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Rp–Hplc Method Development And Validation For The Qualitative Analysis Of Ondansetron And Rabeprazole

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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 Ondansetron and Rabeprazole in pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of ammonium acetate buffer, water, and methanol (25:15:60 v/v/v) at a wavelength of 275 nm. The method exhibited linearity between 50–150 ppm, with correlation coefficients of 1.000 (Ondansetron) and 0.99 (Rabeprazole). Validation as per ICH Q2(R1) confirmed specificity, accuracy, precision, and robustness. The proposed method is suitable for routine quality-control analysis of Ondansetron and Rabeprazole in combined formulations.

Index Terms - HPLC, Ondansetron and Rabeprazole.

I. INTRODUCTION

Pharmaceutical analysis is a vital branch of pharmaceutical sciences concerned with the qualitative and quantitative determination of drugs, excipients, and impurities in bulk drugs and formulations. It ensures the safety, efficacy, and quality of pharmaceutical products, meeting the standards set by regulatory authorities such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Analytical chemistry plays a central role in all stages of drug development, including formulation design, stability testing, quality control, and regulatory compliance. With the increasing complexity of modern drug formulations, advanced analytical techniques have become indispensable tools for accurate drug characterization [1].

Among the various analytical methods available, chromatography occupies a prominent place due to its efficiency, selectivity, and reproducibility. Chromatographic techniques are based on the principle of separation of components in a mixture according to their differential distribution between stationary and mobile phases. The major types of chromatography include thin-layer chromatography (TLC), gas chromatography (GC), ion exchange chromatography, size exclusion chromatography, and high-performance liquid chromatography (HPLC) [2-5]. Of these, HPLC particularly Reverse Phase HPLC (RP-HPLC) is the most widely used method in pharmaceutical industries due to its high resolution, accuracy, and speed. RP-HPLC involves a nonpolar stationary phase and a relatively polar mobile phase, making it suitable for the analysis of polar and nonpolar compounds alike. It is extensively used for the estimation of active pharmaceutical ingredients (APIs), stability studies, and impurity profiling. The advantages of RP-HPLC include improved precision, better reproducibility, minimal sample preparation, and the ability to separate complex mixtures efficiently. However, the method requires careful optimization of parameters such as mobile phase composition, flow rate, detection wavelength, and column temperature to achieve accurate results. [6-10].

Method development in HPLC involves selecting the appropriate stationary phase, mobile phase, and detection wavelength for achieving optimum separation and resolution between drug components. Once developed, the method must be validated according to ICH Q2(R1) guidelines to confirm its reliability for

routine use. Validation parameters include accuracy, precision, linearity, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). A validated analytical method ensures that the procedure consistently produces reliable results within defined limits. In the present investigation, an RP-HPLC method was developed and validated for the simultaneous estimation of Ondansetron and Rabeprazole in pharmaceutical dosage forms. Ondansetron, a selective serotonin 5-HT_3 receptor antagonist, is commonly used to prevent nausea and vomiting associated with chemotherapy, radiotherapy, and postoperative conditions. Rabeprazole, a proton pump inhibitor (PPI), is widely used to treat gastroesophageal reflux disease (GERD), peptic ulcers, and related acid-related disorders. Since both drugs are frequently prescribed together in combined dosage forms to manage gastrointestinal complications associated with chemotherapy, their simultaneous estimation is crucial for quality control and therapeutic consistency [11-13].

The primary objective of this study was to develop a simple, rapid, precise, and cost-effective RP-HPLC method capable of simultaneously quantifying Ondansetron and Rabeprazole. The method was further validated as per ICH guidelines to confirm its suitability for routine analysis in pharmaceutical quality control laboratories. This analytical approach ensures the reliability, reproducibility, and robustness required for maintaining the quality of combined drug formulations [14].

II. MATERIALS AND METHODS

Instruments and Equipment

Pure drug standards of Ondansetron and Rabeprazole were procured from a certified pharmaceutical manufacturer. Methanol and water of HPLC grade were obtained from Merck Ltd. Ammonium acetate (analytical reagent grade) was used for buffer preparation. All other reagents and solvents employed were of analytical or HPLC grade to ensure the highest purity and reproducibility.

