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Original Article

A RELIABLE RP-UPLC-TUV METHOD FOR SIMULTANEOUS ESTIMATION OF CLARITHROMYCIN, AMOXICILLIN, AND VONOPRAZAN IN CO-PACKED PHARMACEUTICAL DOSAGE FORMS: METHOD DEVELOPMENT AND VALIDATION WITH STABILITY INDICATING PROPERTIES

K. ANUSHA, G. SOWJANYA*

GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh-530045, India
*Corresponding author: G. Sowjanya; *Email: sowjanya.tatineni@gitam.edu

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ABSTRACT

Objective: The study aims to develop a reliable RP-UPLC-TUV method for simultaneous estimation of Clarithromycin, Amoxicillin, and Vonoprazan in bulk and combined dosage.

Methods: A simple, specific, and reliable method for determining Clarithromycin, Amoxicillin, and Vonoprazan has been developed using the RP-UPLC method. In order to successfully separate Clarithromycin, Amoxicillin, and Vonoprazan, 1.0 µl of a 100 % level solution was injected into a Hibar C18 (100 x 2.1 mm and 2 µm) column. The mobile phase consisted of Ammonium Acetate and Acetonitrile in equal volumes, and the flow rate was kept at 0.3 ml/min while the detection wavelength was set to 210 nm. Both the column and the injection port were kept at a temperature of 30 °C at all times.

Results: The retention time (RT) of Clarithromycin, Amoxicillin, and Vonoprazan was observed at 1.24 min, 0.97 min and 1.66 min, correspondingly with accepted system suitability. The linear responses were observed for Clarithromycin, Amoxicillin, and Vonoprazan in the range of 25 to 150 µg/ml, 25 to 150 µg/ml and 1 to 6 µg/ml, respectively. The LOD and LOQ values were calculated to 0.07 µg/ml and 0.22 µg/ml for Clarithromycin, 0.81 µg/ml and 2.45 µg/ml for Amoxicillin and 0.03 µg/ml and 0.09 µg/ml for Vonoprazan. The % RSD values of both precision were assessed in the range of 0.8-1.4. The mean recovery of Clarithromycin, Amoxicillin, and Vonoprazan was in the range of 99.66 %-100.88 %. The statistical analysis of the validation parameters confirmed that the approach was reliable in terms of its accuracy, sensitivity, and precision while also exhibiting a high degree of sensitivity. The study of analytes in a variety of stressful situations guarantees the stability of the substances, ensuring that they represent the method's stability indication.

Conclusion: The newly established technique is quite effective in separating Clarithromycin, Amoxicillin, and Vonoprazan from one another. Also separated with excellent resolution were the degradation products that were formed as a result of the stress conditions. The study concluded that the developed method has considerable adoption in the pharmaceutical sector.

Keywords: Clarithromycin, Amoxicillin, and Vonoprazan, RP-UPLC, Hibar C18 column, Specificity, Stability indicating

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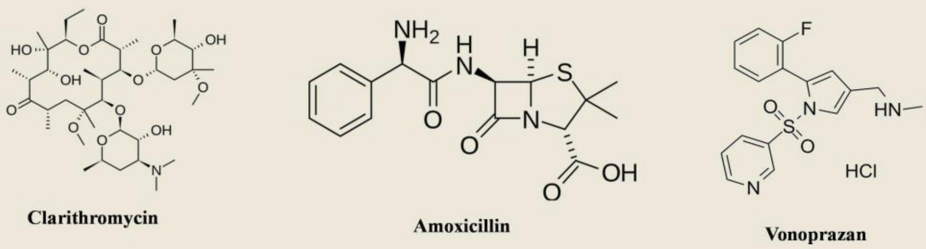
INTRODUCTION

