RESEARCH PAPER

Method Development, Preparation & In Vivo Anti-Ulcer Evaluation of Tegoprazan-Loaded Niosomes

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Abstract
Peptic ulcer disease (PUD) is still a prevalent gastrointestinal condition that calls for better medication administration and efficient acid suppression. Tegoprazan is a new potassium-competitive acid blocker (P-CAB) that inhibits stomach acid production quickly and continuously, while niosomal drug delivery systems may enhance its therapeutic efficacy through improved drug retention and controlled release. The purpose of this work was to create and verify a reverse-phase high-performance liquid chromatography (RP-HPLC) technique for tegoprazan quantitative quantification and to evaluate the gastroprotective efficacy of a tegoprazan-loaded niosomal formulation prepared by the ethanol injection method in a pyloric ligation-induced rat model. The ICH Q2(R1) recommendations for system appropriateness, specificity, accuracy, precision, and linearity were followed in the development and validation of the RP-HPLC technique. The ethanol injection approach was used to create niosomes loaded with tegoprazan, which were then given orally (5 mg/kg) before pyloric ligation. stomach juice volume, stomach pH, free acidity, total acidity, ulcer area, and histological alterations were measured in order to evaluate anti-ulcer activity. The validated RP-HPLC method demonstrated satisfactory analytical performance with excellent specificity, accuracy, precision, and linearity. Compared with unformulated tegoprazan, the niosomal formulation significantly reduced gastric volume, free acidity, total acidity, and ulcer area while increasing gastric pH and preserving gastric mucosal architecture. Overall, the tegoprazan-loaded niosomal formulation exhibited superior gastroprotective activity, and the validated RP-HPLC method proved suitable for routine quantitative analysis and pharmaceutical quality control.

Keywords: Tegoprazan; RP-HPLC; Niosomes; Ethanol injection method; Peptic ulcer disease; Gastroprotection

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1. Introduction

With improvements in diagnosis and pharmacological treatment, peptic ulcer disease (PUD) is still a significant gastrointestinal condition globally. It is brought on by an imbalance between the protective elements of the stomach mucosa and the aggressive elements, including pepsin, H. pylori infection, stomach acid, and non-steroidal anti-inflammatory drugs (NSAIDs). Recurrent mucosal damage can lead to epigastric pain, gastrointestinal bleeding, perforation, gastric outlet obstruction, and recurrent ulceration and might have a major influence on patients' quality of life. The three

primary goals of the current therapy paradigm are mucosal repair, H. pylori eradication, and acid suppression. Still, therapy often fails to prevent recurrence and maintain resolution of symptoms, especially in patients on long-term NSAIDs or those who have ongoing H. pylori infection (Malfertheiner et al., 2022). Proton pump inhibitors (PPIs) are the first-choice medications used to treat acid-related disorders because of their potent acid-suppressive actions. These, however, have hindered their clinical use due to the delayed onset of action, requirement for acidic activation, and meal-related dosing. Their clinical use has, however, been limited by delayed onset of action, dependence on acidic activation, and interindividual variability based on CYP2C19 metabolism. As a result, there is also some acid suppression that is not consistent, and therapeutic response varies. Furthermore, there is growing interest in alternative therapies that are faster acting, more predictable in their pharmacokinetics, and provide long-term acid inhibition, due to concerns about the use of PPIs for extended periods of time (Scarpignato et al., 2016; Hussaini et al., 2025).

Tegoprazan is a novel potassium-competitive acid blocker (P-CAB) that reversibly inhibits the gastric ATPase to quickly and consistently reduce stomach acid flow. Unlike PPIs, tegoprazan doesn't need acid activation to have the intended effect, thus providing earlier onset of action and more uniform intragastric pH maintenance. The foregoing properties result in less dependency on the timing of the medication's administration and better pharmacodynamic properties, which makes tegoprazan a viable therapeutic choice for gastric ulcer, gastroesophageal reflux disease (GERD), erosive esophagitis, and disorders associated with H. pylori (Andreev et al., 2026; Cho et al., 2020; Qiao & Li, 2025). Clinical studies have shown that tegoprazan has a similar efficacy in healing ulcers as conventional PPIs but has a good safety profile. The non-inferior efficacy to lansoprazole in gastric ulcer treatment was demonstrated in randomized clinical trials (Cho et al., 2020). Other more recent systematic reviews and meta-analyses have similarly proved that it is effective in GERD and other acid-mediated conditions, comparable or better than PPIs (Hussaini et al., 2025; Bandyopadhyay et al., 2026). Likewise, H. pylori eradication has shown to be effective with a tegoprazan-based regimen, resulting in better outcomes due to its prolonged acid suppression. Likewise, H. pylori eradication has been shown to be effective when used with a tegoprazan-based regimen, which provides for prolonged acid suppression and improved outcomes for eradication. Recent evidence also indicates that tegoprazan demonstrates a rapid onset of antisecretory activity with sustained intragastric pH control and favourable tolerability, making it an attractive next-generation alternative to conventional acid-suppressive therapies across a broad spectrum of gastrointestinal disorders (Anik et al., 2024; Wang et al., 2025). Although these are all benefits, oral drug delivery is still subject to physiological parameters that have a significant effect on drug dissolution, gastric residence time, and drug availability, which might result in the creation of novel and improved oral medication delivery techniques.

Nanotechnology drug delivery systems are seen as successful methods to enhance therapeutic efficacy of drugs. Both lipophilic and hydrophilic drugs can be absorbed via niosomes, which are vesicular carriers composed of non-ionic surfactants. They shield drugs from degradation, enhance stability, and deliver controlled and sustained drug release, leading to an improvement in therapeutic efficacy (Ge et al., 2019; Ahmad et al., 2022). Niosomes have a number of merits over conventional dosage forms: make the medication more soluble, improve its membrane permeability, allow for a longer release of a drug, decreased systemic toxicity, and increased bioavailability. They are attractive alternatives to liposomal systems (Moghassemi & Hadjizadeh, 2014; Marianecci et al., 2016) that are relatively easy to prepare, biocompatible, and have a relatively low production cost. Niosomes have been thoroughly investigated for the administration of antibacterial, anticancer, anti-inflammatory, and gastrointestinal medications because of these qualities. In recent years, formulation innovations have enhanced the stability, drug loading capacity, and controlled release capabilities of vesicles, further expanding their pharmaceutical applications (Kazi et al., 2010; Ahmad et al., 2022; Teron et al., 2024; Mawazi et al., 2024). Moreover, advances in nanovesicle engineering have demonstrated that oral nanocarriers can improve drug absorption by protecting therapeutic molecules from degradation within the gastrointestinal tract, facilitating epithelial transport, and enhancing systemic bioavailability. Such pharmacokinetic improvements may translate into greater therapeutic effectiveness while reducing dose-related variability (Ren et al., 2022; Gharat et al., 2024).

Tegoprazan has an excellent acid-suppressive activity, but its incorporation into a niosomal delivery system may lead to an increase in therapeutic activity. Niosomal encapsulation may offer protection against degradation, enhance gastric residence, sustain drug release, and increase drug availability at the gastric mucosa. All these benefits can help improve gastroprotection by providing long-lasting acid suppression, minimizing fluctuations in drug levels, and improving patient adherence (Ge et al., 2019; Ahmad et al., 2022). Furthermore, optimizing tegoprazan within an advanced nanocarrier system may improve its pharmacokinetic profile by prolonging drug retention at the target site and promoting more efficient drug distribution, thereby maximizing its therapeutic potential in ulcer management (Gharat et al., 2024). Although tegoprazan has been clinically proven to be effective in acid-related diseases, there is still insufficient data about its use in nanovesicular systems for anti-ulcer applications. Hence, it is rational to design a niosomal tegoprazan formulation to increase the gastroprotective activity. In addition, developing an approved analytical method for tegoprazan quantitative estimation is crucial for the quality, reliability, and repeatability of formulation development based on the existing analytical validation guidelines (Panda et al., 2025). Recently, stability-indicating RP-HPLC methods specifically

developed for tegoprazan have further highlighted the importance of robust analytical validation to ensure accurate quantification during formulation development, stability assessment, and routine pharmaceutical quality control (Gunda et al., 2026).

