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Analytical Techniques Development and Validation For Simultaneous Estimation Of Vonoprazan Fumarate And Itopride Hydrochloride In Synthetic Mixture

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ABSTRACT
Vonoprazan fumarate is a potassium-competitive acid blocker used in the management of acid-related gastrointestinal disorders such as gastroesophageal reflux disease (GERD), peptic ulcer disease, and Helicobacter pylori eradication therapy. Itopride hydrochloride is a prokinetic and anti-emetic agent that improves gastrointestinal motility through dopaminergic antagonism and acetylcholinesterase inhibition. The present study aimed to develop and validate two UV spectrophotometric and RP-HPLC methods for simultaneous estimation of Vonoprazan fumarate and Itopride hydrochloride in a synthetic mixture as per ICH Q2 (R2) guideline. For the both drugs Identification tests including melting point, solubility, FT-IR and UV method. For the UV spectrophotometric method as First-order derivative method and simultaneous equation method in using a solvent as methanol. First-order derivative spectrophotometry and simultaneous equation methods were developed using optimized ZCP at 302 nm for Vonoprazan fumarate and 238 nm for Itopride hydrochloride. In addition, simultaneous UV estimation was performed at 232 nm and 258 nm for both drugs. The RP-HPLC method employed a mobile phase consisting of phosphate buffer (pH 4.5 adjusted with orthophosphoric acid), acetonitrile, and methanol in the ratio of 55:30:15 (%v/v/v), with detection carried out at 212 nm. The method produced sharp and well-resolved peaks with retention time of 3.5 min for Vonoprazan fumarate and 7.0 min for Itopride hydrochloride. For the first-order derivative method, linearity was established in the range of 1-5 µg/mL for Vonoprazan fumarate and 7.5-37.5 µg/mL for Itopride hydrochloride, with correlation coefficients of 0.9973 and 0.9990, respectively. Accuracy studies showed excellent recoveries ranging from 99.50-99.66% for Vonoprazan fumarate and 99.94-99.96% for Itopride hydrochloride. Sensitivity parameters revealed LOD and LOQ values of 0.04 and 0.12 µg/mL for Vonoprazan fumarate and 0.28 and 0.87 µg/mL for Itopride hydrochloride. The percentage assay results were 99.00% and 99.73%, respectively, confirming method accuracy. Overall, the developed methods were simple, precise, accurate, and reproducible. They comply with ICH Q2 (R2) validation requirements and are suitable for routine quality control analysis of combined pharmaceutical formulations containing Vonoprazan fumarate and Itopride hydrochloride.

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
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1. INTRODUCTION:

Vonoprazan fumarate is a novel potassium-competitive acid blocker (P-CAB) that has gained attention for its superior gastric acid suppression compared to traditional proton pump inhibitors (PPIs). It works by reversibly and competitively inhibiting the H^+, K^+-ATPase enzyme in gastric parietal cells, leading to sustained acid suppression. Unlike PPIs, which require activation in an acidic environment, vonoprazan exhibits a rapid onset of action, longer duration of effect, and increased stability in varying pH conditions[1-2].

Itopride hydrochloride is a prokinetic agent primarily used in the management of functional dyspepsia and other gastrointestinal motility disorders. It is a dopamine D_2 receptor antagonist and acetylcholinesterase inhibitor, enhancing gastric emptying and peristalsis. Unlike other prokinetic drugs, Itopride does not cause severe cardiac side effects, making it a safer option for long-term use[3-5]. The combination of Vonoprazan fumarate and Itopride hydrochloride is used for the treatment of acid- related disorders, Including gastroesophageal reflux disease (GERD). Vonoprazan reduces acid production while Itopride enhances gastric emptying, preventing acid retention and reflux. Combination therapy can alleviate epigastric pain, early satiety, and bloating more effectively than monotherapy. Since improved motility can also reduce reflux, lower doses of acid suppressants may be required. Patients who do not respond well to PPIs alone may benefit from the addition of Itopride. Patients who do not respond well to PPIs alone may benefit from the addition of Itopride. Also, this combination is recommended by Subject expert committee (Gastroenterology & Hepatology) In Fixed dose combination division (CDSCO Approved) in 12/09/2024[6]. A comprehensive literature survey revealed several reported analytical methods for the estimation of Vonoprazan fumarate either alone or in combination with other drugs, including UV spectrophotometric methods[7-10], RP-HPLC methods[11-17], UV-HPLC[18-19], stability-indicating HPLC[20], stability-indicating RP-HPLC and UV method[21], stability-indicating RP-UPLC method[22], stability-indicating HPTLC method[23] and LC-MS/MS methods[24]. Despite the availability of these sophisticated and well-established analytical techniques, all reported methods focus on the individual estimation of these drugs or their determination in biological matrices. To the best of our knowledge, no validated spectrophotometric and chromatographic method has been reported for the simultaneous quantification of Vonoprazan fumarate and Itopride hydrochloride in a synthetic mixture. The absence of a unified, cost-effective, and time-efficient analytical approach for their concurrent estimation highlights a significant analytical gap in the literature. Therefore, the present study was undertaken to develop and validate novel, accurate, precise, and robust RP-HPLC and UV spectrophotometric methods (simultaneous equation and first order derivative) for the concurrent estimation of both drugs, in accordance with ICH Q2 (R2)[24] guideline, thereby providing a reliable analytical tool suitable for routine quality control analysis.

