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Rp-Hplc Method Development And Validation For Concurrent Estimation Of Pantoprazole And Levosulpiride

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Abstract: A simple, precise, and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Pantoprazole and Levosulpiride in bulk drugs and combined pharmaceutical dosage forms. Pantoprazole, a proton pump inhibitor, and Levosulpiride, a prokinetic agent, are commonly co-formulated for the management of gastroesophageal reflux disorders, highlighting the need for a reliable analytical method for their concurrent quantification. The chromatographic separation was achieved on a C18 column using an isocratic mobile phase, ensuring sharp and well-resolved peaks with retention times of approximately 4.1 minutes for Pantoprazole and 6.7 minutes for Levosulpiride. The method employed a flow rate of 1.0 mL/min and UV detection at 245 nm, with a total run time of under 10 minutes. Method validation was conducted in line with ICH Q2 (R1) guidelines, evaluating parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method showed excellent linearity ($r^2 \approx 0.999$), recovery rates within 98–102%, and %RSD values below 2%. LOD and LOQ were determined as 0.3 µg/mL and 1.0 µg/mL for Pantoprazole, and 0.6 µg/mL and 2.0 µg/mL for Levosulpiride, respectively. The proposed method is efficient, robust, and suitable for routine quality control analysis of combined formulations.

Index Terms - Pantoprazole, Levosulpiride, Method Validation.

I. INTRODUCTION

Fixed-dose combinations (FDCs) are increasingly employed in the treatment of multifactorial diseases to enhance therapeutic efficacy and patient compliance. Among these, the combination of Pantoprazole and Levosulpiride is widely prescribed for the management of gastroesophageal reflux disease (GERD), functional dyspepsia, and other acid-related gastrointestinal disorders. Pantoprazole is a proton pump inhibitor (PPI) that irreversibly inhibits the H+/K+ ATPase enzyme in the gastric parietal cells, thereby reducing gastric acid secretion [1]. It is characterized by good oral bioavailability, rapid onset of action, and a prolonged duration of acid suppression. Levosulpiride, a substituted benzamide derivative, acts as a selective dopamine D2 receptor antagonist. It exhibits both prokinetic and antipsychotic activity, but in this combination, its primary role is to enhance gastrointestinal motility and alleviate symptoms associated with delayed gastric emptying [2-4].

Given the complementary mechanisms of action, the co-administration of Pantoprazole and Levosulpiride offers an effective approach for treating gastrointestinal disorders that involve both acid hypersecretion and impaired motility. Consequently, the development of reliable analytical methods for the simultaneous estimation of these drugs in pharmaceutical formulations is essential to ensure product quality, safety, and efficacy [5-8]. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) remains one of the most powerful tools in pharmaceutical analysis due to its high sensitivity, reproducibility, and suitability for complex mixture separation. The method allows precise quantification of multiple components in a single run, making it particularly suitable for fixed-dose combinations. Despite the availability of individual methods for

Pantoprazole and Levosulpiride, few validated procedures exist for their concurrent estimation, especially those that meet the regulatory requirements for routine quality control. Method development for RP-HPLC involves systematic optimization of chromatographic conditions, including column selection, mobile phase composition, flow rate, detection wavelength, and injection volume, to achieve satisfactory separation and peak resolution. Once developed, the method must be validated in accordance with International Council for Harmonisation (ICH) Q2 (R1) guidelines [9]. This involves a comprehensive evaluation of parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The present study focuses on the development and validation of a simple, rapid, and accurate RP-HPLC method for the simultaneous estimation of Pantoprazole and Levosulpiride in bulk and combined dosage forms, with the aim of facilitating routine analysis in quality control laboratories [10-13].

II. MATERIALS AND METHODS

The chemicals used in this formulation development are:

Chemicals and Reagents

Pantoprazole and Levosulpiride working standards were obtained from a certified pharmaceutical source with stated purity. All reagents used were of analytical or HPLC grade. HPLC-grade water and acetonitrile were used throughout the study. Triethylamine (LR grade), sodium hydroxide (LR grade), and glacial acetic acid (AR grade) were procured from reliable suppliers. The mobile phase was filtered through a 0.45 µm membrane filter and degassed prior to use.

Instrumentation and Equipment

Chromatographic analysis was performed using a Waters Alliance HPLC system (Model 2690) equipped with a UV detector. pH adjustments were carried out using a Model 152 pH meter (RI). Weighing of all materials was done using a SAB 203 L analytical balance (Scale Tech). Sample and standard preparations utilized Class-A glassware (Borosil). An ultrasonic bath sonicator (Model PSA-10A, Digital Pro) was used for solution degassing and dissolution.

Table 1. List of Apparatus Used

S. No	Equipment	Model	Manufacturer
1	HPLC System	Waters 2690	ALLIANCE
2	pH Meter	Model 152	RI
3	Analytical Balance	SAB 203 L	Scale Tech
4	Glassware (Pipettes etc.)	NA	Borosil Class-A
5	Ultra Sonicator	PSA-10A	Digital Pro

Preparation of Mobile Phase

A mixture of 1400 mL of purified water and 400 mL of acetonitrile was prepared, followed by the addition of 2 mL of triethylamine. The pH was adjusted to 6.0 ± 0.5 using either sodium hydroxide or glacial acetic acid. The final volume was made up to 2000 mL with water. This prepared mobile phase also served as the diluent for all procedures.