Study Objectives:

1. To create and verify an HPLC technique for quantitative quantification of tegoprazan.
2. To prepare a tegoprazan-loaded niosomal formulation and evaluate its anti-ulcer activity in pyloric ligation-induced rats.
3. To compare the gastroprotective efficacy of the formulation with un-formulated tegoprazan using gastric secretory parameters, ulcer area, gross gastric morphology, and histopathology.

2. Methodology

2.1 Materials

The RP-HPLC technique was developed using Milli-Q/HPLC grade water, orthophosphoric acid (AR, ACS grade), methanol (HPLC grade), and potassium dihydrogen phosphate (LR grade). Chromatographic analysis was carried out using a Waters 2690 Alliance HPLC system equipped with a SAB 203 L electronic balance, Borosil Class-A glassware, a PSA-10A Digital Pro ultrasonicator, and a Model 152R pH meter. The mobile phase served as the diluent throughout the analysis. For formulation development, tegoprazan, Span 60, cholesterol, ethanol, distilled water, and phosphate-buffered saline (PBS) were used for the preparation of tegoprazan-loaded niosomes.

2.2 RP-HPLC Method Development

The phosphate buffer was made by dissolving 2.72 g of potassium dihydrogen phosphate in distilled water, raising the volume to 1 L, and using orthophosphoric acid to bring the pH down to 3.8. Methanol and phosphate buffer (30:70, v/v) made up the mobile phase. Before being used, it was filtered through a 0.45 µm membrane filter and sonicated for 15 minutes. The mobile phase was utilised as the diluent in the creation of the standard and sample solutions, with 40 mg of tegoprazan utilised for the standard preparation. For isocratic chromatographic separation, a C18 column (4.6 × 150 mm, 5 µm) was utilized. UV detection at 245 nm was used to provide the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 10 µL, the duration was 10 minutes, and the sample and column temperatures were maintained at 20 ± 5°C and 30 ± 0.5°C, respectively. Method optimization was carried out by carrying out two chromatographic runs, and the optimum condition of the chromatography was chosen based on the symmetry, retention time, theoretical plate number, and resolution.

2.3 RP-HPLC Method Validation

Analytical techniques were used to validate the RP-HPLC optimized procedure. System appropriateness, specificity, assay, accuracy, precision, linearity, range, and bracketing standards were the validation criteria. The system's suitability was evaluated using retention time, tailing factor, number of theoretical plates, and percentage relative standard deviation (%RSD). Specificity was confirmed by checking for interfering peaks through the comparison of blank and sample chromatograms. Accuracy was checked using three different concentrations that is 50%, 100% and 150%. Precision was calculated from analysis of replicate samples while linearity was done by plotting peak area versus analyte concentration in the range 16–48 ppm.

2.4 Preparation of Tegoprazan-Loaded Niosomes by Ethanol Injection Method

The preparation of tegoprazan-loaded niosomes was achieved using the ethanol injection technique. The needed amounts of Span 60, cholesterol, and tegoprazan were carefully weighed and then dissolved in 10–20 mL of ethanol at a moderate temperature of 40–60°CConversely, phosphate-buffered saline (PBS) or distilled water was heated to 60–70°C while being continuously stirred magnetically. Using a syringe with a needle and continuous magnetic stirring at 800–1000 rpm, the organic layer was gradually injected into the heated aqueous layer. After the addition of the entire volume of the organic layer, the stirring was done for 30-60 minutes to allow the evaporation of ethanol as well as the self-assembling of Span 60 and cholesterol to form niosomes entrapping tegoprazan.

2.5 Optimization of Tegoprazan-Loaded Niosomes Using Central Composite Design

The Central Composite Design (CCD) was used to optimize niosomal formulations loaded with tegoprazan. The study included cholesterol and span 60 as independent variables. The investigation of Span 60 was conducted in the range of 50-150 mg, while that of cholesterol was done in the range of 25-100 mg, along with axial points of the experimental design. Altogether, eleven formulations were designed based on the CCD experimental design, and the optimized formulation was used for further in vivo antiulcer studies.

2.6 Experimental Animals

We purchased male Wistar albino rats from the Institutional Animal House, each weighing 180 ± 20 g and housed in polypropylene cages in a typical laboratory setting with a temperature of 25 ± 2°C and a 12-hour light-dark cycle. They had unlimited access to a standard diet and water, with the exception of fasting. The IAEC recommendations served as the basis for the study.

2.7 Dose Calculation

The dose of tegoprazan used experimentally was determined based on the dose that is recommended for humans using the body surface area method of conversion. With the use of a human dose of 50 mg and the human-to-rat conversion factor, five milligrammes per kilogram of body weight was established as the experimental dose.

2.8 Experimental Design

The animals were divided into four experimental groups at random, each consisting of six individuals (n = 6). Group I was given solely the vehicle without pyloric ligation as the standard control. Group II received the vehicle and pyloric ligation as the ulcer control. One hour before to pyloric ligation, Group III was given unformulated tegoprazan orally at a dosage of 5 mg/kg body weight. Group IV received the tegoprazan-loaded niosomal formulation orally at the same dose (5 mg/kg body weight), 1 h before pyloric ligation. All treatments were administered orally, and the anti-ulcer activity was subsequently evaluated using gastric secretory parameters, ulcer area, gross gastric morphology, and histopathological examination.

2.9 Induction of Gastric Ulcer

The animals had access to drink for up to an hour before to the experiment, but they were famished for 24 hours prior. After giving each animal their treatment through the oral route, anesthesia was induced by the administration of sodium pentobarbital (35 mg/kg) and xylazine (10 mg/kg) via the intraperitoneal route. Each animal's belly was shaved, and a 2-centimeter incision was created at the abdomen's midline. The pyloric portion of the stomach was sutured with a 3-0 silk suture without cutting off the blood flow.

2.10 Collection of Gastric Juice

The mice died from cervical dislocation four hours after pyloric ligation. Stomachs were removed, and the gastric fluid was collected in graded tubes to determine the gastric volume. After centrifuging the samples for ten minutes at 3000 rpm, the supernatant was collected and used to determine the pH, free acidity, and total acidity. Stomachs were then opened along the greater curvature, washed with ice-cold normal saline, mounted on cork boards and photographs taken for ulcer scoring.

2.11 Evaluation of Anti-Ulcer Activity

The size of ulcers, the percentage of ulcer inhibition, the volume of gastric juice, the pH of the stomach, and the free and total acidity were all measured in order to evaluate the anti-ulcer impact. Immediately following sample collection, the pH of the stomach was tested. Free acidity was determined by titrating 0.5 mL of gastric juice against 0.01 N sodium hydroxide using Topfer's indicator. After that, total acidity was determined by carrying out titration with phenolphthalein.

2.12 Histopathological Examination

The stomach tissue samples were sectioned at 5 μm, dehydrated using stepwise ethanol, paraffin embedded, xylene cleared, and stained with hematoxylin and eosin (H&E) after being fixed in 10% neutral buffered formalin for a whole day. Histopathological analysis was carried out using the light microscope to study the loss of epithelium, infiltration of inflammatory cells, submucosal edema and gland disruption.

2.13 Statistical Analysis

The mean ± standard deviation (SD) and standard error of the mean (SEM) were used to express the experimental data for stomach pH, gastric juice volume, free acidity, total acidity, ulcer area, ulcer protection, and histological findings. One-way analysis of variance (ANOVA) and a suitable post hoc multiple comparison test were used to statistically compare the experimental groups. Statistical significance was defined as a p-value of less than 0.05.

