the absorbance readings at 230 nm vs the concentrations.

2.7.2.2. Accuracy

The process described in (2.7.1) was repeated in duplicate for the determination of varied concentrations of pure VPZ samples. The concentrations were determined using the respective regression equations, followed by the mean recovery percentages and standard deviations.

2.7.2.3. Precision

2.7.2.3. 1 . Intraday precision

The intraday variation was assessed using the previously described approach (2.7.1) for analysis of 5.00, 10.00, and 15.00 µg/mL of VPZ three times (n=9) on the same day. The concentrations were determined using the respective regression equations, followed by the mean recovery percentages and relative standard deviations.

2.7.2.3.2. Interday precision

The previously mentioned VPZ concentrations were determined on three consecutive days (n=9) using the previously described technique (2.7.1). Following that, the mean recovery percentages and relative standard deviations were computed.

2.7.2.4. Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the corresponding calibration curve according to the ICH guideline Using the associated calibration curve. The estimation was based on the standard deviation of response, as per the ICH guideline for determining LOD and LOQ and calculated as follow,

$LOD = 3.3 \times \sigma/S$.

$LOQ = 10 \times \sigma/S$.

Where (σ) is the response standard deviation and (S) is the calibration curve slope. In this case, the standard deviation of the y -intercept of the regression line can be utilized as the response standard deviation.

2.7.2. 5. Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was tested by varying detection wavelength ±2 nm (228 & 232 nm) of optimized conditions from the λ max (230 nm) [33].

2.7.2.6. Application of the proposed method for the determination of VPZ in pharmaceutical formulation (Topoprazan®)

Ten tablets were weighted, finely powdered and thoroughly mixed well; then an exactly weighed quantity of the powder equivalent to 10.0 mg of VPZ was transferred into 100 mL volumetric flask, sonicated for 30 min and completed to the volume with the same diluent then filtered, further dilution was made using the same diluent. The obtained solution is claimed to contain (100.00 µg /mL) of VPZ which was analyzed adopting the procedure previously mentioned under (2.7.1). From the corresponding regression equation, the nominal content of the pharmaceutical dosage form was estimated. Standard addition technique was also applied by analyzing the pharmaceutical formulation spiked with different concentration of pure standard drugs. These concentrations were calculated from the corresponding regression equation and the mean recovery percentages and standard deviations were then calculated.

3. Results and Discussion

UV-Visible spectrophotometric analysis has the advantages of being widely available, simple, fast, less time consumption with low impacts on environment when compared to the chromatographic techniques and this work concerns with the development and validation of the first UV-spectrophotometric method to determine VPZ as there is no specific monograph in the pharmacopeia to the date with significant economic advantage over the all of previously published methods for its determination therefore, the development of a simple, low cost and rapid protocol for its quantification is

essential to set the circumstances for optimal measurement conditions, several solvents with different compositions and polarities were examined e.g., methanol, ethanol, 1-propanol, ethyl acetate, and water. In addition to different ratios for buffer systems, e.g., phosphate, borate, and citrate were investigated to select the optimal solvent for the proposed procedure and (20mM Phosphate Buffer at pH 6.8: methanol (50:50 v/v) has been selected with respect to solubility and suitable response. The selection of a suitable wavelength has a significant impact on both selectivity and sensitivity. For the selection analytical wavelength, VPZ solution was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm by a Shimadzu UV-1601 PC, UV-visible double-beam Spectrophotometer. The chemical structure of VPZ was shown in (Figure 1). The λmax of 230 nm was chosen for the determination of VPZ and the absorption spectrum was shown in (Figure 2) and this wavelength was adopted for all measurements.

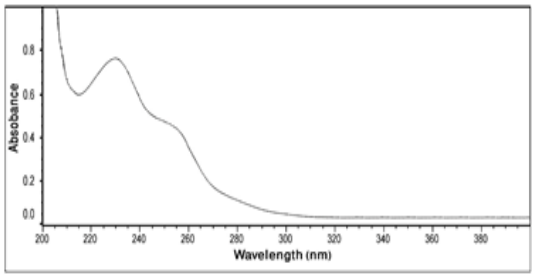

Fig.2. UV-absorption spectrum of VPZ with maximum at 230 nm.

The proposed method has been validated by assessing concentration range, linearity, accuracy, sensitivity precision, stability and robustness in accordance with ICH guidelines as the validation parameters of the proposed analytical method is represented in (Table 1).