Preparation of Standard Solutions

Accurately weighed 40 mg of Pantoprazole and 40 mg of Levosulpiride were transferred into two separate 100 mL volumetric flasks. Each was dissolved in approximately 60 mL of diluent and sonicated for 5 minutes. The solutions were then made up to volume with diluent. From each stock solution, 4 mL was withdrawn and combined in a 50 mL volumetric flask, and the volume was adjusted to the mark with the same diluent.

Preparation of Sample Solutions

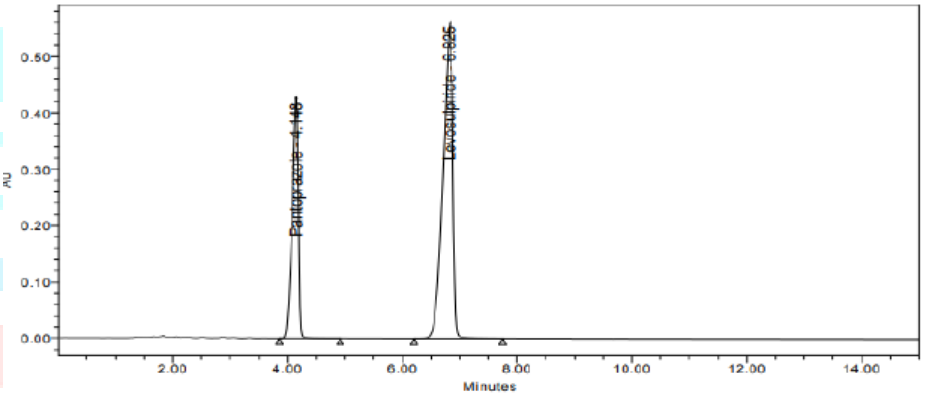
Tablet samples equivalent to 80 mg of Pantoprazole and 80 mg of Levosulpiride were weighed and transferred into individual 100 mL volumetric flasks. Approximately 60 mL of diluent was added, and the mixtures were sonicated for 5 minutes to aid dissolution. The volume was then brought up to 100 mL. From each solution, 4 mL was transferred into a 50 mL volumetric flask, and the volume was made up with diluent.

Chromatographic Conditions

The chromatographic separation was carried out using an isocratic mode on a C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase was delivered at a flow rate of 1.0 mL/min, and the detection was performed at a wavelength of 245 nm. The injection volume was 20 µL, and the run time was set to 15 minutes. Column temperature was maintained at ambient conditions, while the sample compartment was held at 20 ± 5°C.

III. METHOD DEVELOPMENT AND METHOD VALIDATION

Optimized Chromatogram:



	Peak Name	RT	Area	% Area	Height	USP Plate Count
1	Pantoprazole	4.148	3230404	32.42	429603	6010
2	Levosulpiride	6.825	6732754	67.58	568265	6860

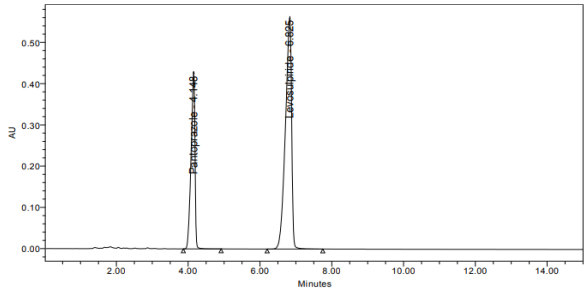
Figure 1: Optimized chromatogram for pantoprazole and levosulpiride

Chromatographic conditions:

Model: LC
Method: Isocratic method
Mobile phase: Take 1300 ml water, 500 ml of Acetonitrile (70:30), and 2 ml of Triethylamine, adjust pH with NaOH (or) glacial acetic acid to 4.5 ± 0.5 and then make up with water up to 2000 ml.
Flow: 1.0 mL/min
Injection volume: 20µl
Wavelength: 245 nm,
Run time: 15 minutes
Column: C18 250mm*4.6mm, 5µm 90
Column temperature: Ambient
Sample temperature: 20 ± 5°C

System suitability:

System suitability testing was performed prior to the analysis to ensure proper performance of the chromatographic system. Parameters such as retention time, peak area, theoretical plates, tailing factor, and %RSD were evaluated using standard solutions of Pantoprazole and Levosulpiride (Figure 10 and Figure 11).
The average retention times were 4.181 min for Pantoprazole and 6.771 min for Levosulpiride. Theoretical plate counts were found to be 6010 and 6860, while tailing factors were 0.8 and 0.7, respectively. The %RSD values were well within the acceptable limit of 2.0%, confirming the precision of the chromatographic system. Thus, the system performance was found suitable for analysis.