2. EXPERIMENTAL MATERIALS AND INSTRUMENTATION

2.1 Chemicals and reagents

Vonoprazan fumarate was obtained as a gift sample from Dham Tech Pharma Ltd. Itopride hydrochloride was procured from Ami Lifescience Ltd. HPLC-grade methanol was purchased from Finar Chemicals Pvt. Ltd., Ahmedabad, Gujarat, India. All other chemicals and reagents used in the study were of analytical reagent (AR) grade or HPLC grade and were used without further purification.

2.2 Instrumentation

UV spectroscopic analysis was performed using a Shimadzu UV-1900 UV–Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with UV Probe 2.7 software, a spectral bandwidth of 1 nm, and 1.0 cm matched quartz cuvettes over the wavelength range of 200-400 nm.

Chromatographic analysis was carried out using a Systronics RP-HPLC system (Model SYS-LC-138, Systronics, India) coupled with a UV detector. The pH of the buffer solutions was measured using a Chemi Line pH meter (Chemi Line Instruments, India). An analytical balance (Scale-Tec, India) was used for accurate weighing of samples. The mobile phase was degassed by sonication using a Digital Pro+ sonicator (Model PS-10A, Broleo, India) prior to use.

2.3 Preparation of Solutions

2.3.1 Preparation of Stock Solution

Precisely weighed quantities of 10 mg each of Vonoprazan fumarate and Itopride hydrochloride were quantitatively transferred into a 100 ml volumetric flask and subsequently diluted to volume

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

with methanol to achieve a final concentration of 100 µg/mL. The solutions were sonicated for 5 mins to ensure complete dissolution.

2.3.2 Preparation of calibration curve

The calibration standards in the concentration range of 1-5 µg/mL for Vonoprazan fumarate for and 7.5-37.5 µg/mL for Itopride hydrochloride, appropriate aliquots of the respective stock solutions were transferred into a series of 10 mL volumetric flasks. For Vonoprazan fumarate, aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL were diluted to volume with methanol to yield final concentrations of 1, 2, 3, 4, and 5 µg/mL, respectively. Similarly, for Itopride hydrochloride, aliquots of 0.75, 1.5, 2.25, 3.0, and 3.75 mL were diluted to volume with methanol to obtain concentrations of 7.5, 15, 22.5, 30, and 37.5 µg/mL, respectively.

The prepared solutions were analyzed under optimized spectrophotometric conditions using a 1 cm matched quartz cuvette. For chromatographic analysis, 20 µL of each working standard solution was injected into the RP-HPLC system under optimized chromatographic conditions.

3. METHODOLOGY

3.1 Method I: UV SEPCTROPHOTOMETRIC METHOD DEVELOPMENT

Pipetted out 0.2 ml solution from stock solution of Vonoprazan fumarate (100 µg/ml) and 1.5 ml Itopride hydrochloride (100 µg/ml) into different 10 ml volumetric flask and diluted upto mark with methanol to get the 2 µg/ml of Vonoprazan fumarate and 15 µg/ml Itopride hydrochloride. Every solution was scanned between 200 to 400 nm.

3.1.1 Simultaneous equation as Vierordt's method

Solutions of Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, the absorption maxima (λmax) were established at 232 nm for Vonoprazan fumarate and 258 nm for Itopride hydrochloride. This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated according to the following mathematical expressions.