In order to treat a wide range of bacterial infections, a popular antibiotic that is used for this purpose is called Clarithromycin [1]. It is an antibiotic that comes from erythromycin and is part of the macrolide family [1-3]. It is via this inhibition of bacterial protein synthesis that Clarithromycin is able to exert its antibiotic actions [1, 2]. It does this by attaching itself to the 50S component of the bacterial ribosome, which prevents the production of protein synthesis complexes that are really useful. Because of this interference with protein synthesis, bacterial growth and reproduction are both stifled as a result [2, 3]. The antibacterial properties of Amoxicillin come from the drug's ability to block the production of cell walls in bacteria [1-4]. It accomplishes this goal by causing disruptions in the cross-linking of peptidoglycan chains, which are necessary constituents of the cell walls of bacteria [4, 5]. The bacterial cell is rendered more vulnerable to osmotic lysis as a result of the effects of Amoxicillin, which works by preventing the production of a cell wall that is capable of maintaining its structural integrity. The germs will perish as a consequence of this action in the end. Clarithromycin and Amoxicillin are the two antibiotics that are indicated as part of a treatment plan for eliminating *Helicobacter pylori* [1-5]. This bacterium is linked to peptic ulcers [5]. In order for Vonoprazan to have its effect, it inhibits in a way that is both selective and irreversible an enzyme known as H+/K+-ATPase [6, 7]. This enzyme is what is ultimately responsible for the formation of acid in the stomach. Because it inhibits the activity of this enzyme, Vonoprazan is superior to other proton pump inhibitors (PPIs) in its ability to lower stomach acid production [6, 7]. It has an acid-suppressing effect that lasts for a longer period of time, which makes it effective for treating diseases where there is a worry for excessive acid generation [6]. Ulcers, also known as sores in the lining of the stomach or intestine, may be caused by a specific kind of bacterium known as H. pylori [6, 7]. The combination of Clarithromycin, Amoxicillin and Vonoprazan is effectively used to treat ulcers and stop their recurrence [8, 9]. The three combined drugs were approved by FDA in May 2023 [9]. The IUPAC names and chemical structures of Clarithromycin, Amoxicillin and Vonoprazan were publicized in fig. 1 [10-12].

A large number of analytical procedures were observed in the review of literature for analysis of Clarithromycin and Amoxicillin as solo entity and in a mixture with other agents [13-15]. In the same way, numerous methods have been pronounced in the literature to estimate Clarithromycin and Amoxicillin combination with other anti-ulcer drugs such as omeprazole, lansoprazole, and rabeprazole [16-19]. Clarithromycin, Amoxicillin and Vonoprazan are a novel combination in the pharmaceutical industry, effectively used to treat ulcers and stop their recurrence than former combinations and hence, a reliable liquid chromatographic approach is necessary in order to evaluate the quality and amount of Clarithromycin, Amoxicillin, and Vonoprazan in bulk as well as formulations. A review of the relevant literature found that, as of this moment, there has not been a single analytical method documented for the examination of Clarithromycin, Amoxicillin, or Vonoprazan. UPLC is a technology that requires shorter run periods and boosts sensitivity, in addition to providing selectivity, sensibility, and a wide range of analysis. As a result, the establishment and validation of a new UPLC technique was chosen and prioritized.



Clarithromycin: 6-(4-dimethylamino-3-hydroxy- 6-methyl-tetrahydropyran-2-yl) oxy-14-ethyl-12,13-dihydroxy- 4-(5-hydroxy-4-methoxy-4,6- dimethyl-tetrahydropyran-2-yl) oxy-7-methoxy-3,5,7,9,11, 13-hexamethyl-1- oxacyclotetradecane-2,10-dione

Amoxicillin: (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Vonoprazan: 1-[5-(2-Fluorophenyl)-1-(3-pyridinylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine

Fig. 1: Molecular structures and IUPAC names of clarithromycin, amoxicillin and vonoprazan

MATERIALS AND METHODS

Materials and methods

Instruments

This chromatographic examination took place using the aid of an Acquity UPLC separation unit that was mounted with a TUV detector. In order to do tests and perform processing on the data that had been generated, the Empower 2 software was employed. The pH meter obtained from BVK enterprises, India.

Chemicals and reagents

Gift samples of Clarithromycin, Amoxicillin, and Vonoprazan were given by Spectrum Research Lab, Hyderabad. Ammonium acetate, acetic acid, and acetonitrile were purchased from Merck, India.