3. Results

3.1 RP-HPLC Method Development

3.1.1 Trial 1 Chromatogram

Using a C18 column (4.6 × 150 mm, 3.5 μm) and an isocratic mobile phase made of methanol and phosphate buffer (40:60, v/v),the first chromatographic parameters were examined. Chromatography revealed a well-resolved peak of tegoprazan with a retention time of about 4.52 min (Figure 1).

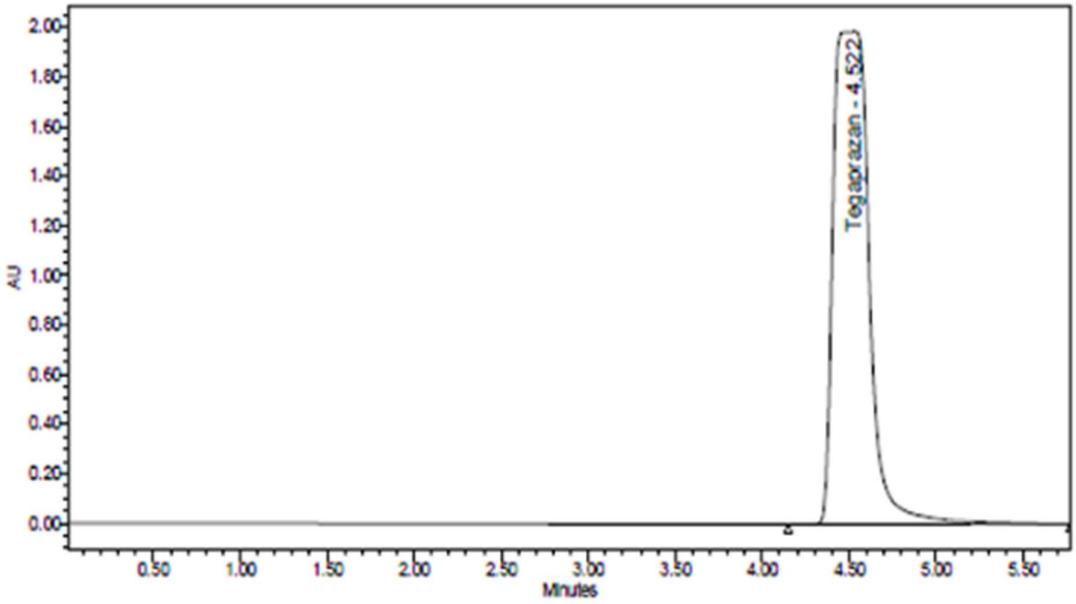

Figure 1. HPLC chromatogram of tegoprazan obtained during Trial 1

3.1.2 Trial 2 Chromatogram

The second experiment conducted using the same mobile phase composition and A C18 column measuring 4.6 x 150 mm and 5 µm produced a well-defined peak at a retention duration of around 4.56 minutes when the time was increased to 20 minutes. This shows better peak shape than the first experiment (Figure 2).

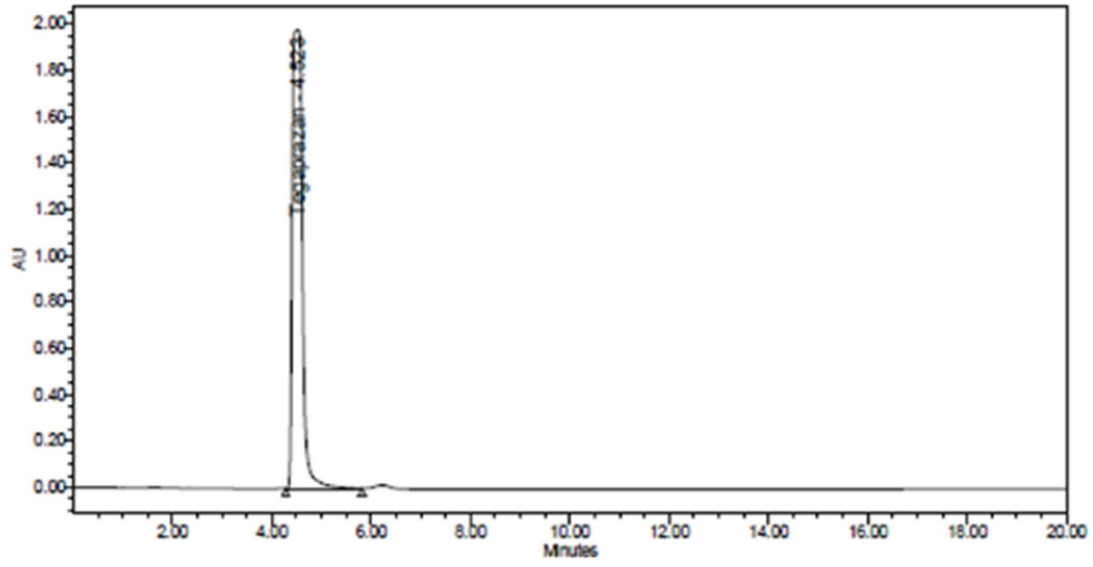

Figure 2. Trial 2 HPLC chromatogram of tegoprazan

3.1.3 Optimized HPLC Chromatogram

Following optimization, separation was carried out using a mobile phase mixture of methanol and phosphate buffer (30:70, v/v) at a flow rate of 1.0 mL/min, an injection volume of 10 µL, and a total run length of 10 minutes. The optimized chromatogram generated a single peak at 5.60 minutes, showing acceptable separation efficiency for further validation (Figure 3).

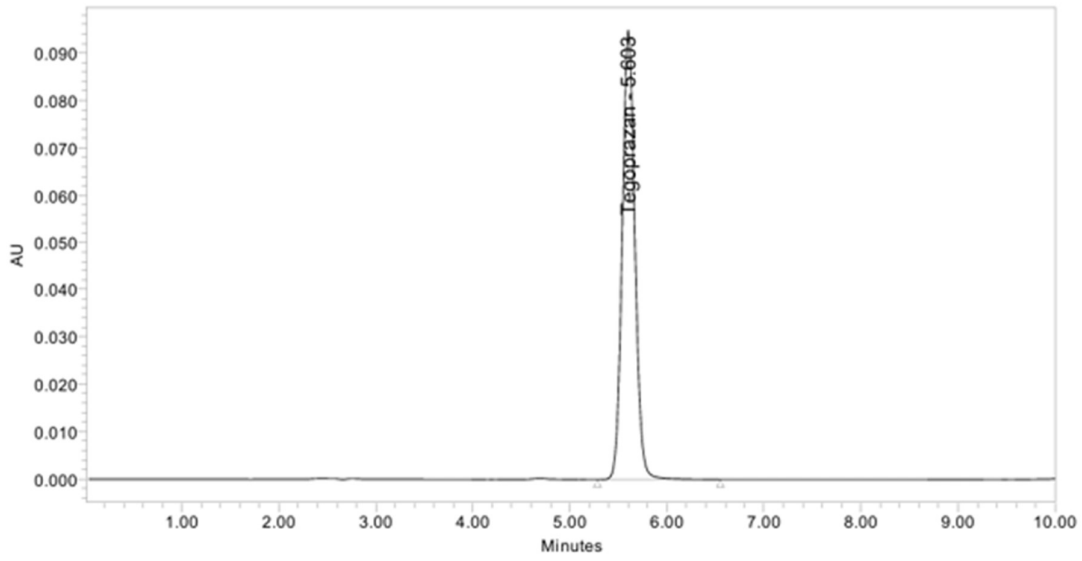

Figure 3. Optimized HPLC chromatogram of tegoprazan

3.2 RP-HPLC Method Validation

3.2.1 Results of System Suitability

Optimized HPLC conditions met all system suitability criteria. Five injections of the standard solution yielded a similar response pattern with a mean retention time of 5.623 minutes, SD of 0.015, and % RSD of 0.27. The theoretical plates number and tailing factor values (8744 and 1.1 respectively) met the pre-set acceptance criteria, showing satisfactory performance of the chromatographic method (Table 1, Figure 4).