Standard Stock solutions of Vonoprazan fumarate and Itopride hydrochloride in the concentration range 1-5 µg/mL and 7.5-37.5 µg/ml were made in the methanol and absorbance of these solutions was measured at 232 nm and 258 nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).

$$\text{At } \lambda 1 \; A1 = ax1bCx + ay1bCy \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{At } \lambda 2 A2 = ax2bCx + ay2\, bCy \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For measurements in 1 cm cells b=1,

Rearrange eq. (2),

$$Cy = A2 - ax2Cx/\, ay2$$

Substituting for Cy in eq (1) and rearranging

$$Cx = A2ay1 - A1\; ay2/ax2\; ay1 - ax1\; ay2 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Cy = A1ax2 - A2\; ax1/ax2\; ay1 - ax1\; ay2 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where C_x and C_y are the concentration of Vonoprazan fumarate and Itopride hydrochloride, respectively, A_1 and A_2 are absorbance at 232 nm and 258 nm, respectively, ax_1 and ax_2 are absorptivity of Vonoprazan fumarate at 232 nm and 258 nm, respectively; ay_1 and ay_2 are absorptivity of Itopride hydrochloride at 258 nm and 232 nm, respectively. By solving the two simultaneous equations, the concentrations of Vonoprazan fumarate and Itopride hydrochloride in sample solutions were obtained.

3.1.2 First Order Derivative Method

Solutions of Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, each solution was scanned in the range of 200-400 nm. All Zero-order Spectrum (D0) were converted to First Derivative Spectrum (D1) using delta lambda 2.0 and scaling factor 4. The overlain first derivative spectrum of Vonoprazan fumarate and Itopride hydrochloride at different concentration were recorded. The Zero-crossing point (ZCP) of Vonoprazan fumarate was found to be 302 nm and ZCP of Itopride hydrochloride was found to be 238 nm.

Standard Stock solutions of Vonoprazan fumarate and Itopride hydrochloride in the concentration range 1-5 µg/mL and 7.5-37.5 µg/ml were made in the methanol and absorbance of these solutions was measured at 238 nm and 302 nm. Graph of Absorbance v/s Concentration was plotted.

3.2 Method II: Reverse Phase High Performance Liquid Chromatography Method

Chromatographic analysis was performed via isocratic elution, wherein various mobile phase configurations including Methanol: Water, Acetonitrile: Phosphate Buffer (pH 5), Phosphate Buffer (pH 4.5): ACN were evaluated in varying ratios. Optimal resolution of both analyte peaks was achieved using a mixture of Phosphate Buffer (pH

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

4.5 adjusted with 10% orthophosphoric acid): ACN: Methanol (55: 30: 15 %v/v/v) at a consistent flow rate of 1 mL/min. All solvents underwent filtration through a 0.45 µm membrane and were degassed via sonication for 30 minutes before use. Separation was executed on a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) stationary phase, with the eluent monitored using a UV Detector and chromatograms specifically extracted at 212 nm. Calibration curves were subsequently established by plotting the measured peak areas against their respective concentrations to derive the corresponding linear regression equations.

3.3 METHOD VALIDATION

The analytical methodologies employed in this research were rigorously validated in accordance with the regulatory standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) under the ICH Q2 (R2)[24] guideline for analytical procedure validation.

3.3.1 Specificity

Specificity denotes the capacity of an analytical procedure to accurately and distinctly quantify the target analyte despite the potential interference of co-existing substances. Within a complex sample, these extraneous components commonly encompass synthesis impurities, degradation products, or various matrix constituents that could otherwise confound the measurement.

3.3.2. Linearity and Range (n=6)

The linearity of the analytical procedure was evaluated through the preparation of five distinct concentrations of standard solutions. Vonoprazan fumarate and Itopride hydrochloride demonstrated linear responses within the concentration ranges of 1-5 µg/mL and 7.5-37.5 µg/mL, respectively. The proportionality of both analytes was statistically assessed by calculating the slope, y-intercept, and correlation coefficient (R^2) from the resulting calibration curves.

3.3.3. Precision

The precision of both analytical methodologies was evaluated across three distinct parameters: repeatability, intraday (intermediate) precision, and interday (reproducibility) precision. To assess intraday precision, standard solutions of Vonoprazan fumarate (1, 2, 3 µg/mL) and Itopride hydrochloride (7.5, 15, 22.5 µg/mL) were analyzed in triplicate at three separate time intervals within a single day. Interday precision was similarly established by evaluating the same concentration levels over three consecutive days. Furthermore, repeatability was rigorously determined through six replicate injections of a single concentration level 2 µg/mL for Vonoprazan fumarate and 15 µg/mL for Itopride hydrochloride. All precision data were statistically quantified and reported as the percentage relative standard deviation (%RSD) to ensure compliance with ICH Q2 (R2) guideline.

3.3.4 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

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

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.6 Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure denotes the proximity of the experimental result to the accepted reference value or conventional true value. To confirm the accuracy of the proposed method, recovery studies were conducted in accordance with ICH Q2(R2) Guidelines at three distinct concentration levels: 50%, 100%, and 150%. These evaluations targeted. Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) using the standard addition technique, with each level analyzed in triplicate. The methodology's accuracy was subsequently established by calculating the percentage recovery of both analytes across these fortified concentrations.