Preparation of solutions

Standard preparation

50 mg Clarithromycin, 50 mg Amoxicillin, and 2 mg Vonoprazan pure powders were accurately weighed and transferred into a 50 ml volumetric flask, where they were dissolved with diluent to obtain a solution with concentrations of 1000, 1000, and 40 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan, respectively. It is illustrated as stock solution. 1 ml of the above-mentioned stock solution was diluted to 100 ml to get concentrations of 100, 100, and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan, respectively (100 % solution).

Preparation of sample solution

The tablets and capsule powder equivalent to 50 mg of Clarithromycin, 2 mg of Vonoprazan and 50 mg of Amoxicillin was precisely weighed and taken in a 50 ml volumetric flask, where they were dissolved with diluent to obtain a solution with concentrations of 1000 µg/ml, 1000 µg/ml, and 40 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan, respectively. After filtration, 1 ml of the above-mentioned stock solution was diluted to 100 ml to get concentrations of 100, 100, and 4µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan, respectively. Before the sample solution was introduced into the device, it was filtered via a 0.45 µ nylon filter so that any particulate matter could be removed.

Method development

This was accomplished by a variety of trials (table 1), which involved modifying the mobile system, columns, and buffers, among other things. Initially, method development was started with SB 100 C18 column and mobile phase of OPA: Acetonitrile (40:60 v/v), where peak shapes were not good along with the less plate count for Vonoprazan. Further, OPA was replaced with KH_2PO_4 in mobile system and column was changed to HSS C18 and Hibar C18. Successful separation of Clarithromycin, Amoxicillin, and Vonoprazan were not done with the altered conditions. Finally, with Hibar C18 (100 mm x 2.1 mm and 2 µm) column, Ammonium Acetate and Acetonitrile (60:40 v/v), all three drugs separated effectively.

Method validation

Validation of the technique was performed in accordance with the Q2 standards of the ICH guidelines [30].

System suitability

Standard solution was thereafter injected in six separate duplicates in order to verify that the present procedure is suitability for the system. System suitability parameters such as USP plates, resolution, tailing and % RSD were assessed computed from the resultant chromatograms.

Linearity

The linear response of the current approach was assessed by analyzing a series of concentrations of 25, 50, 75, 100, 125, 150 µg/ml for both Clarithromycin, Amoxicillin and 1, 2, 3, 4, 5, and 6 µg/ml for Vonoprazan. At last, a calibration curve was plotted for each Clarithromycin, Amoxicillin, and Vonoprazan in between given concentrations and resultant peak responses to compute the regression coefficient (R^2). The identical process was repeated for two more time to calculate the standard deviation (SD) of y-intercept and mean of slope.

Sensitivity

Sensitivity in terms of limit of detection (LOD) and limit of quantification (LOQ) were computed by following equations.

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ-SD of the y-intercepts of linear plot (n=3)

S–Slope linear curves (n=3)

Specificity

The approach is considered as specific when the drug under investigation can be identified by the method to be present in the presence of other matter efficiently and without any interference. Injecting a volume of 1.0 µl of each of the separate solutions of blank, standard preparation, and standard solution spiked with excipients was needed. It was determined if there were any interferences between the retention times (RT) of the blank and the placebo and the RTs of the Clarithromycin, Amoxicillin, and Vonoprazan in the chromatograms that were recorded. The approach was able to achieve a higher level of specificity by comparing the chromatograms of the standard and forced degradation solutions.

This was done in order to check for interferences between the RTs of the degradation products and active ingredients Clarithromycin, Amoxicillin, and Vonoprazan.

Precision

The findings of the homogeneous solution on multiple analyses under similar environments are shown to have an excellent correlation with one another; one may assert that the methodology in concern possesses high levels of precision. Analyzing the 100 % level six times brought the system precision of the anticipated method up to the expected level while injecting the sample solution six times brought the method precision up to the expected level. Calculations were done to determine the % RSD values of observed peak regions (system precision) as well as % assay (method precision).

