RESEARCH PAPER

Development and Validation of Novel Analytical methods for Simultaneous estimation of Vonoprazan fumarate and Levosulpiride in Synthetic mixture

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ABSTRACT

Objectives: The present study reports the development and validation of efficient, sensitive, and reliable UV spectrophotometric and RP-HPLC methods for the simultaneous quantification of Vonoprazan fumarate and Levosulpiride in a synthetic mixture. Method and Results: The first-order derivative UV spectrophotometric technique was employed by measuring the amplitudes at 308nm (zero-crossing point of Vonoprazan fumarate) for the quantification of Levosulpiride and at 269nm (zero-crossing point of Levosulpiride) for Vonoprazan fumarate. The method demonstrated excellent linearity within the concentration ranges of 1-5 µg/mL for Vonoprazan fumarate and 3.75-18.75 µg/mL for Levosulpiride, yielding correlation coefficients (R^2) of 0.9989 and 1 For Vonoprazan fumarate and Levosulpiride for both drugs. The percentage recovery values ranged from 99.33%-99.80% for Vonoprazan fumarate and 99.73%-99.94% for Levosulpiride., confirming the accuracy and precision of the method. For RP-HPLC analysis, chromatographic separation was performed on a Kromstar Vertex C_{18} column (250 × 4.6 mm, 5 µm) under isocratic conditions using a mobile phase composed of Phosphate Buffer: Acetonitrile (58:42 %v/v), adjusted to pH 4.5 with orthophosphoric acid. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 233 nm. The retention times were found to be 3.6 min for Vonoprazan fumarate and 6.3 min for Levosulpiride. The calibration curves exhibited good linearity over the ranges of 1-5 µg/mL for Vonoprazan fumarate and 3.75-18.75 µg/mL for Levosulpiride, with recovery values between 99.73%-99.94% and 99.92%-99.96%, respectively. Validation results established that both the UV spectrophotometric and RP-HPLC methods are suitable, accurate, and reliable for the simultaneous quantitative determination of Vonoprazan fumarate and Levosulpiride in synthetic mixture. Conclusion: Validation performed in accordance with ICH Q2 (R2) guideline established the suitability and reliability of the developed methods for the simultaneous determination of Vonoprazan fumarate and Levosulpiride in synthetic mixture.

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1. INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic and relapsing gastrointestinal disorder characterized by the retrograde flow of gastric contents into the esophagus, resulting in symptoms such as heartburn, acid regurgitation, chest discomfort, and epigastric pain. The pathogenesis of GERD is multifactorial and includes increased gastric acid secretion, impaired esophageal clearance, delayed gastric emptying, and transient relaxation of the lower esophageal sphincter (LES). Although proton pump inhibitors (PPIs) remain the standard therapy, a considerable proportion of patients continue to experience incomplete symptom relief, particularly in cases associated with motility dysfunction, bloating, and non-acid reflux[1].

Vonoprazan fumarate (Figure 1 A) chemically known as1-(5-(2-fluorophenyl)-1-(pyridin-3 yl sulfonyl)-1H-pyrrol-3-yl)-N-methylmethanamine fumarate is a potassium-competitive acid blocker used in the treatment of acid-related gastrointestinal disorders. It is a novel class of drug that suppresses gastric acid secretion by inhibiting H^+/K^+-ATPase in the stomach. It shows rapid onset of action and provides effective acid suppression compared to conventional therapies[1-3].Levosulpiride (Figure 1 B) chemically known as *N*-[[(2*S*)-1-ethylpyrrolidin-2-yl] methyl]-2-methoxy-5-sulfamoylbenzamide is a substituted benzamide derivative used as a prokinetic agent in gastrointestinal disorders. It enhances gastrointestinal motility by blocking dopamine D_2 receptors and increasing acetylcholine release. It is widely used in the treatment of dyspepsia and reflux-related conditions[4-6].

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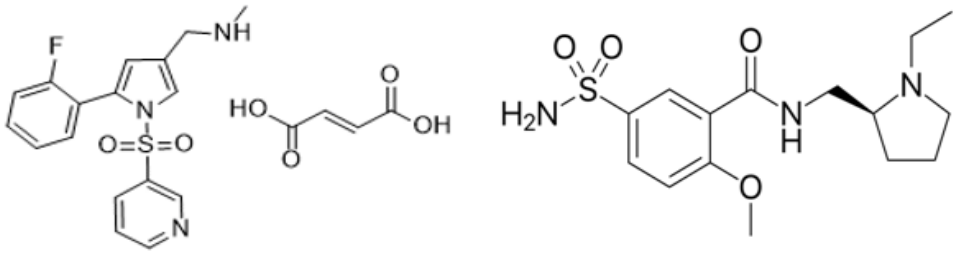