3.3.7 Assay as analysis of Synthetic Mixture

A synthetic mixture containing. Vonoprazan fumarate and Itopride hydrochloride in the ratio of 1:7.5 was prepared. Accurately weighed quantities of Vonoprazan fumarate (2 mg) and Itopride hydrochloride (15 mg) were blended with commonly used excipients, namely Microcrystalline cellulose (10 mg), Poly vinyl pyrrolidone (8 mg), Mannitol (7 mg), Fumaric acid (2.5 mg), Aerosil (1.5 mg), Magnesium Stearate (1 mg), using a mortar and pestle to obtain a homogeneous mixture. An accurately weighed portion of the prepared blend equivalent to 20 mg of Vonoprazan fumarate and 150 mg of Itopride hydrochloride was transferred into a 100 mL volumetric flask. Approximately 70 mL of methanol was added, and the mixture was sonicated to ensure complete dissolution of the drugs. The volume was then made up to the mark

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

with methanol and mixed thoroughly. The resulting solution was filtered through Whatman filter paper to remove insoluble excipients. The obtained stock solution contained 200 µg/mL of Vonoprazan fumarate and 1500 µg/mL of Itopride hydrochloride. For sample analysis, 0.1 mL of this stock solution was accurately transferred into a 10 mL volumetric flask and diluted to volume with methanol to yield final concentrations of 2 µg/mL of Vonoprazan fumarate and 15 µg/mL of Itopride hydrochloride. The prepared sample solution was analyzed using the optimized RP-HPLC and UV spectrophotometric methods, and the percentage assay of both drugs was calculated.

3.3.8 Robustness

Robustness of the developed RP-HPLC and UV spectrophotometric methods was evaluated by deliberately introducing small and systematic variations in analytical conditions and assessing their effect on the assay results. For the RP-HPLC method, robustness was examined by varying the flow rate (±0.1 mL/min from 1.0 mL/min), mobile phase composition (±2% variation in organic phase), and detection wavelength (±2 nm from 212 nm). The effects of these changes on retention time, peak area, tailing factor, and resolution were studied. For the UV spectrophotometric method, robustness was assessed by varying the detection wavelength (±2 nm from 232 nm for Vonoprazan fumarate and 258 nm for Itopride hydrochloride) and evaluating the effect of slight variations in solvent composition.

3.3.9 System Suitability Tests

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates (table 1).

4. RESULTS AND DISCUSSION:

4.1 Selection of wavelength

For the simultaneous equation method, standard solutions of Vonoprazan fumarate (2 µg/mL) and Itopride hydrochloride (15 µg/mL) in methanol were subjected to spectral scanning between 200 and 400 nm at medium speed, with methanol employed as the blank solution. For the analytical determination, the absorption maxima (λmax) were established at 232 nm for Vonoprazan fumarate and 258 nm for Itopride hydrochloride (Figure 1). This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated.

To determine the wavelength for measurement, Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) solutions were scanned between 200-400 nm. Absorbance maximum was obtained at their λmax 232 nm and 258 nm for measurement of Vonoprazan fumarate and Itopride hydrochloride, respectively. For first order derivative technique, Measurement and determination of Vonoprazan fumarate and Itopride hydrochloride were observed at 238 nm and 302 nm, respectively. (Figure 2).

For RP-HPLC method, coupled with UV detection, is fundamentally dependent upon the strategic selection of an optimal detection wavelength. Both analytes exhibited significant molar absorptivity at 212 nm, leading to its selection for the simultaneous quantification of Vonoprazan fumarate and Itopride hydrochloride within the synthetic mixture. The spectral rationale for this choice of detection wavelength is showed in Figure 1.

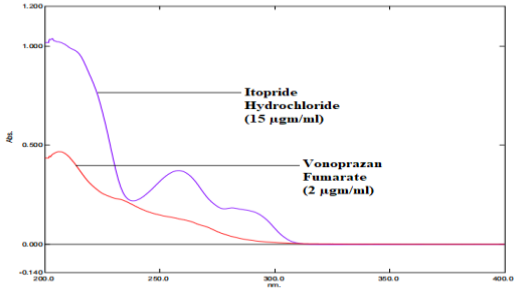

Figure 1: Overlain UV Spectra of Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) In Methanol (Zero-Order)