Table 1: Assay parameters and method validation for the determination of pure sample of VPZ by the proposed spectrophotometric method

Parameters	
$\lambda\ (nm)$	230.0
Concentration $(\mu g/mL)$	2.00 – 30.00
Slope	0.0321
Intercept	0.0042
Correlation (r) coefficient	0.9997
Accuracy (mean ± S.D.)	99.48 ± 0.347
Precision (% RSD) Repeatability a	1.221
Precision (% RSD) Intermediate precision b	1.301
LOD c $(\mu g/mL)$	0.24
LOQ c $(\mu g/mL)$	0.70

[a]; The intraday (n=3), average of three different concentrations three times within day.
[b]; The intraday (n=3), average of three different concentrations in three successive days.
[c]; Limit of detection and limit of quantification.

Linearity was studied and the proposed method obeyed Beer's law in the concentration range of (2.00 - 30.00 µg/mL) with a good correlation coefficient of r = 0.9998. Beer's law range was confirmed by the linearity of the calibration curve of VPZ as shown in (Figure 3) and it was found that the linear regression equation; y = 0.0321 X + 0.0042 r = 0.999 8VPZ as shown in (Figure 3) and it was found that the linear regression equation;

y = 0.0321 X + 0.004 r = 0.9998

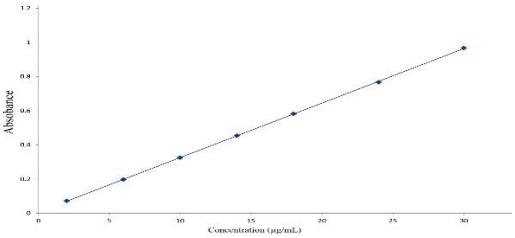

Fig.3. Calibration curve for UV-visible spectrophotometric determination of VPZ (2.00 – 30.00µg/(mL)) at 230.0 nm

Where (X) represents the VPZ concentration in µg/mL, (y) is the response and (r) is the correlation coefficient.

Recovery studies were performed to validate the accuracy of developed method (Tables 1,2). The proposed method was accurate to determine VPZ in pure powder with a mean recovery percentage of 99.48 ±0.947. The precision of the developed method was analysed by performing the intraday and interday analysis of test sample at λmax. The precision of intraday and interday precision was found to be good with % RSD less than 2 which indicates that the method was precise. The LOD and LOQ were found to be 0.24 µg/mL and 0.70 µg/mL, respectively which shows that this method was very sensitive.

Table 2: Results of accuracy for the determination of pure sample of VPZ by the proposed method

VPZ		
Taken $(\mu g/mL)$	Found $(\mu g/mL)$	% Recovery*
5	4.981	99.62
10	9.927	99.27
15	14.734	98.23
20	19.884	99.42
25	25.221	100.88
Mean ± S.D.	99.48 ± 0.347	

* Average of three determinations.

The present work is specific to determine VPZ in pharmaceutical dosage form and its validity has been evaluated by using the standard addition method as the sample recovery in tablet formulation was in good agreement with the label claim and thus suggested the validity of the method and the absence of formulation excipient interference (Table 3).

Table 3: Determination of VPZ in its formulation by the proposed method and application of standard addition technique

Dosage form	Drug					
Topoprazan® labelled to contain 26.72 mg Vonoprazan fumarate(VPZ) equivalent to 20 mg Vonoprazan per tablet.	VPZ	Taken ($\mu g/mL$) 10	Found (%) ±S.D. 99.11 ± 0.138	Added ($\mu g/mL$) 5	Found ($\mu g/mL$) 4.907	% Recovery* 98.14
				10	9.889	98.89
				15	14.812	98.75
			Mean ± S.D.			98.59 ±0.399

* Average of three determinations.

The results obtained were compared to the reported method statistically by Student's t -test and F -test (ICH -Q1A (R1),2005).

The calculated t - and F - values did not exceed the tabulated values at the 95% confidence level indicating close similarity as no significant difference between the proposed and the reference method with respect to accuracy and precision (Table 4).

Table 4: Statistical comparison of the results obtained by applying the proposed procedure with the reported method

Value	Proposed method	Reported method [30]
Mean	99.48	98.73
SD	0.347	0.356
RSD%	0.352	0.361
n	5	3
Variance	0.897	0.127
Student's t-test a	1.29 (2.447)	
F value b	7.081 (39.25)	

[a, b]; Values in parenthesis are the corresponding theoretical of t and F

Furthermore, for stability study of the proposed method at room temperature 25°C, the prepared solutions were stored and analyzed after 24 h. It was found that VPZ is stable for the period of analysis in diluent.

The evaluation of robustness should take place throughout the development phase and is dependent on the type of method under consideration with showing the reliability of an analysis with respect to deliberate variations in method parameters. To test the robustness of UV spectrophotometric method, parameters such as detector wavelength variations are altered and the quantitative influence of the variables is determined with respect to %RSD results for [34]. Robustness was performed by changing two different wavelengths in low and high limits for the optimum wavelength and % RSD was less than 2 which indicates that the method was robust (Table 5).