Accuracy

To validate the accuracy parameter, the percentage recovery procedure was chosen as the approach to use. During the course of this procedure, a predetermined quantity of sample solution was mixed in with three distinct percentages of standard solution (50, 100, and 150 %). Each solution that had been spiked was examined three times. At each of the three levels that were indicated, the typical % recovery of Clarithromycin, Amoxicillin, and Vonoprazan was calculated.

Robustness

The ability of the technique to generate similar outcomes after changing the method conditions deliberately to some amount is termed as "robustness." A number of minor modifications were made to the flow rate (±0.1 ml/min), Temperature (±5 °C), and mobile phase ratio (±1 part) in order to verify the reliability of the approach that is now being used.

Forced degradation (FD) studies

In order to assess the stability indicating quality of the procedure, it was decided to purposefully subject the drug solution to circumstances that would cause its degradation. Research may readily make accurate predictions about the pathways of degradation and the necessary storage conditions for drug material and drug product by using these investigations [20-22]. FD studies were done in accordance with the requirements of ICH's Q1A, QIB, and Q2B [28-30].

Acid degradation

In the process of acid degradation, stock solution of blended Clarithromycin, Amoxicillin, and Vonoprazan and 2N HCl solution were equally mixed together and allowed to reflux for thirty minutes at a temperature of 60 °C. Further, the mixed solution was neutralized with 2N NaOH and again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution [23].

Alkali degradation

In the process of Alkali degradation, stock solution of blended Clarithromycin, Amoxicillin, and Vonoprazan and 2N NaOH solution were equally mixed together and allowed to reflux for thirty minutes at a temperature of 60 °C. Further, the mixed solution was neutralized with 2N HCl and again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution [24].

Oxidative degradation

In the process of Oxidative degradation, stock solution of blended Clarithromycin, Amoxicillin, and Vonoprazan and 20 % H_2O_2 solution were equally mixed together and allowed to reflux for thirty minutes at a temperature of 60°C. The solution again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution [25].

Photodegradation

During the course of the Photodegradation process, the stock solution of blended Clarithromycin, Amoxicillin, and Vonoprazan was placed in an UV cabinet at shorter wavelength (254 nm) over a period of seven days while a dark control was maintained. The solution again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution [26].

Dry heat degradation or thermal degradation

During the course of the dry heat degradation process, a standard stock solution volume about 10 ml was kept in oven at 105 °C for 6 h. The solution again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution [27].

Neutral degradation by water

In the process of oxidative degradation, stock solution of blended Clarithromycin, Amoxicillin, and Vonoprazan and 2N NaOH solution were equally mixed together and allowed to reflux for thirty minutes at a temperature of 60°C. The solution again diluted to obtain a solution of 100, 100 and 4 µg/ml for Clarithromycin, Amoxicillin, and Vonoprazan correspondingly. After injecting a volume of 10 µl of the prepared solution above, chromatograms were obtained and then assessed to determine the amounts of Clarithromycin, Amoxicillin, and Vonoprazan that had been degraded by comparing them to the control solution.

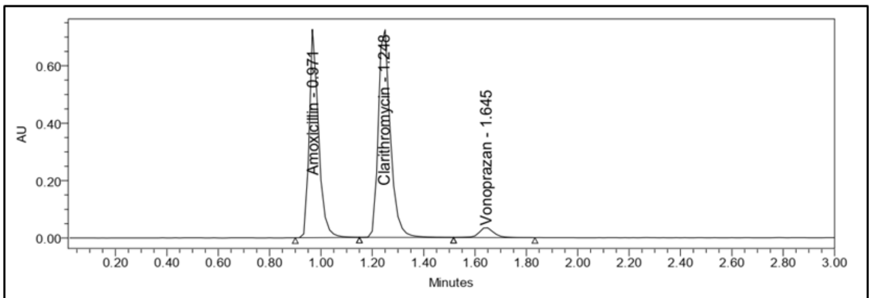
Application of current method for assay of commercial dosage form

The stated approach was utilized to determine the % purity of Clarithromycin, Amoxicillin, and Vonoprazan in commercially available formulation. This was accomplished by performing the analysis of same concentrations of standard and sample solution. The % purities of Clarithromycin, Amoxicillin, and Vonoprazan were determined by comparing the peak responses of Clarithromycin, Amoxicillin, and Vonoprazan in both the standard solution and the sample solution.