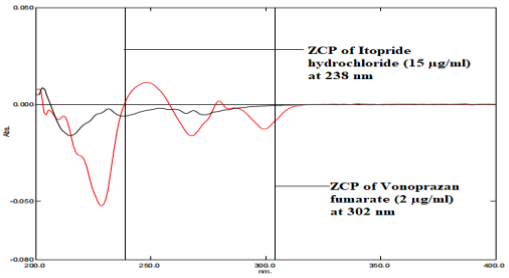

Figure 2: Overlain UV Spectra of Vonoprazan fumarate (2 µg/ml) and Itopride hydrochloride (15 µg/ml) In Methanol (First-Order)

4.2 Simultaneous equation (Vierordt's) method

For multi-component UV analysis, Vierordt's method is named after the German scientist Karl Vierordt. UV Spectra of Vonoprazan fumarate (1-5 µg/mL) and Itopride hydrochloride (7.5-37.5 µg/mL) over the linearity and range had been showed in Figure 3 and 4, respectively.

Journal of Molecular Science
Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

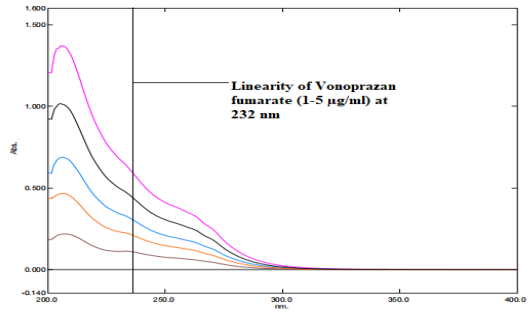

Figure 3: Linearity Spectra for Vonoprazan fumarate (1-5 µg/ml) at 232 nm in methanol

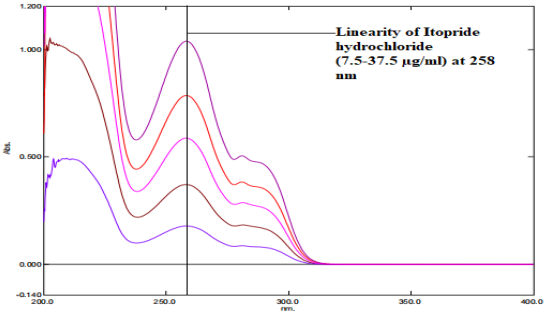

Figure 4: Linearity Spectra for Itopride hydrochloride (7.5-37.5 µg/ml) at 258 nm in methanol

4.3 Simultaneous equation as First Order Derivative Method

Overlain UV Spectra of Vonoprazan fumarate (1-5 µg/ml) and Itopride hydrochloride (7.5-37.5 µg/ml) In methanol (First Order) have been shown in Figure no. 5 and 6 at the wavelength of 238 nm and 302 nm.

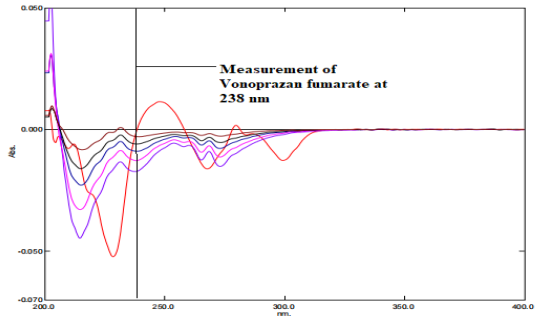

Figure 5: Overlain UV Spectra of Vonoprazan fumarate (1-5 µg/ml) at 238 nm

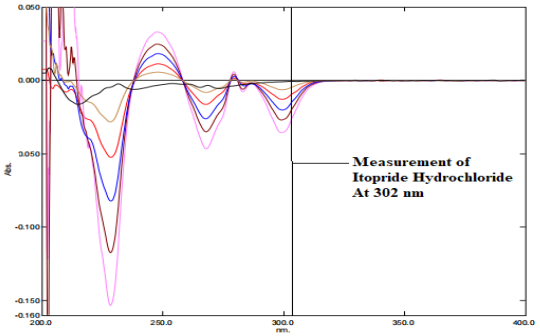

Figure 6: Overlain Spectra of Itopride hydrochloride (7.5-37.5 µg/ml) at 302 nm

4.4 RP-HPLC Method Development

An RP-HPLC method coupled with UV detection was developed for the concurrent quantification of Vonoprazan fumarate and Itopride hydrochloride, with the primary objective of achieving optimal peak symmetry and high theoretical plate counts within an efficient analytical runtime. Chromatographic parameters were refined through the systematic evaluation of various stationary and mobile phase compositions. Among the reversed-phase C_8 and C_{18} columns assessed, the Kromstar C_{18} (250 × 4.6 mm, 5 µm) demonstrated superior performance, yielding highly symmetric peaks and the most favorable retention times. The optimal mobile phase was identified as a mixture of Phosphate Buffer (pH 4.5 adjusted with 10% orthophosphoric acid): ACN: Methanol (55: 30: 15 %v/v/v) at 212 nm. Although alternative ratios of this buffer and solvent were investigated, they resulted in undesirable peak tailing and excessive retention for both analytes.