Figure 1: Chemical Structure of (A) Vonoprazan fumarate and (B) Levosulpiride

The fixed-dose combination of Vonoprazan fumarate and Levosulpiride offers a dual synergistic therapeutic approach for the management of GERD. Vonoprazan primarily targets the acid-related component of the disease by suppressing gastric acid secretion, whereas Levosulpiride addresses the motility-related component by enhancing gastrointestinal transit and reducing gastric stasis. This complementary mechanism results in improved symptom control, reduced frequency of reflux episodes, and better overall patient outcomes compared to monotherapy with acid suppression alone. The combination therefore represents a rational therapeutic strategy for patients with mixed acid and motility-related GERD symptoms[7].

Recently, this combination has gained clinical importance and was approved in India by the Central Drugs Standard Control Organization (CDSCO) in 2024[7], reflecting its growing acceptance in contemporary GERD management. Despite its increasing clinical use, there is limited analytical data available for its simultaneous estimation in combined forms.

A comprehensive review of the available literature reveals that a variety of analytical techniques, including UV spectrophotometry[8-10], Voltammetry[11], Fluorimetry[12], High-performance liquid chromatography (HPLC)[13-17], RP-UPLC-TUV[18], stability-indicating HPLC method[19-21,] eco-friendly liquid chromatography[22-23], have been extensively reported for the individual estimation of pharmaceutical agents or their combinations with other drugs. These methods demonstrate the versatility and sensitivity of modern analytical platforms for drug quantification. However, most of the reported methods are either limited to single-drug analysis or applied in combinations different from the present therapeutic pairing. From an analytical perspective, the development of a simple, accurate, precise, and validated method for simultaneous estimation of Vonoprazan fumarate and Levosulpiride is essential to ensure quality control of pharmaceutical formulations. Regulatory guidelines such as ICH Q2 (R2) guideline[24] emphasize the importance of method validation parameters including linearity, accuracy, precision, specificity, robustness, and sensitivity for reliable analytical performance.

The present study aims to develop and validate a robust and sensitive analytical method for the simultaneous estimation of Vonoprazan fumarate and Levosulpiride in bulk and synthetic forms using a suitable UV spectrophotometric technique and RP-HPLC as chromatographic technique in accordance with ICH Q2 (R2) guideline[24]. The novelty of this work lies in the development of a simple, cost-effective, and reproducible analytical method for a recently introduced fixed-dose combination, which has limited published analytical data. The proposed methods ensure rapid analysis with high sensitivity and can be effectively applied for routine quality control analysis in pharmaceutical industries.

2. MATERIALS AND ANALYTICAL CONDITIONS

2.1 Chemicals and Reagents

Vonoprazan fumarate was received as a bulk drug sample from Dhamtec Pharma and Consultants, Navi Mumbai, whereas Levosulpiride was kindly provided as a gift sample by Troikaa Pharmaceuticals Ltd., Ahmedabad. All solvents used in the study were procured from Finar Chemicals, and AR-grade potassium dihydrogen phosphate was obtained from Astron Chemical Ltd. Freshly prepared solutions were used throughout the analysis.

2.2 Spectrophotometric and Chromatographic Conditions

UV spectrophotometric measurements were conducted using a Shimadzu UV-1800 equipped with UV-Probe software, with methanol employed as the diluent. Chromatographic separation was performed on a Systronics LC-138 system comprising a photodiode array detector, manual injector, and a Kromstar Vertex C18 column (250 × 4.6 mm, 5 µm). Data acquisition and processing were carried out using Clarify software. The analysis was executed under isocratic conditions using a mobile phase of Acetonitrile: Phosphate Buffer: Acetonitrile (58:42 %v/v), adjusted to pH 4.5 with orthophosphoric acid. The flow rate was maintained appropriately, detection was set at 233 nm,

and the total run time was 10 minutes. Under the optimized chromatographic conditions, the retention times were approximately 3.6 minute for Vonoprazan fumarate and 6.3 minute for Levosulpiride.

2.3 Preparation of Analytical Solutions

2.3.1 Stock and standard Solution

Precisely weigh 10 mg each of Vonoprazan fumarate and Levosulpiride and transfer them into separate volumetric flasks. Add an adequate volume of methanol and sonicate for approximately 30 minutes to ensure complete dissolution. Dilute to volume with methanol to obtain standard stock solutions having a concentration of 100 µg/mL, and label them appropriately as standard stock solutions.