Table 1: List of Instruments used in the Study

S. No.	Instrument	Model/Make
1	HPLC System	Waters Alliance e2690
3	Weighing balance	SAB 203 L
4	Sonicator	PSA10A
5	pH Meter	Model 152

Table 2: List of Chemicals and Reagents

S. No.	Chemical/Reagent	Grade/Source
1	Water (Milli Q / HPLC Grade water)	CMS Laboratories, Hyderabad
2	Ammonium acetate	CMS Laboratories, Hyderabad
3	Methanol	HPLC Grade, Merck Pvt. Ltd.

Selection of Mobile Phase:

Various trials were conducted using combinations of aqueous buffer and organic solvents to optimize peak symmetry, resolution, and retention time. Based on solubility and chromatographic performance, a mixture of ammonium acetate buffer, water, and methanol in the ratio of 25:15:60 (v/v/v) was selected as the final mobile phase. The flow rate was maintained at 1.0 mL/min, and the elution was carried out in isocratic mode to achieve sharp, well-resolved peaks for both analytes. The UV absorption spectra of Ondansetron and Rabeprazole were scanned between 200–400 nm. The overlapping maximum absorbance for both drugs was observed at 275 nm, which was chosen as the detection wavelength to ensure optimum sensitivity and accuracy.

Preparation of Standard Solutions:

Stock solutions of Ondansetron and Rabeprazole (1000 µg/mL each) were prepared separately by dissolving accurately weighed quantities of each drug in methanol. From these, working standard solutions of varying concentrations (50–150 ppm) were prepared by serial dilution with the mobile phase to establish calibration curves.

Sample Preparation:

Tablet formulations containing Ondansetron and Rabeprazole were finely powdered. An accurately weighed portion equivalent to the average weight of one tablet was transferred into a volumetric flask, dissolved in methanol, sonicated for 10 minutes, and filtered through a 0.45 µm membrane filter. The filtrate was suitably diluted with the mobile phase to obtain the desired working concentration.

III. RESULTS AND DISCUSSION

The developed RP-HPLC method successfully achieved sharp, well-resolved, and symmetrical peaks for both Ondansetron and Rabeprazole under optimized chromatographic conditions. The separation was performed using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of Ammonium acetate buffer : Water : Methanol (25:15:60 v/v/v) at a flow rate of 1.0 mL/min, and detection wavelength of 275 nm.

Under these conditions, Ondansetron and Rabeprazole showed well-defined peaks at retention times of approximately 3.5 minutes and 5.9 minutes, respectively, with no interference from blank or excipient peaks, demonstrating excellent specificity.

Table 3: Optimized Chromatographic Conditions

Parameter	Observation
Column	C18 250mm x 4.6mm, 5µm particle size
Mobile Phase	Ammonium acetate buffer: water: methanol (35:15:50)
Flow Rate	1 mL/min
Injection Volume	20 µL
Wavelength	275 nm
Runtime	15 min
Temperature	Ambient (25°C)
Mode	Isocratic