Table 5: Results for Robustness study

Wavelength(nm)	Sample absorbance	Standard absorbance	Mean absorbance ± S.D.	%RSD
228	0.610	0.589	0.609 ± 0.002	0.342
	0.610			
	0.610			
232	0.618	0.595	0.619 ± 0.001	0.247
	0.621			
	0.620			

3.1. Assessment of the analytical method greenness using analytical eco -scale and green analytical procedure index (GAPI)

The greenness profile of the proposed method was assessed by analytical Eco -scale and GAPI tools [35, 36]. The Eco -Scale method is a semi -quantitative tool based on calculating penalty points of two main parameters of the analytical procedure [37].

These two parameters include a reagent parameter that can be calculated by concerning amounts, physical, environmental, and health hazards of the reagents. The other parameter is related to the instrumentation including the instrument's energy utilized during its usage, occupational hazards, and amount of the waste eliminated by the device [38]. After calculating the penalty points using these parameters, the results are subtracted from 100, indicating that the method is considered as an excellent or acceptable or inadequate green method. The higher the analytical eco -scale scores, the more the greenness of the analytical process. Analytical eco -scale scores more than 75 are considered as excellent green method. Points are the number of pictograms in material safety data sheet of the chemical X the score for the signal word (safe = 1, danger = 2) X amount penalty points so, the penalty points score for methanol as follow; [(3) pictograms x (1) signal word x (2) amount] = 6 penalty points. Phosphate buffer has no pictograms with zero penalty points. The instrumental energy consumption also has penalty points as following [(0) for methods using less than 0.1 kWh per sample, (1) for methods using 0.1 –1.5 kWh per sample, (2) for methods using more than 1.5 kWh per sample]. UV spectrophotometric method is assigned zero penalty point for the instrumental energy consumption. Waste is assigned penalty points = 6 points as of its amount and no

further treatment has been applied for recycling. The occupational hazard with value equal zero as there were no vapors or gases. Hence, the analytical procedure of our proposed method showed that the applied approach gives only 12 penalty points indicating excellent green analysis with less waste and hazardous reagents (Table 6).

Table 6: Assessment of the method greenness utilizing eco-scale tool

Parameter	Penalty points
Methanol	6
Phosphate buffer	0
Instrument	0
Occupational hazards	0
Waste	6
Total penalty points	12
Analytical eco-scale total score a, b	88

[a]; Analytical Eco-Scale total score = 100 – total penalty points.
[b]; If the score is > 75, it represents excellent green analysis.

Another tool for estimating the greenness of analytical procedure called green analytical procedure index (GAPI) was used [39]. Each step of the analytical procedure is evaluated using terms of four major categories with 15 parameters, covering the sample collection, transportation, preservation, preparation, reagents, and compounds used, and instrumentation along with the purpose of the method [40]. The GAPI tool assessment is represented by five pentagrams using three color -specific symbols (green, yellow and red) to illustrate the low, medium, and high environmental impact of each analytical methodology step [41]. There is a color code; green which indicates low influence on the environment, yellow and red signifying medium and high influence on the environment, respectively.

In the developed method, there is no extraction step in the sample preparation (direct method) and the solvent system was without any additional treatments.

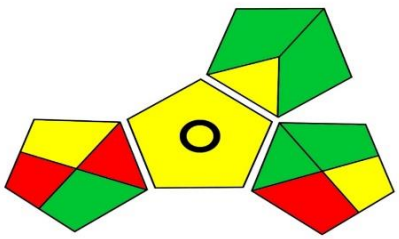

Fig.4. GAPI assessment for green profile analysis of the proposed spectrophotometric method.

The analytical procedure shows 3 red zones appearing at their corresponding parts (1&3&15) due to the offline sampling and transport to QC laboratories which is mandatory due to the separation between pharmaceutical production sites and QC sites. The last red zone is due to no waste treatment has been developed. The yellow pictograms on GAPI represent the medium impacts during sample storage, reagents and waste disposal. The green pictograms are indicative of the greenness of the developed method. Therefore, the proposed method provides simple procedure with the minimum amount of waste and hazardous compounds. According to (Figure 4), the proposed method was a direct eco -friendly method with no need for extraction procedures with excellent green profile score according to Eco -scale and GAPI guidelines.

4. Conclusion

The developed method offers advantage as it is the first spectrophotometric method to estimate vonoprazan fumarate in pure powder and pharmaceutical dosage form. The developed method was validated as per ICH Q2 (R1) guidelines and found to be simple, accurate, linear, precise, robust and specific. Therefore, the proposed method can be applied for routine analysis of VPZ with advantages of simple, specificity, low cost and excellent green profile with low impacts on environment.

5. Conflicts of interest:

There is no conflicts of interest to declare

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