Table 1. Outcomes of System suitability for the optimized HPLC method

Parameter	Observed value	Acceptance criteria
Mean retention time (min)	5.623	
Standard deviation	0.015	
%RSD	0.27	≤2.0
Theoretical plates	8744	≥2000
Tailing factor	1.1	≤2.0

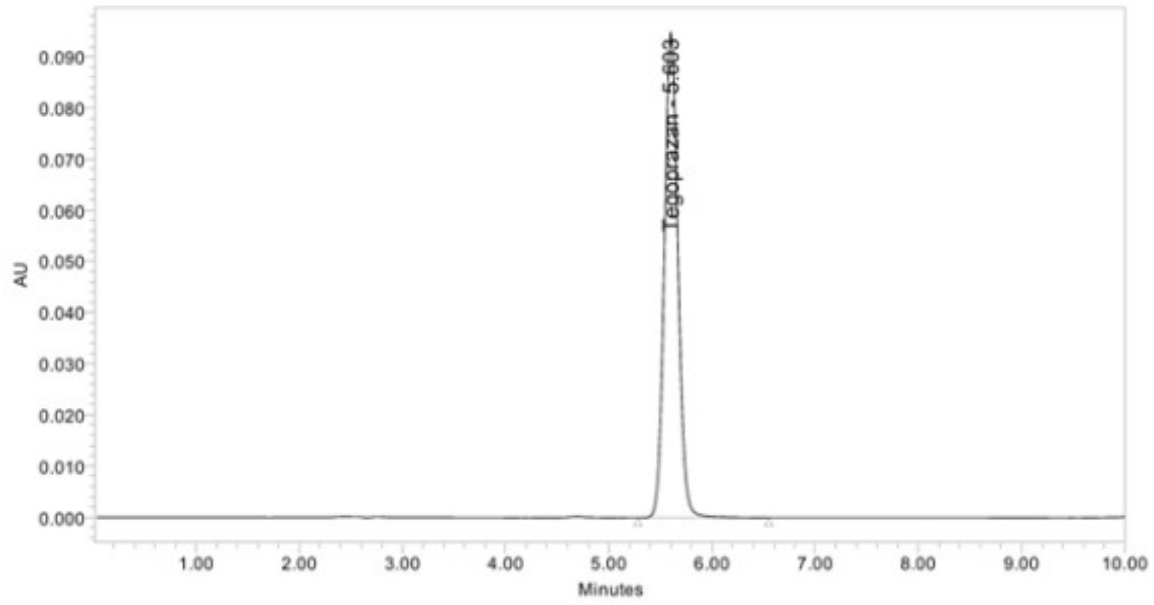

Figure 4. System suitability chromatograms of tegoprazan

3.2.2 Specificity Results

The assessment of specificity through blank chromatograms indicated the lack of any interfering peak at the retention time of tegoprazan. This lack of interference proved the specificity of the developed method for determination of tegoprazan and compliance with the acceptance criteria (Figure 5).

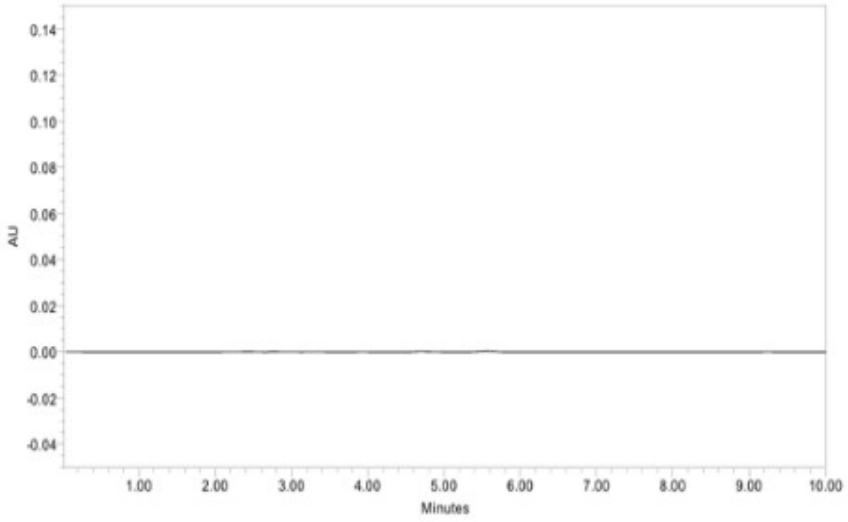

Figure 5. Blank HPLC chromatogram showing the absence of interfering peaks

3.2.3 Batch Assay Results

The results obtained from the batch assay analysis on the two samples, each prepared separately, gave the retention time values of 5.650 min and 5.658 min with the assay values being 100.27% and 100.70%, respectively. The average assay value obtained was 100.48% that was within the acceptable limit of 98-102%, indicating that the methodology used was suitable for quantification of tegoprazan (Table 2, Figure 6).

Table 2. Batch assay results of tegoprazan

Sample	Retention Time (min)	Assay (%)
Sample 1	5.650	100.27
Sample 2	5.658	100.70

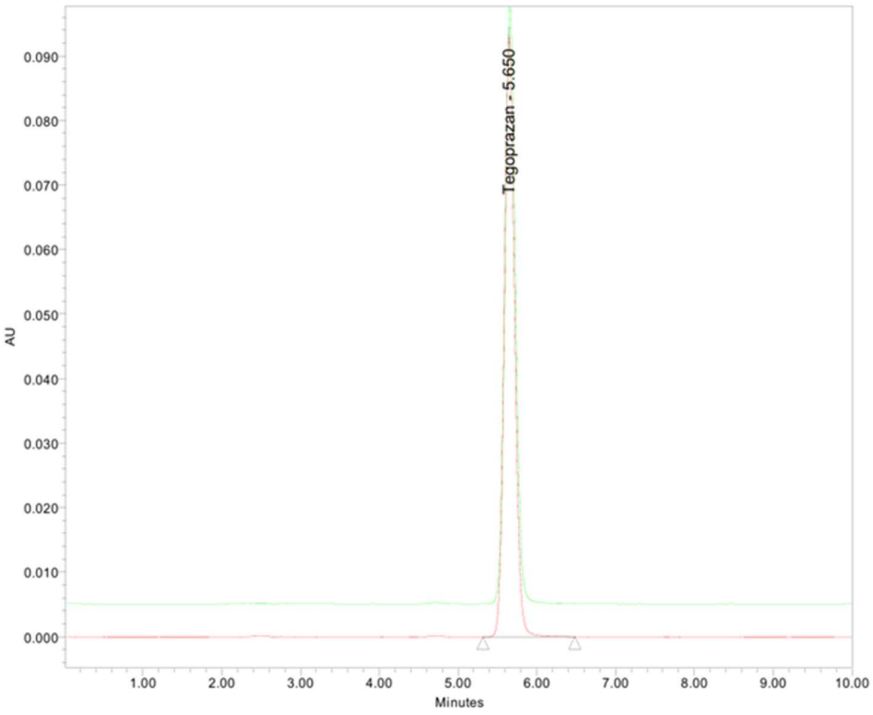

Figure 6. Batch assay chromatograms of tegoprazan sample solutions

3.2 HPLC Method Validation

3.2.4 Accuracy Results

The established HPLC method's accuracy was assessed at concentrations of 50%, 100%, and 150%. The mean recovery values calculated were 100.38%, 100.48%, and 96.69%, respectively. Corresponding to the aforementioned values, the %RSD values were found to be 0.45%, 0.36%, and 0.05%. The chromatographic representations at different concentrations are given in Figures 7–9, in contrast, Table 3 displays the recovery values.