2.3.2 Preparation of Sample Solution

An accurately weighed quantity of Vonoprazan fumarate (20 mg) and Levosulpiride (75 mg) was transferred into a 100 mL volumetric flask. Methanol was added up to approximately half the volume, and the mixture was sonicated to ensure complete dissolution of the drugs. The solution was then diluted to the mark with methanol and filtered through Whatman filter paper to obtain stock concentrations of 200 µg/mL for Vonoprazan fumarate and 750 µg/mL for Levosulpiride. Subsequently, 0.1 mL of this solution was transferred into a 10 mL volumetric flask and diluted to volume with methanol to obtain final concentrations of 2 µg/mL for Vonoprazan fumarate and 7.5 µg/mL for Levosulpiride.

2.3.3 Preparation of Mobile phase

A mixture of Phosphate Buffer: Acetonitrile in the ratio of 58:42%v/v, was prepared, thoroughly mixed, and the pH was adjusted to 4.5 using 10% orthophosphoric acid.

3 METHODOLOGIES AND VALIDATION

3.1 Selection of Suitable Analytical Wavelength: The blank solution was scanned for absorbance over the range of 200-400 nm. The analytes were detected at a wavelength of 233 nm for RP-HPLC, at which both drugs exhibited satisfactory absorbance characteristics, as shown in Figure 2.

3.2 Linearity

For UV method, Precisely measured aliquots of Vonoprazan fumarate (100 µg/ml) (0.1, 0.2, 0.3, 0.4 and 0.5 ml) and Levosulpiride (100 µg/ml) (0.375, 0.75, 1.125, 1.5 and 1.875 ml) were pipetted out in same five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 1, 2, 3, 4, and 5 µg/ml for Vonoprazan fumarate and 3.75, 7.5, 11.25, 15 and 18.75 µg/ml for Levosulpiride. The absorbance of the prepared solutions was recorded at 269 nm for Vonoprazan fumarate and 308 nm for Levosulpiride, using methanol as the blank. The corresponding calibration curves are presented in Figures 4. For RP-HPLC method, accurately measured aliquots of Vonoprazan fumarate stock solution (100 µg mL^{-1}), specifically 0.1, 0.2, 0.3, 0.4, and 0.5 mL, and Levosulpiride stock solution (100 µg mL^{-1}), namely 0.375, 0.75, 1.125, 1.5, and 1.875 mL, were transferred into five separate 10 mL volumetric flasks. Each flask was diluted to volume with the mobile phase consisting of Phosphate Buffer: Acetonitrile (pH 4.5) in the ratio of 58:42 (%v/v), to obtain final concentrations of 1, 2, 3, 4, and 5 µg mL^{-1} for Vonoprazan fumarate and 3.75, 7.5, 11.25, 15, and 18.75 µg mL^{-1} forLevosulpiride. Subsequently, 20 µL of each prepared solution was injected into the RP-HPLC system using a Hamilton syringe, and the chromatographic analysis was performed. The corresponding chromatograms are presented in Figure 5.

3.3 Precision

Method precision was assessed through intraday, interday, and repeatability studies. For intraday precision, Vonoprazan fumarate solutions at concentrations of 1, 2, and 3 µg mL^{-1} and Levosulpiride solutions at 3.75,7.5,11.25 µg mL^{-1} were analyzed in triplicate within the same day. Interday precision was evaluated by examining the same concentration levels on three consecutive days. Repeatability was determined by analyzing Vonoprazan fumarate (2 µg mL^{-1}) and Levosulpiride (7.5µg mL^{-1}) six times under identical conditions. The precision of the method was expressed in terms of percentage relative standard deviation (%RSD) for both methods.

3.4 Accuracy: The pre-analyzed solution was spiked with known quantities of Vonoprazan fumarate and Levosulpiride at three concentration levels (50%, 100%, and 150%), and the mean percentage recovery of both drugs was calculated for both methods.

3.5 Detection Limit and Quantification Limit:

In accordance with ICH Q2 (R2) guideline, the Limits of Detection (LOD) and Quantification (LOQ) were calculated using standard equations for both methods.

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

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.

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

3.6 Specificity: The degradation behavior and potential analytical interferences evaluated by injecting the sample solutions containing Vonoprazan fumarate and Levosulpiride were prepared and injected into RP-

HPLC system. Method specificity was established by examining any interference through analysis of the blank chromatogram, as well as the individual and combined chromatograms of Vonoprazan fumarate and Levosulpiride.

3.7 Robustness:
The Robustness of RP-HPLC was evaluated by deliberately introducing small, controlled variations in analytical parameters such as detection wavelength and flow rate, and verifying that the system suitability criteria were consistently met. Method robustness was further confirmed through repeated analysis under these modified conditions.