RESULTS AND DISCUSSION

Optimized method conditions

In the aim of successful separation of Clarithromycin, Amoxicillin, and Vonoprazan, 1.0 µl of a 100 % level solution was injected into a Hibar C18 column with dimensions of (100 mm x 2.1 mm and 2 µm). The mobile system consisted of ammonium acetate and acetonitrile in equal volumes, and the flow rate was kept at 0.3 ml/min while the detection wavelength was set to 210 nm. As compared to the reported methods [16-19], this method has more organic phase, which was good for the column life. Both the column and the injection port were kept at a temperature of 30 °C at all times. The mobile phase and all prepared solutions were filtered through the 0.45 microns filters before being introduced to the instrument. This was done to eliminate any particulate debris. The RT of Clarithromycin, Amoxicillin, and Vonoprazan observed at 1.24 min, 0.97 min and 1.66 min, correspondingly with accepted system suitability (fig. 2). The total run time was 3 min. Which was very short when compared to the reported methods [16, 19].



Drug name	Peak area	Plate Count	Tailing Factor	Resolution
Amoxicillin	2044631	3282	1.85	
Clarithromycin	2628562	3306	1.19	3.4
Vonoprazan	154310	3989	1.09	4.2

Fig. 2: Optimized method chromatogram representing retention times and system suitability data

Method validation

The system suitability indicators, such as % RSD, R, N, and T, followed the previously established acceptable limits established by various regulatory bodies. Table 1 displays the acquired results together with the allowed ranges for the system suitability parameters. The R^2 values assessed from regression analysis for the Clarithromycin (25 to 150 µg/ml), Amoxicillin (25 to 150 µg/ml), and Vonoprazan (1 to 6 µg/ml) concentrations have been calculated to be 0.999, depicting the linearity of the procedure with a significant impact (fig. 3). Clarithromycin, Amoxicillin, and Vonoprazan had mean slopes of 26188, 20093, and 38082, respectively. The SD values of the y-intercepts of Clarithromycin, Amoxicillin, and Vonoprazan were determined to be 9193, 1079.7, and 621.9, respectively. From the reported methods, it was concluded that, present method was very sensitive to estimate the drugs at low concentrations. Whereas the reported methods were having the linearity range at higher concentrations [19].

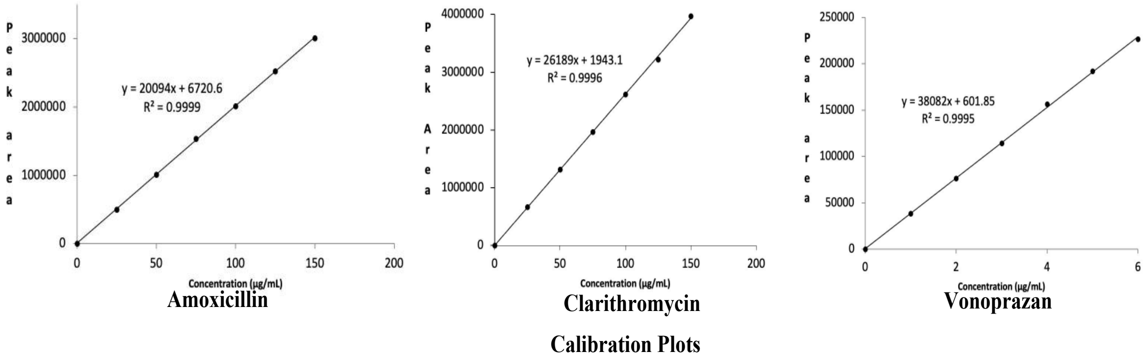


Fig. 3: Linearity plots of Clarithromycin (25 to 150 µg/ml), Amoxicillin (25 to 150 µg/ml), and Vonoprazan (1 to 6 µg/ml)