4.4 VALIDATION OF THE PROPOSED METHODS

4.4.1 Specificity

Specificity is defined as the ability of an analytical method to unequivocally assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The specificity of the developed RP-HPLC method was evaluated by comparing chromatograms of the mobile phase (blank), placebo (excipients), and the test preparation solution. The chromatogram of the blank showed no peaks at the retention times corresponding to Vonoprazan fumarate and Itopride hydrochloride. Similarly, no interfering peaks from excipients were observed at the respective retention times of the analytes in the sample chromatogram. These results demonstrate that the developed method is specific and free from interference due to mobile phase components or formulation excipients, thereby confirming its suitability for the simultaneous estimation of Vonoprazan fumarate and Itopride hydrochloride. Retention time was found to be 3.5 min and 7.0 min for Vonoprazan fumarate and Itopride hydrochloride, respectively showed in table 1.

Table 1: System suitability parameters

Parameters	Retention Time	Tailing Factor	Number of Theoretical plate	Resolution
Vonoprazan fumarate	3.5 min	0.94	8756	4.5
Itopride hydrochloride	7.0 min	0.56	6981	

Journal of Molecular Science
Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

4.4.2 Linearity and range

Simultaneous equation as Vierordt's method, UV Spectra of Vonoprazan fumarate (1-5 µg/ml) and Itopride hydrochloride (7.5-37.5 µg/ml) over the linearity and range had been showed in Figure 3 and 4, respectively. For UV, Vonoprazan fumarate exhibited a linear response in the concentration range of 1-5 µg/mL at 232 nm and 258 nm. The correlation coefficients (r^2) were found to be 0.9997 and 0.9979 at 232 nm and 258 nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 2 %, demonstrating good precision and repeatability. Itopride hydrochloride showed linearity over the concentration range of 7.5-37.5 µg/mL at 258 nm and 232 nm, with correlation coefficients (r^2) of 0.999 and 0.998, respectively.

For, First Order Derivative Method UV Spectra of Vonoprazan fumarate (1-5 µg/ml) and Itopride hydrochloride (7.5-37.5 µg/ml) over the linearity and range had been showed in Figure 5 and 6, respectively. For UV, Vonoprazan fumarate exhibited a linear response in the concentration range of 1-5 µg/mL at 238 nm. The correlation coefficients (r^2) were found to be 0.9973 at 238 nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 2 %, demonstrating good precision and repeatability. Itopride hydrochloride showed linearity over the concentration range of 7.5-37.5 µg/mL at 302 nm, with correlation coefficients (r^2) of 0.999, respectively.

The RP-HPLC chromatogram of Vonoprazan fumarate (1-5 µg/mL) and Itopride hydrochloride (7.5-37.5 µg/mL). The Peak Area was found. Calibration graphs were plotted between concentrations and peak areas were observed. The regression equation of calibration curve was generated and Correlation Coefficient for Vonoprazan fumarate 0.9993 and for Itopride hydrochloride 0.9993, respectively. The %RSD values were less than 2.0%, confirming acceptable precision and reproducibility of the developed method. The linearity data are summarized in Table 2.

Table 2: Linearity and sensitivity data

Parameters	Simultaneous equation as Vierordt's method				First Order Derivative Method		HPLC	
	VONO		ITO		VONO	ITO	VONO	ITO
Wavelength (nm)	232	258	258	232	238	302	212	
Beer's Law Limit (µg/mL)	1-5	1-5	7.5-37.5	7.5-37.5	1-5	7.5-37.5	1-5	7.5-37.5
Correlation Coefficient (r^2)	0.99977	0.99979	0.9999	0.9998	0.99973	0.999	0.9993	0.99993
LOD (µg/ml)	0.03	0.048	0.22	0.27	0.04	0.28	0.026	0.24
LOQ	0.10	0.145	0.66	0.82	0.12	0.87	0.077	0.72

4.4.2.1 Calculation for Simultaneous Equation Method for Vonoprazan fumarate and Itopride hydrochloride in Synthetic Mixture.