3.8 System Suitability
System suitability was assessed by performing six replicate injections of freshly prepared standard solutions of Vonoprazan fumarate and Levosulpiride. Chromatographic parameters such as retention time, number of theoretical plates, and tailing factor were determined from the resulting standard chromatograms.

4. RESULTS

4.1 Selection of Analytical Wavelength for RP-HPLC: The Vonoprazan fumarate (2 µg/ml) and Levosulpiride (7.5 µg/ml) solution was scanned for absorbance over the range of 200-400 nm. The analytes were detected at a wavelength of 233 nm, at which both drugs exhibited satisfactory absorbance characteristics, as shown in Figure 2.

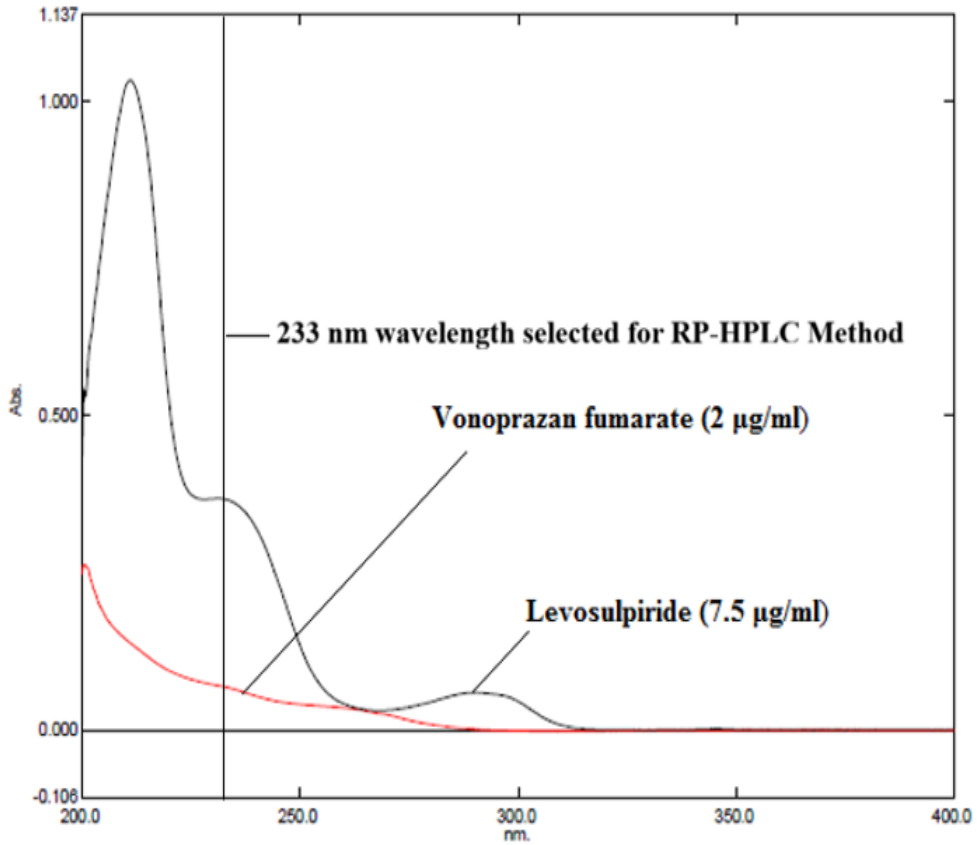

Figure 2: Overlain UV Spectra of Vonoprazan fumarate (2 µg/ml) and Levosulpiride (7.5 µg/ml) in Methanol

4.2 Selection of Analytical Wavelength for UV Spectrophotometric Method
A reliable first order derivative Spectrophotometric method was developed for simultaneous estimation of Vonoprazan fumarate and Levosulpiride in synthetic mixture by UV Spectrophotometry. For the selection of analytical wavelength, Solutions of Vonoprazan fumarate (2 µg/ml) and Levosulpiride (7.5 µg/ml**)** were scanned within the spectral range of 200–400 nm. Vonoprazan fumarate and Levosulpiride showed maximum absorbance (λmax) at 232 nm and 289 nm, respectively, as illustrated in Figure 3.