The LOD and LOQ were assessed as 0.07 µg/ml and 0.22 µg/ml for Clarithromycin, 0.81µg/ml and 2.45 µg/ml for Amoxicillin and 0.03 µg/ml and 0.09 µg/ml for Vonoprazan. At the RT of Clarithromycin, Amoxicillin, and Vonoprazan, could not observe any interference from other solutions. It demonstrates the high degree of specificity of stated method determining the Clarithromycin, Amoxicillin, and Vonoprazan present in given bulk and formulation samples (fig. 4 and 5).

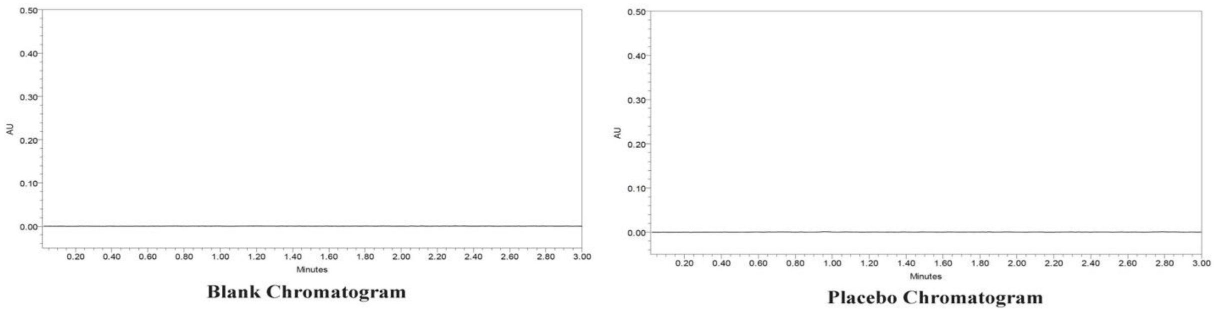


Fig. 4: Chromatograms representing the specificity of the method

The % RSD of the peak area responses of Clarithromycin, Amoxicillin, and Vonoprazan in successive consecutive injections of 100 % level or standard solution was less than two (table 1). This is a major representation of the methods' precision.

Table 1: Precision data of clarithromycin, amoxicillin and vonoprazan

Inter-precision			Method precision		
Drug name	**% Assay mean±SD**	**% RSD**	**Drug name**	**% Assay mean±SD**	**% RSD**
Clarithromycin	99.28±1.12	1.12	Clarithromycin	100.34±0.94	0.90
Amoxicillin	99.66±0.82	0.82	Amoxicillin	101.18±1.46	1.40
Vonoprazan	99.75±0.87	0.87	Vonoprazan	100.05±0.81	0.80

All the values were expressed in (n=3) mean±SD

The % recovery (n=3) of Clarithromycin, Amoxicillin, and Vonoprazan in stated spiked sample solutions was 98-102 % (table 2), confirming the accuracy of the approach as per ICH provisions. Significant changes were not observed in the % RSD and remaining system suitability indicators (table 3), demonstrating the robustness of the approaches. Present method has good recovery values as compared with the existing methods.

Table 2: Accuracy data of clarithromycin, amoxicillin, and vonoprazan at various levels

Drug name	% Level	Amount added*	Amount recovered*	Mean % recovery
Clarithromycin	50	50	49.88	99.76
	100	100	100.02	100.02
	150	150	150.20	100.14
Amoxicillin	50	50	50.08	100.16
	100	100	99.66	99.67
	150	150	150.86	100.58
Vonoprazan	50	2	1.99	99.66
	100	4	4.03	100.88
	150	6	6.01	100.20