Vonoprazan fumarate (2 µg/ml) and Itopride hydrchloride (15 µg/ml) in methanol, both the solutions were scanned over range of 200-400nm against methanol as blank, using medium scan speed. The sampling wavelength for analysis includes 232 nm for Vonoprazan fumarate and 258 nm for Itopride hydrochloride. The method employs Simultaneous Equation as per Vierodt's method and the concentrations of drugs in sample solution were determined by using the following formula:

Vonoprazan fumarate,

$$C_x = \frac{A2 \times ay1 - A1 \times ay2}{ax2 \times ay1 - ax1 \times ay2}$$

Where ax_1 and ax_2 represented the absorptivity of Vonoprazan fumarate at 232 nm and 258 nm, respectively; ay_1 and ay_2 denoted the absorptivity of Itopride hydrochloride at 258 nm and 232 nm, respectively; and A_1 and A_2 corresponded to the absorbance of the sample measured at 232 nm and 258 nm, respectively.

C (Vonoprazan fumarate) = 1.8 µg/mL; The concentration of Vonoprazan fumarate (Cx), calculated using Vierordt's simultaneous equation method, was found to be 1.8 µg/mL.

For itopride hydrochloride,

$$C_y = \frac{A1 \times ax2 - A2 \times ax1}{ax2 \times ay1 - ax1 \times ay2}$$

where a_{x1} and a_{x2} are the absorptivity values of Vonoprazan fumarate at 232 nm and 258 nm, respectively; a_{y1} and a_{y2} represent the absorptivity of Itopride hydrochloride at 258 nm and 232 nm, respectively; and A_1 and A_2 are the absorbance values of the sample measured at 258 nm and 232 nm, respectively.

C (Itopride hydrochloride) = 17.8 µg/mL; The concentration of Itopride hydrochloride (Cy), calculated using Vierordt's simultaneous equation method, was found to be 17.8 µg/mL.

Journal of Molecular Science
Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

4.4.3 Precision

Methodological precision was evaluated through intraday, inter-day, and repeatability assessments using triplicate analyses of Vonoprazan fumarate (1, 2 and 3 µg/ml) and Itopride hydrochloride (7.5, 15.0 and 22.5 µg/ml) across three consecutive days and within a single diurnal period. Absorbance values were recorded for these concentrations to establish intermediate precision, while repeatability was specifically determined using concentrations of 2 µg/ml for vonoprazan fumarate and 15 µg/ml for Itopride hydrochloride. The outcomes, expressed as Relative Standard Deviation (% RSD) for each precision parameters were less than 2.0%.

4.4.4 LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) are calculated using the standard deviation responses and slopes obtained from the calibration curves of each drug at their specific wavelengths. The results of LOD and LOQ were displayed in Table 2.

4.4.5 Accuracy

To evaluate the accuracy of the proposed methodology, recovery studies were performed using the standard addition technique, in which pre-analyzed samples were spiked with known concentrations of pure Vonoprazan fumarate and Itopride hydrochloride. These assessments were executed at three levels 50%, 100%, and 150% and conducted in triplicate to ensure statistical reliability. The accuracy was expressed as the percentage recovery of the added standards. For the UV spectrophotometric approach, as a Simultaneous equation as Vierordt's method the percentage recovery was found to be within range of 99.00%-99.75% for Vonoprazan fumarate and 99.60%-100.16% for Itopride hydrochloride. For first order derivative method the percentage recovery was found to be within range of 99.50 %– 99.66% for Vonoprazan fumarate and 99.94%-99.96% for Itopride hydrochloride. For RP-HPLC method, the percentage recovery was found to be within the range of 99.76%-99.94% for Vonoprazan fumarate and 99.96%-100.18% for Itopride hydrochloride with detailed results provided in Table 3.

Table 3: Recovery study data for UV and RP-HPLC Method

Vierordt's Method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/mL)**	**Amount of drug taken (µg/mL)**	**Total Std Amt (µg/mL)**	**Total amount Recovered (µg/mL)**	**% Mean Recovery ± SD(n=3)**
Vonoprazan fumarate	50	2	1	c	2.97	99.00 ± 0.0170
	100	2	2	4	3.99	99.75 ± 0.245
	150	2	3	5	4.96	99.20 ± 0.0082
Itopride hydrochloride	50	15	7.5	22.5	22.45	99.77 ± 0.0125
	100	15	15	30	29.88	99.60 ± 0.0049
	150	15	22.5	37.5	37.56	100.16 ± 0.0093
First Order Derivative Method						
Vonoprazan fumarate	50	2	1	3	2.99	99.66 ± 0.0232
	100	2	2	4	3.98	99.50 ± 0.0170
	150	2	3	5	4.98	99.60 ± 0.0163
Itopride hydrochloride	50	15	7.5	22.5	22.49	99.95 ± 0.0125
	100	15	15	30	29.99	99.96± 0.0205
	150	15	22.5	37.5	37.48	99.94 ± 0.0216
RP-HPLC Method						
Vonoprazan fumarate	50	2	1	3	2.993	99.76±0.63
	100	2	2	4	3.995	99.87±0.85
	150	2	3	5	4.997	99.94±0.25
Itopride hydrochloride	50	15	7.5	22.5	22.491	99.96 ± 0.12
	100	15	15	30	29.994	99.98± 0.25
	150	15	22.5	37.5	37.57	100.18 ± 0.26