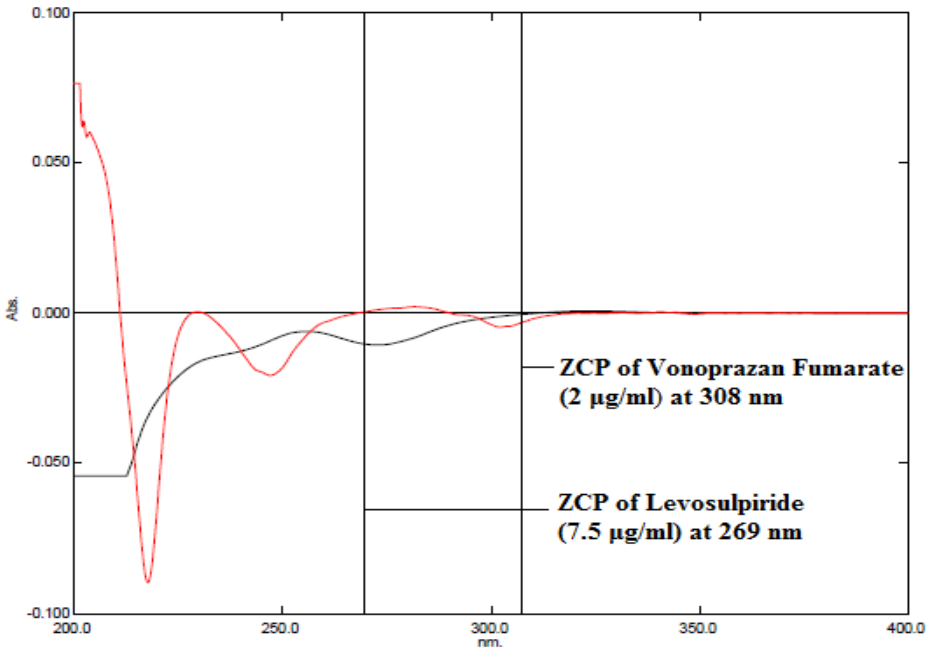

Figure 3: Overlain UV Spectra of Vonoprazan fumarate (2 µg/ml) and Levosulpiride (7.5 µg/ml) in Methanol (First order)

4.3 Method Validation for RP-HPLC Method

4.3.1 Linearity: The method demonstrated excellent linearity over the concentration ranges of 1–5 µg mL^{-1} for Vonoprazan fumarate and 3.75-18.75µg mL^{-1} for Levosulpiride showed in figure 4. The correlation coefficients were found to be 0.998 for Vonoprazan fumarate and 1for Levosulpiride. The detailed results are summarized in Table 1.

Table 1: Linearity data for RP-HPLC Method

Concentration (µg/ml)		Area ± SD (n=6)		%RSD	
Vonoprazan fumarate	Levosulpiride	Vonoprazan fumarate	Levosulpiride	Vonoprazan fumarate	Levosulpiride
1	3.75	|-0.004| ± 0.000045	|-0.002| ± 0.000023	1.12	1.15
2	7.5	|-0.009| ± 0.000094	|-0.003| ± 0.000029	1.04	0.97
3	11.25	|-0.015| ± 0.000146	|-0.004| ± 0.000030	0.97	0.75
4	15	|-0.020| ± 0.000180	|-0.005| ± 0.000025	0.90	0.50
5	18.75	|-0.025| ± 0.000205	|-0.006| ± 0.000020	0.82	0.33

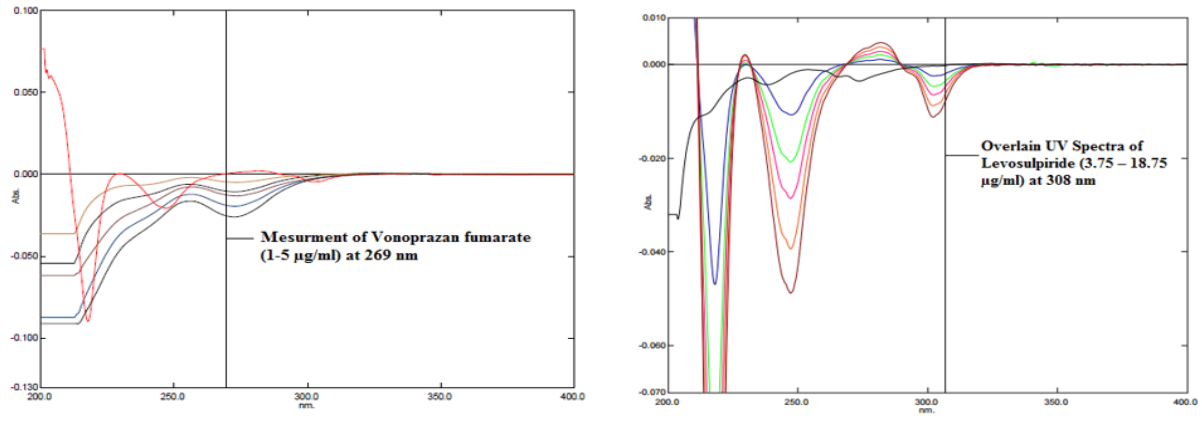

Figure 4: Linearity of 1st order Derivative UV Spectra of (A) Vonoprazan fumarate (1-5 µg/ml) at 269 nm (B) Levosulpiride (3.75-18.75 µg/ml) at 308 nm

4.3.2 Precision

Precision refers to the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample. Method precision was evaluated through intraday, interday, and repeatability studies. The %RSD values for system precision are presented in Table 2 for Vonoprazan fumarate and Levosulpiride, respectively. Since all %RSD values were below 2%, the method was confirmed to be precise, reproducible, and repeatable.