Table 3. Accuracy study of the developed HPLC method

Concentration Level	Average Recovery (%)	SD	%RSD
50%	100.38	0.45	0.45
100%	100.48	0.36	0.36
150%	96.69	0.05	0.05

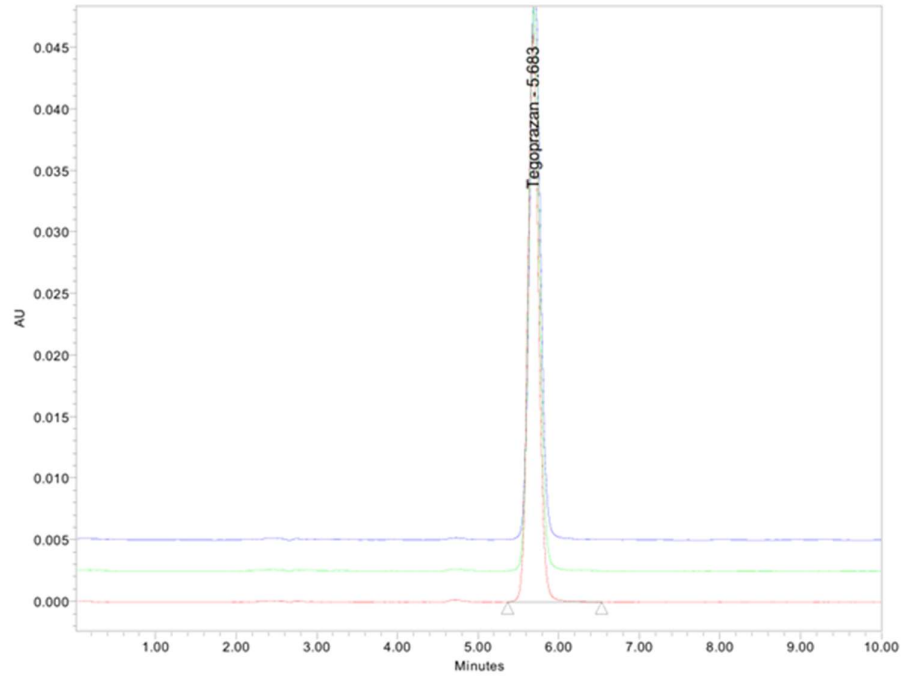

Figure 7. Tegoprazan overlay chromatograms at a 50% accuracy level

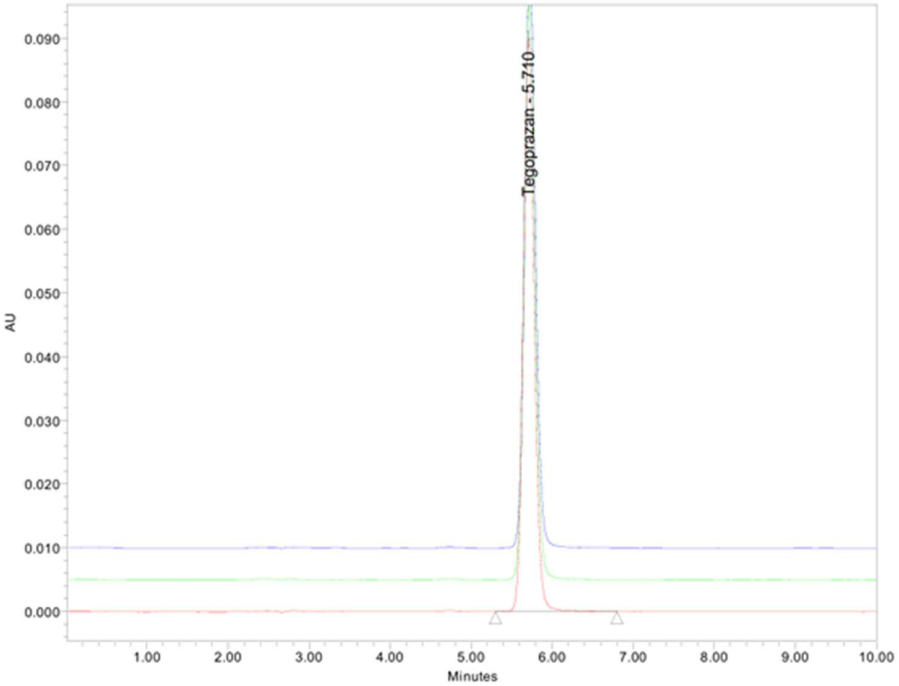

Figure 8. Tegoprazan overlay chromatograms at a 100% accuracy level

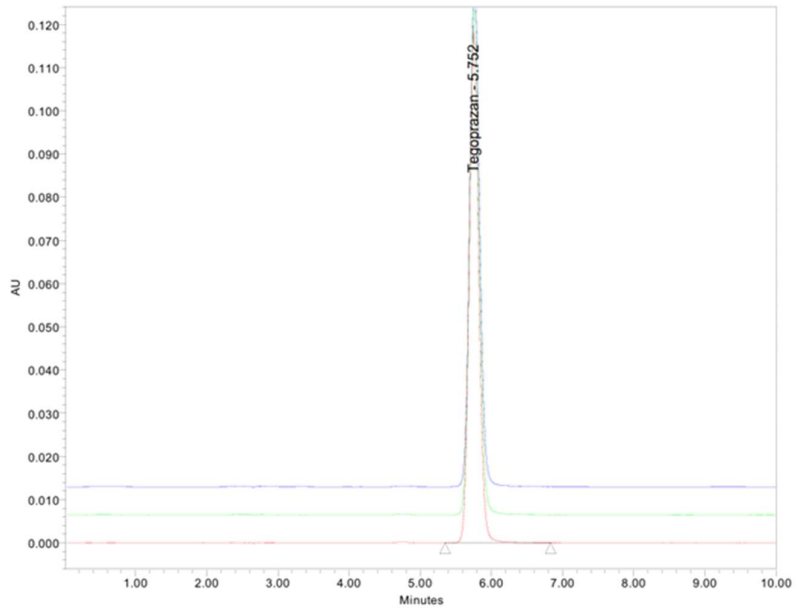

Figure 9. Tegoprazan overlay chromatograms at a 150% accuracy level

3.2.5 Precision Results

The method of accuracy was performed based on the analysis of six sample solutions that were independently prepared and analyzed under the same chromatographic conditions. The result of the analysis gave an average assay percentage of 100.30% with a standard deviation of 0.37% and %RSD of 0.40% and met the required acceptance criteria of 2.0% or less (Table 4, Figure 10).

Table 4. Method precision results

Parameter	Value
Average assay (%)	100.30
Standard deviation	0.37
%RSD	0.40
Acceptance criterion	≤2.0%
Observation	Passed

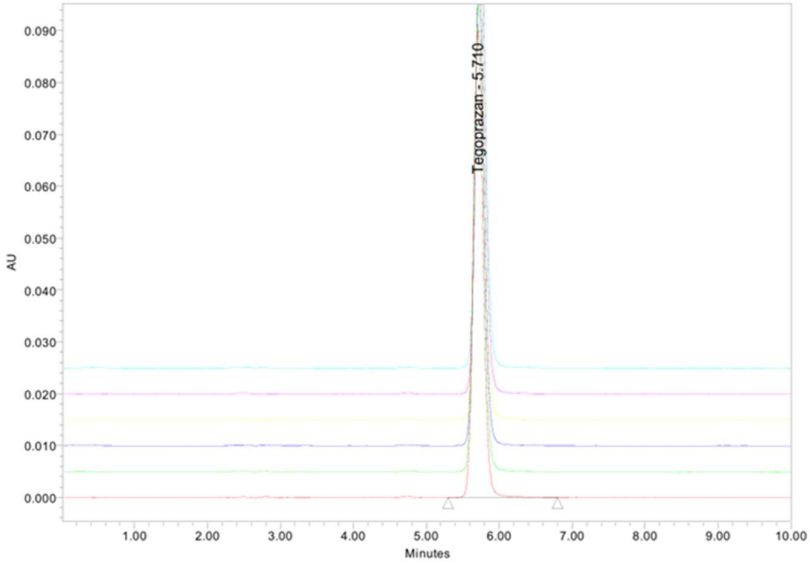

Figure 10. Overlay chromatograms of tegoprazan obtained during the precision study

3.2.6 Linearity Results

Tegoprazan concentrations at 16, 24, 32, 40, and 48 ppm were measured in order to evaluate the linearity of the suggested HPLC approach. Increase in concentration resulted to an increase in the detector signal in a proportional manner, thus generating a calibration graph having a correlation coefficient (r) value of 0.99, which satisfies the criteria for linearity of the process. Table 5 summarises the calibration findings, while Figures 11 display the resultant chromatograms at 50, 75, 100, 125, and 150 ppm.