All the values were expressed in (n=3) mean±SD

Table 3: Results of robustness of the method

Drug name	Variation in parameter		Peak area (mean±SD)	% RSD
Clarithromycin	Flow rate	Low	128821±1335.7	1.0
	(±0.1 ml)	High	128821±1335.7	1.0
	Mobile phase	Low	2034776±9874.1	0.5
	ratio (±1 ml)	High	2690316±15747.1	0.6
	Temperature	Low	2724427±4778.7	0.2
	(±5 °C)	High	2664227±8033.1	0.3
Amoxicillin	Flow rate	Low	2021612±13388.5	0.7
	(±0.1 ml)	High	2021612±13388.5	0.7
	Mobile phase	Low	1542351±6896.4	0.4
	ratio (±1 ml)	High	2114375±17051.1	0.8
	Temperature	Low	2112495±5474.1	0.3
	(±5 °C)	High	2096342±8734.1	0.4
Vonoprazan	Flow rate	Low	2520374±6475.2	0.3
	(±0.1 ml)	High	2520374±6475.2	0.3
	Mobile phase	Low	114803±1603.6	1.4
	ratio (±1 ml)	High	149428±2414.8	1.6
	Temperature	Low	154527±2587.9	1.7
	(±5 °C)	High	151310±2551.5	1.7

All the values were expressed in (n=3) mean±SD

According to the opinions of experts, it is often acceptable for stability-indicating approaches to tolerate up to 10 % degradation of the drugs material in most instances [23]. Comparing the peak regions in the resulted chromatograms of control with those of the standard solution under stressful circumstances allowed for the calculation of an estimate of the proportion of Clarithromycin, Amoxicillin, and Vonoprazan that had degraded. The findings that were observed and calculated are shown in table 4. All the forced samples chromatograms were shown in fig. 5. The purity threshold values were greater than the purity angles of the produced degradants and intended drug substances. According to the findings of the observation, the procedure has a feature that indicates stability. Because degradation of any of the three analytes was not seen under settings of neutral pH, this indicates that the analytes were all stable under these conditions.

Table 4: Results of force degradation studies clarithromycin, amoxicillin, and vonoprazan

Degradation	Drug names	Theoretical plates	Tailing factor	Resolution	% Degradation
Acid	Clarithromycin	2246	1.3	2.8	3.17
	Amoxicillin	2064	1.2	-	3.45
	Vonoprazan	2574	1.2	3.3	4.61
Base	Clarithromycin	2262	1.3	2.8	6.34
	Amoxicillin	2950	1.3	-	4.34
	Vonoprazan	2344	1.1	3.3	5.75

Degradation	Drug names	Theoretical plates	Tailing factor	Resolution	% Degradation
Oxidative	Clarithromycin	2442	1.3	2.8	5.22
	Amoxicillin	2136	1.3	-	4.38
	Vonoprazan	2624	1.2	3.3	5.00
Photolytic	Clarithromycin	2442	1.3	2.9	1.45
	Amoxicillin	2165	1.2	-	1.80
	Vonoprazan	2693	1.2	3.4	0.41
Thermal	Clarithromycin	2409	1.3	2.8	1.92
	Amoxicillin	2170	1.2	-	2.98
	Vonoprazan	2764	1.2	3.3	3.73
Water	Clarithromycin	2436	1.3	2.9	0.10
	Amoxicillin	2177	1.2	-	0.43
	Vonoprazan	2752	1.2	3.4	0.41