Table 2: Precision study for RP-HPLC Method

Drug	Precision Study	Conc. (µg/ml)	Mean Absorbance ± SD	% RSD
Vonoprazan fumarate at 269 nm	Intraday (n=3)	1	|-0.004| ±0.00003	1.13
		2	|-0.009| ±0.00008	1.00
		3	|-0.015| ±0.00012	0.95
	Interday (n=3)	1	|-0.004| ±0.00004	1.14
		2	|-0.009| ±0.00009	1.05
		3	|-0.015| ±0.00013	0.98
	Repeatability (n=6)	2	|-0.009| ±0.00009	1.05
Levosulpiride at 308 nm	Intraday (n=3)	3.75	|-0.002| ± 0.000022	1.10
		7.5	|-0.003| ± 0.000028	0.93
		11.25	|-0.004| ± 0.000028	0.70
	Interday (n=3)	3.75	|-0.002| ± 0.000023	1.15
		7.5	|-0.003| ± 0.000029	0.96
		11.25	|-0.004| ± 0.000029	0.72
	Repeatability (n=6)	7.5	|-0.003| ± 0.000029	1.55

4.3.3 Accuracy

Recovery studies were conducted at three concentration levels (50%, 100%, and 150%). Three replicates at each level were analyzed, and the mean percentage recoveries were calculated. As shown in Table 3, the recovery values for Vonoprazan fumarate and Levosulpiride ranged from 99.33% to 99.80% and 99.73% to 99.94%, respectively. Since all recovery values were within the acceptable range of 98.0%–102%, the method was confirmed to be accurate. These satisfactory recovery results further demonstrate the suitability of the method for routine quality control analysis.

Table 3: Recovery data for RP-HPLC 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	3	2.98	99.33±0.005
	100	2	2	4	3.98	99.50±0.008
	150	2	3	5	4.99	99.80±0.012
Levosulpiride	50	7.5	3.75	11.25	11.24	99.91±0.076
	100	7.5	7.5	15	14.96	99.73±0.092
	150	7.5	11.25	18.75	18.74	99.94±0.146

4.3.4 Detection Limit and Quantitation Limit

The Limit of Detection (LOD) indicates the lowest concentration of analyte that can be detected, while the Limit of Quantification (LOQ) represents the lowest concentration that can be quantified with acceptable accuracy and precision, making it useful for the assessment of impurities or degradation products. As shown in Table 4, the LOD and LOQ values were 0.03 µg mL^{-1} and 0.08µg mL^{-1} for Vonoprazan fumarate, and 0.25 µg mL^{-1} and 0.77 µg mL^{-1} for Levosulpiride, respectively.

Table 4: LOD and LOQ data

For RP-HPLC Method		
Drug Name	**Vonoprazan fumarate**	**Levosulpiride**
LOD (µg/ml)	0.02	0.27
LOQ (µg/ml)	0.08	0.82
For UV Method		
LOD (µg/ml)	0.03	0.25

LOQ (µg/ml)	0.08	0.77

4.3.5 Assay

Three replicate injections of the same sample solution were analyzed, and the resulting chromatograms were recorded. Vonoprazan fumarate and Levosulpiride showed mean recoveries of 99.00% and 99.73%, respectively, as presented in Table 5.

Table 5: Assay for Vonoprazan fumarate and Levosulpiride

For RP-HPLC Method				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Mean Assay ± SD (n=3)**	**% RSD**
Vonoprazan fumarate	2	1.98	99.00±0.05	0.05
Levosulpiride	7.5	7.48	99.73±0.08	0.08
For UV Method				
Vonoprazan fumarate	2	1.997	99.85±0.173	0.17
Levosulpiride	7.5	7.498	99.97±0.316	0.31

4.4 Validation of RP-HPLC Method

A simple and reliable isocratic RP-HPLC method was developed and validated for the simultaneous quantification of Vonoprazan fumarate and Levosulpiride in a synthetic mixture (figure 2). The proposed method demonstrated satisfactory accuracy, precision, and rapid analysis. Both analytes exhibited significant absorbance at 233 nm, which was selected as the optimal detection wavelength. Efficient chromatographic separation with well-resolved and symmetrical peaks was achieved using a mobile phase composed of Phosphate Buffer: Acetonitrile (pH 4.5) in the ratio of 58:42 % v/v, delivered at a flow rate of 1.0 mL min^{-1}. The separation was carried out on a Kromstar Vertex C_{18} column (250 × 4.6 mm, 5 µm) maintained at ambient temperature, with an injection volume of 20 µL, providing consistent reproducibility and repeatability.