Table 5. Linearity results of the developed HPLC method

Concentration (ppm)	Mean Peak Area
16	434438
24	603791
32	855830
40	969033
48	1135242
Correlation coefficient (r)	0.99

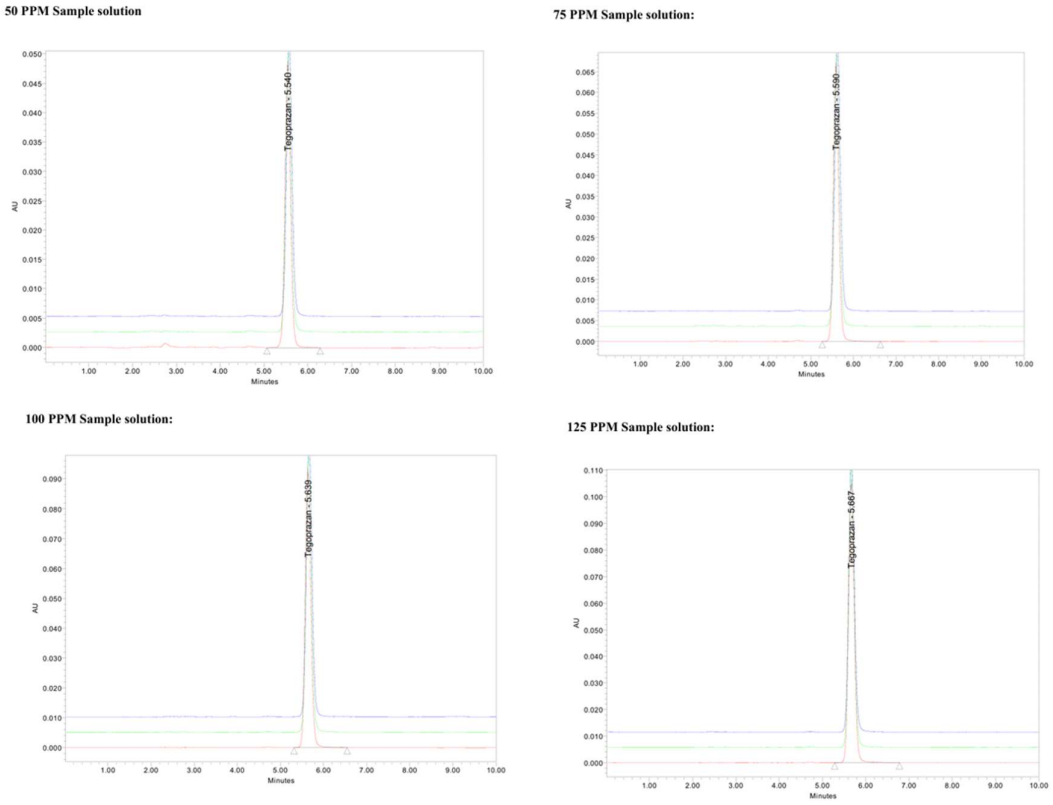

Figure 11. Representative HPLC chromatograms of tegoprazan at different concentration levels (50, 75, 100, and 125 ppm) used for linearity evaluation

3.2.7 Range Results

The concentrations utilized in recovery studies were used to assess the analytical range of the suggested approach. The values of %RSD determined at 50%, 100% and 150% of concentration levels were 0.87%, 0.59% and 0.85%, correspondingly (Table 6), which is a satisfactory value of analytical performance in the range under consideration.

Table 6. Analytical range of the developed HPLC method

Concentration Level	%RSD
50%	0.87
100%	0.59
150%	0.85

3.2.8 Bracketing Standard Results

Bracketing standard chromatogram illustrated consistency in the performance of the system under optimal analytical conditions. Tegoprazan eluted at 5.688 minutes with an area of 862342, peak height of 91830, USP plate count of 8354, and USP tailing factor of 1.1. All the chromatographic data indicated satisfactory performance of the system during the analysis process. Representative chromatographic data is summarized in Table 7.

Table 7 Bracketing standard chromatographic parameters of tegoprazan

Peak Name	Retention Time (min)	Peak Area	Peak Area (%)	Peak Height	USP Plate Count	USP Tailing Factor
Tegoprazan	5.688	862342	100.00	91830	8354	1.1

The resulting representative chromatogram of the bracketing standard method showed a well-formed and symmetrical peak of tegoprazan under optimized chromatographic conditions (Figure 12).

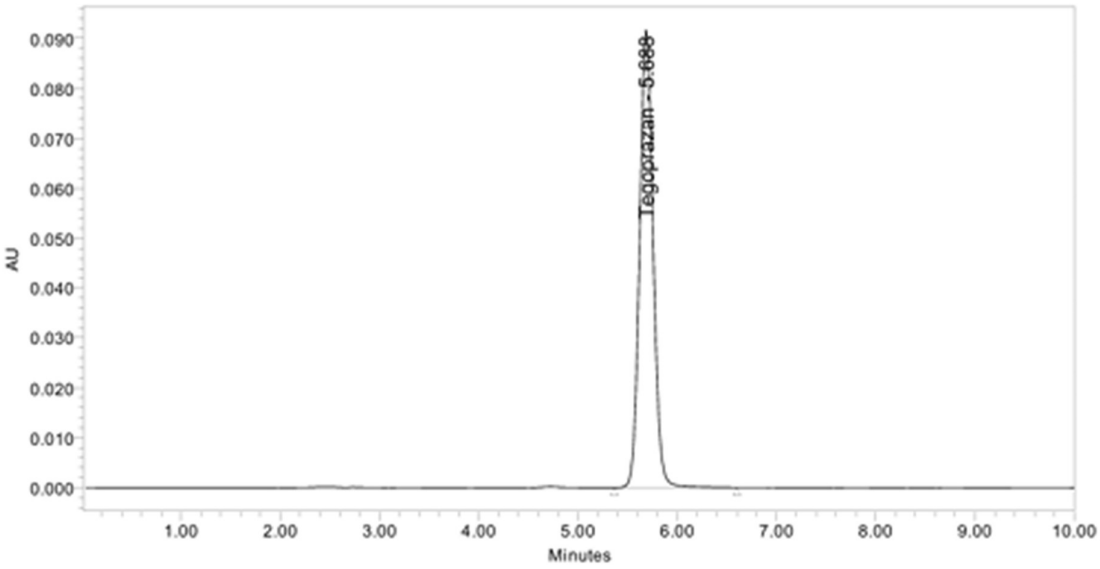

Figure 12. Representative bracketing standard chromatogram of tegoprazan

3.3 In Vivo Anti-Ulcer Activity

3.3.1 Effect on Gastric Volume, Gastric pH, Total and Free Acidity

The rats who underwent pyloric ligation showed a considerable change in the stomach secretory characteristics. The ulcer control group showed high levels of gastric volume (5.48 ± 0.26 mL), free acidity (55.2 ± 1.16), total acidity (84.0 ± 1.22), and the least value of gastric pH (1.28 ± 0.10). Administration of un-formulated tegoprazan (5 mg/kg) resulted in low values of gastric volume (4.18 ± 0.08 mL), free acidity (43.8 ± 2.27), and total acidity (59.6 ± 1.96), along with high gastric pH (2.14 ± 0.19). Tegoprazan loaded formulation (5 mg/kg) resulted in even lower gastric volume (3.38 ± 0.16 mL), free acidity (33.6 ± 0.96), total acidity (50.2 ± 1.71), and the highest gastric pH (3.70 ± 0.12) (Table 8).