4.4.6 Assay as Analysis of Synthetic mixture

From assay, the concentration of Vonoprazan fumarate 2 µg/mL and Itopride hydrochloride 15 µg/mL were run into UV and RP-HPLC. The Percentage assay of Vonoprazan fumarate and Itopride hydrochloride were found to be 99.50% and 99.80% respectively in UV for Vierordt's Method. And The Percentage assay of Vonoprazan fumarate and Itopride hydrochloride were found to be 99.00% and 99.73% respectively in UV for first order derivative method. For RP-HPLC the Percentage assay of Vonoprazan fumarate and Itopride hydrochloride were found to be 99.90% and 99.93%, respectively. Its results showed in Table 4.

Table 4: Assay results for UV and RP-HPLC Method

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 189-198
DoI-10.004687/1000-9035.2026.024

Vierordt's Method				
Name of Drug	**Amount in synthetic mixture (µg/mL)**	**Mean Amount found (µg/mL)**	**% Assay ± SD (n=3)**	**%RSD**
Vonoprazan fumarate	2	1.99	99.50 ± 0.0082	0.41
Itopride hydrochloride	15	14.97	99.80 ± 0.0143	0.10
First Order Derivative Method				
Vonoprazan fumarate	2	1.98	99.00 ± 0.4082	0.41
Itopride hydrochloride	15	14.96	99.73 ± 0.0262	0.18
RP-HPLC Method				
Vonoprazan fumarate	2	1.998	99.90±0.63	0.41
Itopride hydrochloride	15	14.99	99.93±0.96	0.12

4.4.7 Robustness

Chromatographic analysis was used to analyses the effects of changes in analysts, and the results showed that there was no statistically significant difference in the % RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential. The results indicated that minor deliberate variations in method parameters did not produce significant changes in analytical responses. The percentage relative standard deviation (%RSD) values were found to be within acceptable limits (<2%), demonstrating that the developed methods are robust and reliable for routine analysis.

5. CONCLUSION

The current investigation focused on the development and validation of streamlined, cost-effective, and precise analytical protocols for the concomitant quantification of Vonoprazan fumarate and Itopride hydrochloride in a synthetic mixture. While previous literature documents various techniques for these analytes in isolation, a literature gap was identified regarding their simultaneous determination. Consequently, UV-Spectrophotometric and RP-HPLC methodologies were established and validated in strict accordance with ICH Q2 (R2) regulatory standards. For the UV-Spectrophotometric approach, the Vierordt's (simultaneous equation) method and First Order Derivative Method was employed, utilizing analytical wavelengths of 232 nm and 258 nm for Vonoprazan fumarate and Itopride hydrochloride, respectively for Vierordt's (simultaneous equation) method. This method exhibited robust linearity over concentration intervals of 1-5 µg/ml and 7.5-37.5 µg/ml, respectively, yielding correlation coefficients nearer to 0.9997 and 0.9979. Comprehensive validation encompassing accuracy, precision, repeatability, and sensitivity (LOD/LOQ) yielded results within established acceptance criteria. Furthermore, recovery experiments and assay data substantiated the method's reliability for estimating both components within the synthetic mixture. For First Order Derivative Method utilizing analytical wavelength 238 and 302 for vonoprazan fumarate and Itopride hydrochloride, This method exhibited robust linearity over concentration intervals of 1-5 µg/ml and 7.5-37.5 µg/ml, respectively, yielding correlation coefficients nearer to 0.9973 and 0.999.Additionally, a highly sensitive RP-HPLC method was optimized using a C_{18} stationary phase and a mobile phase comprised of Phosphate Buffer (pH 4.5 adjusted with 10% orthophosphoric acid): ACN: Methanol (55: 30: 15 %v/v/v). The system operated at a flow rate of 1 ml/min with UV detection at 212 nm, resulting in well-resolved peaks and favorable system suitability metrics. The chromatographic technique demonstrated superior linearity, precision, and robustness. The obtained assay percentages confirmed that this method is highly suitable for standardized quantitative assessments. In conclusion, both newly developed analytical platforms proved to be efficient, accurate, and reproducible. These validated methods are highly recommended for routine quality control and the simultaneous monitoring of Vonoprazan fumarate and Itopride hydrochloride in both synthetic mixtures and commercial pharmaceutical formulations.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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