4.4.1 Linearity

The method demonstrated excellent linearity over the concentration ranges of 1-5 µg mL^{-1} for Vonoprazan fumarate and 3.75-18.75µg mL^{-1} for Levosulpiride showed in figure 5. The correlation coefficients were found to be 0.9986 for Vonoprazan fumarate and 0.999 for Levosulpiride. The detailed results are summarized in Table 6.

Table 6: Linearity of Vonoprazan fumarate and Levosulpiride

Concentration (µg/ml)		**Area ± SD (n=6)**		**%RSD**	
VONO	**LEVO**	**VONO**	**LEVO**	**VONO**	**LEVO**
1	3.75	205.51±2.2939	556.53±5.1774	1.12	0.93
2	7.5	482.09±5.2213	773.11±5.8368	1.08	0.75
3	11.25	792.66±6.5611	1002.57±5.6174	0.83	0.56
4	15	1133.02±6.2978	1257.95±6.6081	0.56	0.53
5	18.75	1394.88±3.7193	1501.43±7.4386	0.27	0.50

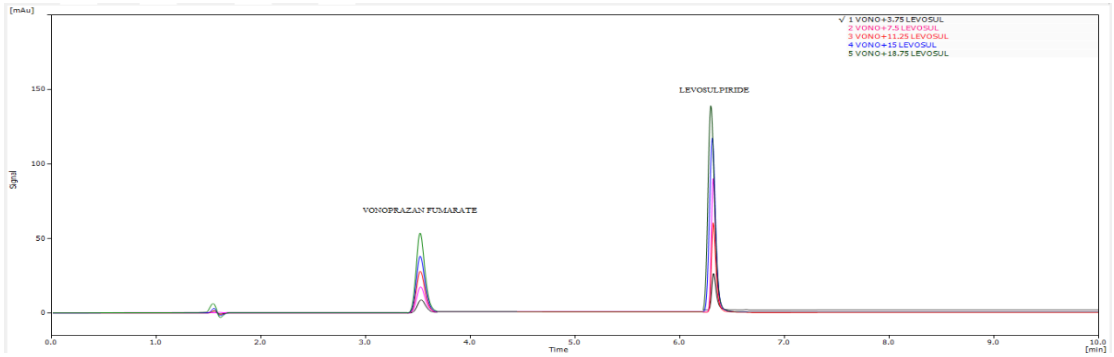

Figure 5: Overlain RP-HPLC Chromatogram of Vonoprazan fumarate (1-5 µg/ml) and Levosulpiride (3.75-18.75 µg/ml) at 233 nm {Run Time: 10 min, Flow Rate: 1.0 ml/min}

4.4.2 Precision

Precision refers to the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample. Method precision was evaluated through intraday, interday, and repeatability studies. The %RSD values for system precision are presented in Tables 7 for Vonoprazan fumarate and Levosulpiride, respectively. Since all %RSD values were below 2%, the method was confirmed to be precise, reproducible, and repeatable.

Table 7: Combined Precision Study of Vonoprazan Fumarate and Levosulpiride for RP-HPLC method at 233 nm

Drug	Study Type	Conc. (µg/ml)	Mean Peak Area (µV·sec) ± SD	% RSD
Vonoprazan fumarate at 233 nm	Intraday (n=3)	1	205.51 ±2.3921	1.16
		2	479.99 ±4.8176	1.00
		3	792.99 ±6.3770	0.80
	Interday (n=3)	1	205.44 ±2.4536	1.19
		2	480.32 ±5.1260	1.07
		3	793.66 ±6.8475	0.86
	Repeatability (n=6)	2	481.22 ± 5.2213	1.09
Levosulpiride at 233 nm	Intraday (n=3)	3.75	556.70 ± 5.099	0.92
		7.5	769.96 ± 5.361	0.70
		11.25	1000.57 ± 5.437	0.54
	Interday (n=3)	3.75	556.36 ± 5.249	0.94
		7.5	769.59 ± 5.845	0.76
		11.25	1000.23 ± 5.793	0.58
	Repeatability (n=6)	7.5	772.61 ± 5.7010	0.74

4.4.3 Accuracy

Recovery studies were conducted at three concentration levels (50%, 100%, and 150%). Three replicates at each level were analyzed, and the mean percentage recoveries were calculated. As shown in Table 8, the recovery values for Vonoprazan fumarate and Levosulpiride ranged from 99.73% to 99.94% and 99.92% to 99.98%, respectively. Since all recovery values were within the acceptable range of 98.0%–102%, the method was confirmed to be accurate. These satisfactory recovery results further demonstrate the suitability of the method for routine quality control analysis.