Table 8. Effect of tegoprazan treatments on gastric secretory parameters in pyloric ligation-induced rats

Treatment	Gastric Volume (mL)	Free Acidity	Total Acidity	Gastric pH
Ulcer control	5.48 ± 0.26	55.2 ± 1.16	84.0 ± 1.22	1.28 ± 0.10
Un-formulated tegoprazan (5 mg/kg)	4.18 ± 0.08	43.8 ± 2.27	59.6 ± 1.96	2.14 ± 0.19
Tegoprazan-loaded formulation (5 mg/kg)	3.38 ± 0.16	33.6 ± 0.96	50.2 ± 1.71	3.70 ± 0.12

Values are expressed as mean ± SEM (n = 5).

3.3.2 Effect on Ulcer Area and Percentage Ulcer Protection

The stomach mucosa's macroscopic examination showed notable variations across the different experimental groups. There was no formation of ulcers in the case of normal controls. On the other hand, there were three primary ulcers along with petechial hemorrhage in the ulcer control, resulting in a total of 363.06 mm² area of ulcers. The administration of un-formulated tegoprazan (5 mg/kg) significantly prevented ulceration; a small number of ulcers were found with an area of 87.02 mm² which corresponds to 76.00% protection from ulcers. Tegoprazan loaded formulation treated animals exhibited less ulcer formation, with a significant reduction in the area of ulcers of 18.90 mm² and 94.80% ulcer protection (Table 9).

Table 9. Effect of tegoprazan treatments on gastric ulcer area and ulcer protection

Treatment	Gross Gastric Lesions	Ulcer Area (mm^2)	Ulcer Protection (%)
Normal control	No lesions	0.00	—
Ulcer control	Three major lesions with petechiae	363.06	0.00
Un-formulated tegoprazan (5 mg/kg)	Few small erosions	87.02	76.00
Tegoprazan-loaded formulation (5 mg/kg)	Minimal lesions	18.90	94.80

3.3.3 Gross Gastric Morphology

Macroscopic analysis of the gastric mucosa revealed considerable differences between the experimental groups (Figure 13). The normal control group had an undamaged gastric mucosal surface with normal stomach folds and no lesions. However, the ulcer control group showed significant damage to the stomach, represented by numerous ulcerative lesions, petechial hemorrhages (marked by arrows), and substantial disintegration of the mucosal surface, which proved successful induction of ulcers. The treatment with unformulated tegoprazan (5 mg/kg b.w.) showed a considerable reduction in the extent of the mucosal damage, and only some erosions were found. The highest gastroprotective activity was revealed for the tegoprazan-loaded niosome formulation (5 mg/kg b.w.), because there were few lesions and the mucosa appeared nearly normal, maintaining the integrity of the stomach mucosal structure.

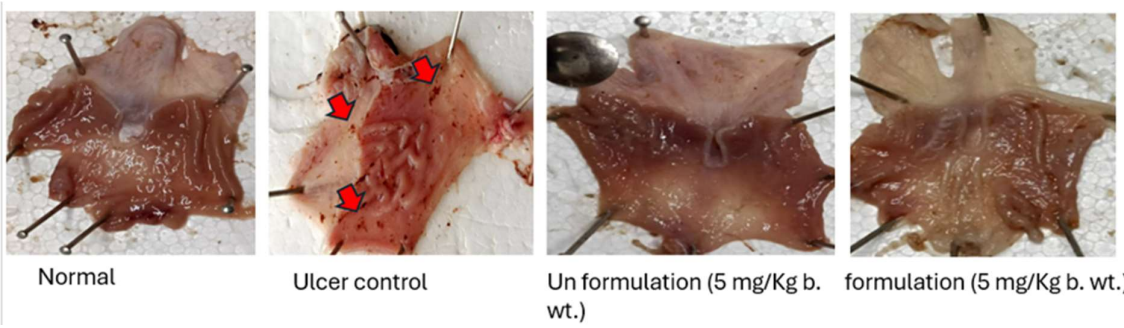

Figure 13. Gross gastric morphology of the normal control, ulcer control, un-formulated tegoprazan, and tegoprazan-loaded niosomal formulation groups

3.3.4 Histopathological Evaluation

Histopathological study further corroborated the macroscopic results obtained through the gross examination of the stomach (Figure 14). Gastric mucosa of the normal control showed no erosion in the gastric mucosa with organized epithelial structure and normal gastric glands. On the other hand, gastric mucosa in the ulcer control showed severe erosion in the epithelial structure, infiltration by inflammatory cells, edema in the sub-mucosal layer and disorganization of the gastric glands which reflected severe ulceration in the mucosa. Un-formulated tegoprazan at 5 mg/kg showed partial recovery in the gastric mucosa with less inflammatory cell infiltration whereas tegoprazan loaded formulation at 5 mg/kg showed almost normal gastric mucosa architecture with no damage to the mucosa.

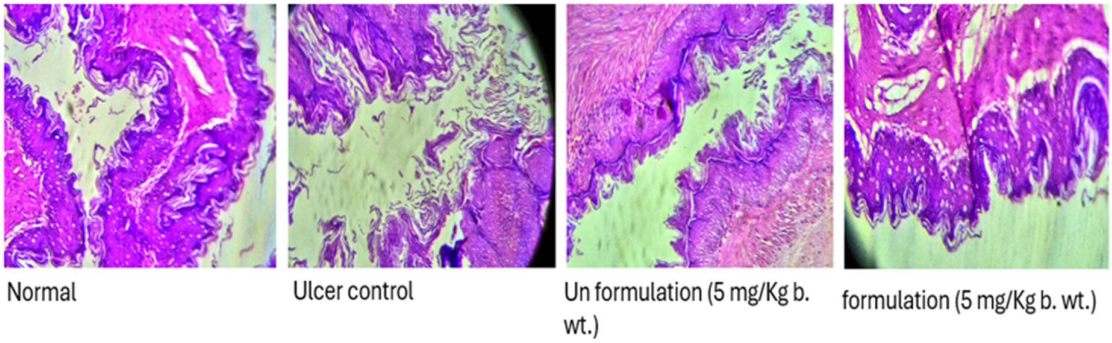

Figure 19. Histopathological sections of gastric tissues in the experimental groups

4. Discussion

The development and evaluation of pharmaceutical formulations depend on trustworthy analytical methods. For the present study, HPLC conditions were optimized to get a sharp symmetrical tegoprazan peak without any chromatographic interference by maintaining a good retention time. The mobile phase, pH, and chromatographic conditions were optimized systematically for efficient separation and reproducible analysis. The results are in line with the principle of method optimization followed by method validation as it is the standard practice in analytical method development for achieving accurate and reproducible quantitative analysis (Panda et al., 2025). The successful validation of the present RP-HPLC method is also consistent with recent analytical investigations reporting stability-indicating chromatographic methods for tegoprazan, emphasizing that robust validation parameters are fundamental for reliable quantification during formulation development, quality assurance, and stability assessment of pharmaceutical products (Gunda et al., 2026).