	Peak Name	RT	Area	% Area	Height	USP Plate Count
1	Pantoprazole	4.148	3230404	32.42	429603	6010
2	Levosulpiride	6.825	6732754	67.58	568265	6860

Figure 2: System suitability for standard chromatogram

Specificity was evaluated to ensure the method's ability to distinctly identify and quantify each analyte in the presence of excipients and other components. Chromatograms of blank and placebo samples exhibited no interfering peaks at the retention times corresponding to Pantoprazole (4.10 min) and Levosulpiride (6.80 min). This confirmed that the method is highly specific and can accurately identify and separate the analytes without interference from formulation excipients. Assay results of the marketed formulation (Pantocid-L) indicated that the method accurately quantified the active ingredients. The mean assay values were 100.46% for Pantoprazole and 99.67% for Levosulpiride, both of which fall within the acceptance range of 98–102%. These results confirm the reliability and accuracy of the developed method for quantitative analysis of the formulation.

Accuracy was determined by recovery studies conducted at three concentration levels — 50%, 100%, and 150% of the target concentration. The percentage recoveries for Pantoprazole ranged from 99.24% to 100.69%, and for Levosulpiride from 99.08% to 100.66%, with %RSD values below 2.0%. The low RSD values (0.21–0.78%) at all concentration levels indicate excellent repeatability and reliability of the analytical method. Therefore, the method was found to be accurate within the specified limits. Method precision was evaluated by performing six replicate injections of the standard solution under identical conditions. The %RSD for Pantoprazole and Levosulpiride was found to be 0.3% for both, confirming the repeatability and precision of the method. These results indicate that the method provides consistent results upon repeated analysis of the same sample.

Linearity studies were performed over the concentration range of 50–150 µg/mL for both drugs. The calibration curves demonstrated a strong linear relationship between peak area and concentration, with correlation coefficients (r^2) of 0.99 for both Pantoprazole and Levosulpiride. This confirms that detector response was directly proportional to analyte concentration within the tested range, ensuring accurate quantification.

Table 2: Selection of Medium for preparation of standard Graph

Pantoprazole Linearity		Levosulpiride Linearity	
PPM	**Areas**	**PPM**	**Areas**
16	1490031	16	3125077
24	2508650	24	5261369
32	3202773	32	6728217
40	3722231	40	7824889
48	4861824	48	10213244
Corr	0.99	Corr	0.99

The validated range for both analytes was established between 50% and 150% of the test concentration. Within this range, the %RSD values for Pantoprazole (0.16–0.79%) and Levosulpiride (0.15–0.32%) were well below 2.0%, confirming that the method maintains precision, accuracy, and linearity across the tested concentrations.

Range for Pantoprazole & Levosulpiride		
Percentage of solution	**RSD for Pantoprazole**	**RSD for Levosulpiride**
50%	0.79%	0.32%
100%	0.19%	0.20%
150%	0.16%	0.15%

Robustness was evaluated by introducing small, deliberate variations in chromatographic parameters, such as flow rate (±0.1 mL/min) and organic phase composition (±2%). These changes did not significantly affect retention time, plate count, or tailing factor. The tailing factors for Pantoprazole and Levosulpiride remained below 2.0, demonstrating that the method is robust and can withstand minor operational variations without compromising analytical performance. The sensitivity of the method was assessed by calculating LOD and LOQ based on the standard deviation of the response and the slope of the calibration curve. The LOD values were 0.3 µg/mL for Pantoprazole and 0.6 µg/mL for Levosulpiride, while the LOQ values were 1.0 µg/mL and 2.0 µg/mL, respectively. These results demonstrate the method's adequate sensitivity for detecting and quantifying low levels of both analytes. All validation parameters - including system suitability, specificity, accuracy, precision, linearity, range, robustness, LOD, and LOQ - complied with the ICH acceptance criteria. The developed RP-HPLC method proved to be simple, accurate, precise, specific, and robust for the simultaneous estimation of Pantoprazole and Levosulpiride in pharmaceutical formulations. The results confirm that the method is suitable for routine quality control analysis of these drugs in combined dosage forms.

IV. CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Pantoprazole and Levosulpiride was successfully validated in accordance with ICH Q2(R1) guidelines. All validation parameters including system suitability, specificity, linearity, accuracy, precision, range, robustness, and sensitivity were found to be within the acceptable limits. The chromatograms demonstrated well-resolved peaks with no interference, confirming the method's selectivity and reliability. The obtained results showed excellent linearity over the tested concentration range, high recovery rates close to 100%, and low %RSD values, indicating the method's accuracy and precision. Moreover, the low LOD and LOQ values confirmed that the method is sufficiently sensitive for detecting and quantifying both analytes at trace levels. Overall, the study concludes that the proposed RP-HPLC method is simple, rapid, accurate, precise, robust, and cost-effective, making it highly

suitable for routine quality control, assay, and stability testing of Pantoprazole and Levosulpiride in combined pharmaceutical dosage forms.

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