Table 8: Recovery of Vonoprazan fumarate and Levosulpiride

Name of Drug	% Level of recovery	Amt. of drug Taken (µg/ml)	Spiked Amt. taken (µg/ml)	Total Std amt. (µg/ml)	Total amt. recovered (µg/ml)	% Recovery ± S.D. (n=3)
Vonoprazan fumarate	50	2	1	3	2.992	99.73±0.0631
	100	2	2	4	3.994	99.85±0.0852
	150	2	3	5	4.997	99.94±0.1257
Levosulpiride	50	7.5	3.75	11.25	11.241	99.92±0.091
	100	7.5	7.5	15	14.991	99.94±0.012
	150	7.5	11.25	18.75	18.743	99.96±0.016

4.4.4 Detection Limit and Quantitation Limit

The Limit of Detection (LOD) indicates the lowest concentration of analyte that can be detected, while the Limit of Quantification (LOQ) represents the lowest concentration that can be quantified with acceptable accuracy and precision, making it useful for the assessment of impurities or degradation products. As shown in Table 4, the LOD and LOQ values were 0.02 µg mL^{-1} and 0.08 µg mL^{-1} for Vonoprazan fumarate, and 0.27 µg mL^{-1} and 0.82 µg mL^{-1} for Levosulpiride, respectively.

4.4.5 Robustness

Deliberate variations in flow rate and detection wavelength were introduced, and the results are summarized in Table 9. The findings indicated that these minor changes did not significantly affect the analytical performance, thereby confirming the robustness of the method.

Table 9: Robustness data for Vonoprazan fumarate and Levosulpiride

Condition	Variation	Vonoprazan fumarate	Levosulpiride
		% Assay ± SD (n=3)	% Assay ± SD (n=3)
Flow rate (1 ml±0.2 ml/min)	0.8 ml/min	99.75±2.28	99.53±1.25
	1.0 ml/min	99.88±7.01	99.96±2.22
	1.2 ml/min	99.95±5.24	99.97±0.75
Detection wavelength (233 nm±2 nm)	231	99.63±2.03	99.74±4.52
	233	100.02±5.54	100.04±2.88
	235	99.28±3.15	99.76±1.63
Mobile Phase Phosphate Buffer (pH 4.5): ACN (58:42 ±2 %v/v)	56:44	99.92±1.64	99.26±2.15
	58:42	99.98±6.24	99.93±1.24
	60:40	99.95±1.43	99.99±2.45

4.4.6 Assay

Three replicate injections of the same sample solution were analyzed, and the resulting chromatograms were recorded Vonoprazan fumarate and Levosulpiride showed mean recoveries of 99.85% and 99.97%, respectively, as presented in Table 5.

5. DISCUSSION

The method was systematically optimized to enhance its sensitivity and selectivity. Validation performed in compliance with ICH Q2 (R2) guideline confirmed acceptable linearity, precision, accuracy, and robustness. Further investigations may explore the applicability of the method to different pharmaceutical formulations and dosage forms.

6. CONCLUSION

The findings of the present investigation demonstrate that the developed UV spectrophotometric and RP-HPLC methods are simple, rapid, accurate, and economical for the simultaneous determination of Vonoprazan fumarate and Levosulpiride in a synthetic mixture. Statistical analysis revealed excellent repeatability, precision, and selectivity, fulfilling all ICH validation criteria. The methods also showed consistent performance under deliberate variations in chromatographic conditions, confirming their robustness and suitability for routine analytical applications. Moreover, the high percentage recovery values along with low %RSD further validate the reliability of the methods for quality control purposes and their capability to detect minor fluctuations in drug concentration. The satisfactory LOD and LOQ values indicate adequate sensitivity, supporting the applicability of the methods in impurity profiling and stability-indicating studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS: ICH: International Council for Harmonization; UV: Ultraviolet, RP-HPLC: Reverse phase High Performance liquid chromatography; API: Active Pharmaceutical Ingredient; LOD: Limit of Detection; LOQ: Limit of Quantification; RSD: Relative Standard deviation

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