The optimized HPLC method met all the major criteria for HPLC method validation including system suitability, specificity, precision, accuracy, linearity, range, batch assay and bracketing standard evaluation. The %RSD values were very low, representing the high repeatability of the methods, and satisfactory recovery rates confirmed the reliability of the methods. Similarly, the linear response was excellent, allowing for good quantitative response over the concentration range investigated, and no interfering peaks were present, verifying the method's specificity. The developed HPLC method is reliable and suitable for the routine estimation of tegoprazan and quality assessment of the formulation as per the analytical method validation guidelines provided by ICH Q2(R1) guideline (Panda et al., 2025). The current investigation has shown that the niosomal formulation with tegoprazan has shown better gastroprotective activity when compared with the un-formulated drug. Animals treated with the formulation showed higher reduction in gastric secretion, ulcer area and gastric tissue damage with the improvement of stomach tissue architecture. The results herein suggest that the niosomal encapsulation resulted in better therapeutic performance of tegoprazan because of better delivery of the drug to the gastric mucosa. Similar results have been reported in previous reviews regarding the enhancements of drugs stability, sustained release and enhanced drug availability at the targeted site and thereby improving the therapeutic efficacy of drugs in niosomes (Teron et al., 2024; Nayak et al., 2022). Similarly, Khatoon et al. (2017) and Pinto et al. (2016) have reported similar enhancements in the performance of drugs encapsulated in niosomes. The superior gastroprotective activity noticed in this study is matching with the known pharmacologic action of tegoprazan. Tegoprazan rapidly blocks gastric acid, resulting in long-lasting acid suppression and not requiring prior activation of acid (Kim et al., 2019; Qiao & Li, 2025). It has been proven to be effective for treatment of gastric ulcers and other acid-related diseases with a favorable safety profile in clinical studies (Cho et al., 2020; Jung et al., 2022; Park et al., 2026). The potent acid-suppressive activity observed with tegoprazan is in agreement with the pharmacokinetic and pharmacodynamic characteristics reported for potassium-competitive acid blockers, which, without requiring acid-mediated activation, quickly and effectively suppress the production of stomach acid. These properties contribute to improved intragastric pH control and may offer therapeutic advantages over conventional proton pump inhibitors in acid-related disorders (Echizen, 2016).

Following pyloric ligation, stomach volume, free acidity, and total acidity all rose while gastric pH dramatically dropped, indicating that the ulcer was successfully produced. Both tegoprazan treatments led to an improvement of these parameters with the niosomal formulation resulting in a bigger rise in stomach pH and a greater decrease in gastric secretion. The results suggest that niosomal administration improves stomach acid secretion inhibition. The observed results are in accordance with the known mechanism of tegoprazan, which inhibits the gastric proton pump (Kim et al., 2019; Qiao & Li, 2025) in a reversible manner, thereby reducing the production of gastric acid. Tegoprazan, in contrast with conventional PPIs, is able to cause rapid acid suppression without the need for acidic activation, resulting in sustained intragastric pH control (Scarpignato et al., 2016). These clinical effects are also reported for patients suffering from gastric ulcer and GERD (Cho et al., 2020; Hussaini et al., 2025). This improvement seen in the niosomal formulation may be due to the sustained release of the drug and gastric retention time of the vesicular carrier (Teron et al., 2024; Mawazi et al., 2024). The capacity of nanovesicular carriers to enhance oral drug transport, extend residence duration at the absorption site, and enable regulated drug release may also contribute to improved therapeutic results after niosomal encapsulation. Such characteristics can increase the fraction of drug reaching the target tissue while reducing fluctuations in systemic exposure (Riya et al., 2022; Deulkar et al., 2024).

The results indicate that gastroprotective efficacy was increased with the tegoprazan loaded formulation as there was a marked decrease in ulcer area and a greater percentage of ulcer protection. Continuous acid suppression and better preservation of the gastric mucosa may have been due to sustained drug release and better localization at the gastric mucosa by tegoprazan. Improvements in therapeutic performance have also been observed in the case of niosomal drug delivery system due to its ability to extend the duration of action of the drug, and its potential to enhance drug localization (Khatoon et al., 2017; Teron et al., 2024). Furthermore, the results of the current investigation are consistent with those of Kim et al. (2019), who demonstrated comparable anti-ulcer efficacy of tegoprazan in experimental stomach ulcer models. Previous investigations have further demonstrated that niosomal systems improve drug localization and prolong therapeutic activity through their vesicular bilayer structure, allowing for the effective encapsulation of lipophilic and hydrophilic medications and promoting prolonged drug release. These characteristics enhance drug stability, increase residence

time at the target site, and improve therapeutic performance, making niosomes promising carriers for the management of chronic gastrointestinal disorders and other diseases requiring controlled drug delivery (Rai et al., 2017; Mandal et al., 2024).

The histopathological data confirmed the biochemical and gross morphological data. The ulcer control group showed severe erosion of the epithelial surface, inflammatory infiltration, oedema and loss of gastric architecture, while the tegoprazan formulation with the loaded delivery system significantly maintained the integrity of the epithelium and preserved the glandular organization with limited inflammatory changes. This study shows positive mucosal healing and protection. The same gastric histology improvement has been seen after drug delivery using tegoprazan (Cho et al., 2020; Kim et al., 2019) and increased protection is seen with vesicular drug delivery (Nayak et al., 2022; Pinto et al., 2016). The increased therapeutic activity of the tegoprazan-loaded formulation is probably a result of the synergistic impact of strong proton pump inhibition and the effect of the drug delivery. Niosomal encapsulation can increase the gastric residence time, enhance the mucosal adhesion, protect the drug from degradation and release it in a sustained manner into the site of action. Other studies had noted the enhanced stability and bioavailability of drugs in niosomes, which could be used as carriers for gastrointestinal drug delivery (Saed et al., 2025;Teron et al., 2024; Mawazi et al., 2024; Sengar, 2024). The current study's findings on histology preservation lend more credence to the idea that regulated drug delivery systems might improve local drug availability at damaged gastric tissue, thereby facilitating mucosal recovery while limiting additional tissue injury. Similar conclusions have been reported for niosomal drug delivery platforms developed for targeted therapeutic applications, where improved tissue exposure and prolonged drug action contributed to superior biological responses (Deulkar et al., 2024; Riya et al., 2022).

This study was performed on a single experimental model of ulcer and with a single dose of tegoprazan. Pharmacokinetic, drug release kinetic, long-term stability, entrapment effectiveness, vesicle size distribution, and zeta potential were not examined. Furthermore, no molecular markers related to oxidative stress and inflammation were studied. Tegoprazan loaded niosomes could be characterized for its physicochemical properties and its pharmacokinetic profile in further studies and the molecular mechanisms could be explored through the use of oxidative stress and inflammatory biomarkers. Optimization of dose, studies in various ulcer models and extended safety evaluation are also recommended to facilitate the clinical translation of this promising gastroprotective formulation.

5. Conclusion

The present work effectively created and validated a dependable RP-HPLC method for the quantitative determination of tegoprazan, which was utilized for a standard pharmaceutical analysis. The optimized analytical method showed good system suitability, specificity, accuracy, precision, linearity and reproducibility characteristics and thus proved to be applicable for formulation development and quality control. Moreover, the gastroprotective effect of a tegoprazan-loaded niosomal formulation was found to be superior to un-formulated drug in a pyloric ligation-induced gastric ulcer experimental model. While the gastrointestinal pH was raised and the gastric mucosal architecture was maintained, the therapy considerably reduced the ulcer area, free acidity, total acidity, and stomach volume. Additionally, the niosomal formulation's enhanced ability to prevent injury to the stomach mucosa was verified by gross morphological and histopathological assessments. Long-term medication retention in the stomach and continuous drug release and increased drug availability is probably responsible for its improved therapeutic performance as a niosomal carrier system. Overall, the findings point to niosomal encapsulation as a potentially effective method for enhancing anti-ulcer efficacy of tegoprazan. The developed tegoprazan loaded niosomal formulation has a great potential to be used as an advanced oral medication delivery method for improved administration of PUD as further detailed physicochemical characterization, pharmacokinetic study, molecular study and long-term safety assessment studies are needed.

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