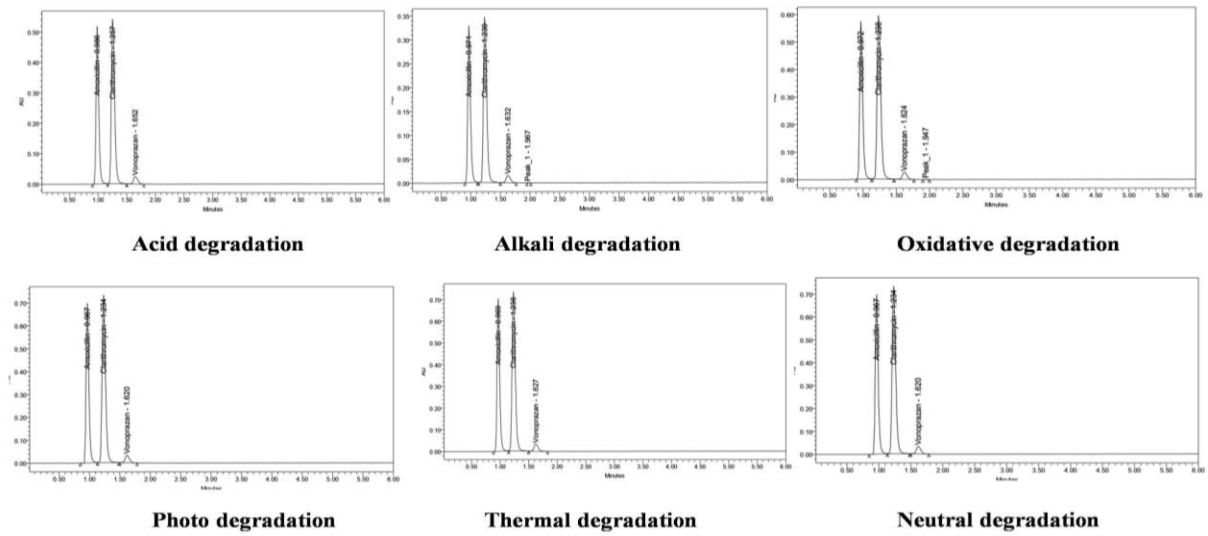


Fig. 5: Chromatograms representing forced degradation studies of the method

Application of current method for assay of marketed formulation

It was discovered that the % purity of the Clarithromycin, Vonoprazan, and Amoxicillin, marketed combination tablet and capsule, was 99.85–100.14 % (table 5). This indicates that the assay values of the Clarithromycin, Amoxicillin, and Vonoprazan were in accordance with the guidelines set by the ICH.

There was no RP-UPLC approach that could be maintained with a simple solvent system and a low RT. Therefore, we attempted to find a technique that indicates stability using a simple solvent system (equal parts of ammonium acetate and acetonitrile) and a shorter amount of time at room temperature (less than two minutes). The RTs of Clarithromycin, Amoxicillin, and Vonoprazan were very low, which is considered to be cost-effective owing to the reduction in elution time and volume of solvent consumption. As a result, the amount of time spent analyzing samples is reduced, and the total number of samples that are tested may be raised. The FD investigations were helpful in determining the quantity of the medication that had been degraded, which was necessary in order to stipulate the stability-indicating characteristic of the approach. The parameters of the established method validation were in accordance with the requirements of ICH's Q2 standard.

Table 5: Assay of clarithromycin, amoxicillin, and vonoprazan in the combined formulation

Drug	Peak	Peak area	Label claim (mg)	Amount found (mg)	Assay (% w/w)
Amoxicillin	Standard Peak	2044331	500	499.23±6.67	99.85±8.9
	Test Peak	2043256			
Clarithromycin	Standard Peak	2629642	500	499.56±7.4	99.91±6.3
	Test Peak	2629991			
Vonoprazan	Standard Peak	154614	20	20.02±2.4	100.14±1.6
	Test Peak	154987			

All the values were expressed in (n=3) mean±SD

CONCLUSION

To investigate Clarithromycin, Amoxicillin, and Vonoprazan simultaneously in mixed bulk form and in dosage form, an economical, low-cost, precise, and specific RP-UPLC technique with high sensitivity has been established. This approach has excellent sensitivity. Investigating analytes under different types of stress makes sure that the stability indication of the method is reflected in the stability of the analytes. The technique that was developed was successful in separating Clarithromycin, Amoxicillin, and Vonoprazan in addition to any potential degradation products of all three drugs. When it came to the newly developed approach, Clarithromycin, Amoxicillin, and Vonoprazan all had quicker separation times. This Method can be experimented for the bioanalysis of these drugs and indicates that the approach anticipated can often be used in commercial markets.

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Nil

AUTHORS CONTRIBUTIONS

All the researchers participated in the research and production of the text in an equal capacity throughout all phases.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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