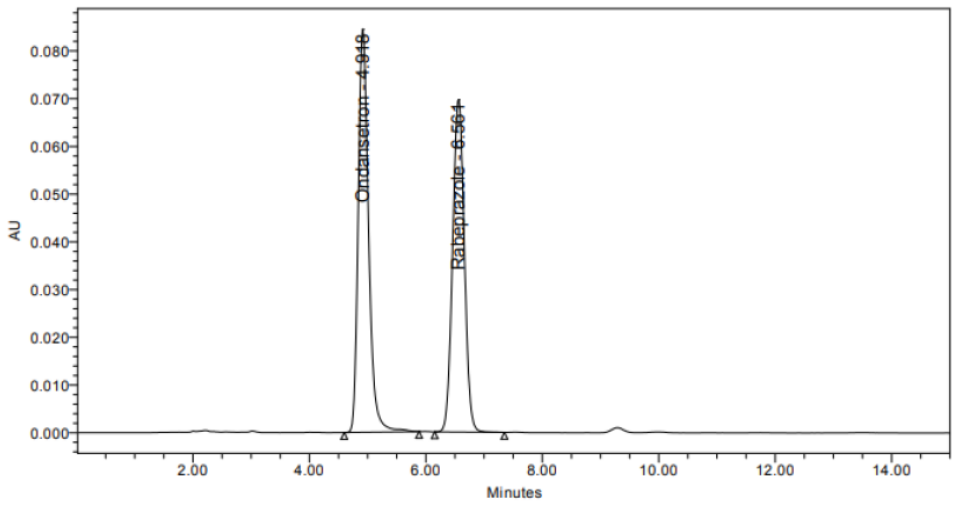

Figure 1: Optimized chromatogram of Ondansetron & Rabeprazole

System Suitability

System suitability parameters, including retention time, theoretical plates, and tailing factor, were evaluated before analysis. Theoretical plate counts exceeded 3000, and tailing factors were less than 1.5, indicating satisfactory column efficiency and peak symmetry.

Table 4: System Suitability Parameters

Parameter	Ondansetron	Rabeprazole
Retention Time (min)	4.98	6.561
Plate Count	3821	4984
Tailing Factor	1.3	1.0
%RSD	1.2	0.8

The limits of detection (LOD) and quantification (LOQ) were found to be 0.16 µg/mL and 0.49 µg/mL for Ondansetron and 0.09 µg/mL and 0.28 µg/mL for Rabeprazole, respectively, demonstrating the method's high sensitivity. Accuracy was assessed through recovery studies at three concentration levels—50%, 100%, and 150%—and the mean percentage recovery was found to be between 98.5% and 101.2% for both drugs, which lies within acceptable limits. Precision studies, including intra-day and inter-day variations, showed %RSD values below 2%, establishing the method's reproducibility and reliability. The calibration plots of peak area versus concentration demonstrated excellent linearity over the range of 50–150 ppm for both drugs. The correlation coefficients (r^2) were 1.000 for Ondansetron and 0.999 for Rabeprazole, confirming the linear relationship between concentration and detector response.

Discussion

The developed RP-HPLC method proved to be simple, accurate, and highly reproducible for the simultaneous estimation of Ondansetron and Rabeprazole in combined pharmaceutical formulations. Optimization of chromatographic conditions, including the use of a C18 column and a mobile phase of ammonium acetate buffer, water, and methanol (25:15:60 v/v/v), resulted in sharp, well-resolved peaks at retention times of 3.5 minutes and 5.9 minutes, respectively. The method exhibited excellent linearity within the concentration range of 50–150 ppm, with correlation coefficients above 0.999, confirming a strong relationship between concentration and peak area. Accuracy studies showed recovery values between 98.5% and 101.2%, while precision results displayed %RSD values below 2%, demonstrating the method's reliability. System suitability parameters such as theoretical plate count and tailing factor were within acceptable limits, confirming consistent column performance. The LOD and LOQ values indicated high sensitivity for both drugs.

IV. CONCLUSION

The present study successfully developed and validated a simple, precise, and robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Ondansetron and Rabeprazole in combined pharmaceutical formulations. The optimized chromatographic conditions—using a C18 column with a mobile phase of ammonium acetate buffer, water, and methanol (25:15:60 v/v/v) ensured excellent resolution, symmetry, and reproducibility of peaks at 3.54 minutes and 5.94 minutes, respectively. The method demonstrated outstanding linearity, accuracy, and precision, with correlation coefficients exceeding 0.999 and recovery values within the acceptable range of 98–102%. System suitability, LOD, and LOQ results confirmed that the method is sensitive, reliable, and efficient for quality-control analysis. All validation parameters complied with ICH Q2(R1) guidelines, confirming the method's suitability for routine analytical applications. The advantages of the developed method include minimal solvent usage, short analysis time, and ease of operation. Therefore, this RP-HPLC method can be effectively applied for routine assay, stability testing, and quality assurance of Ondansetron and Rabeprazole in bulk and combined dosage forms, ensuring consistent pharmaceutical product quality and therapeutic efficacy.

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