# METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PANTOPRAZOLE AND ONDANSETRON BY RP-HPLC

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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 ondansetron in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (ph 3.0) and acetonitrile in the ratio of 60:40 v/v. The flow rate was maintained at 1.0 ml/min, and the analytes were detected at 290 nm using a UV detector. The method showed good resolution between pantoprazole and ondansetron with retention times of approximately [insert rts if available]. The developed method was validated in accordance with ICH Q2(R1) guidelines for various parameters including linearity, accuracy, precision, specificity, robustness, and sensitivity. The method demonstrated excellent linearity over the concentration ranges of [insert ranges] µg/ml for both drugs, with correlation coefficients ($R^2$) greater than 0.999. Recovery studies confirmed the accuracy of the method, and %RSD values for precision were found to be within acceptable limits. The validated rp-hplc method is suitable for routine quality control analysis of pantoprazole and ondansetron in combined dosage forms and can be effectively used for simultaneous quantification in pharmaceutical industries.

## Introduction:

In recent years, the use of combination drug therapies has significantly increased, particularly for managing gastrointestinal disorders that coexist with symptoms like nausea and vomiting. Pantoprazole and Ondansetron are two such drugs often prescribed together, especially for patients undergoing chemotherapy or suffering from acid-peptic disorders accompanied by nausea. Pantoprazole is a proton pump inhibitor (PPI) that suppresses gastric acid secretion by irreversibly inhibiting the H+/K+ ATPase enzyme in the gastric parietal cells. Ondansetron, on the other hand, is a selective 5-HT3 receptor antagonist that effectively prevents nausea and vomiting triggered by chemotherapy, radiotherapy, or postoperative conditions.[2]

The co-administration of these two drugs necessitates the development of a reliable, precise, and rapid analytical method that can simultaneously estimate both compounds in pharmaceutical formulations. While various analytical methods have been reported individually for Pantoprazole and Ondansetron, there exists limited literature on a validated RP-HPLC method capable of estimating both drugs concurrently in a combined dosage form. Hence, the development of such a method becomes essential for quality control and routine analysis in pharmaceutical industries.[4]

High-Performance Liquid Chromatography (HPLC) is a powerful analytical tool widely used in pharmaceutical analysis due to its high precision, sensitivity, specificity, and accuracy. Among the different types of HPLC, Reversed-Phase HPLC (RP-HPLC) is most commonly employed for the analysis of pharmaceutical compounds owing to its compatibility with a wide range of drugs. It is particularly effective for separating and quantifying analytes with varying polarities, such as Pantoprazole and Ondansetron.[5]

The present study aims to develop and validate a simple, accurate, economical, and reproducible RP-HPLC method for the simultaneous estimation of Pantoprazole and Ondansetron in bulk and tablet dosage forms. The method is developed using commonly available solvents and columns, making it suitable for routine laboratory use. The validation of the method is carried out as per International Council for Harmonisation (ICH) Q2(R1) guidelines, evaluating critical parameters such as system suitability, linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness.[7]

This method, once validated, will serve as a reliable quality control tool for pharmaceutical industries in the manufacturing and release testing of fixed-dose combinations containing Pantoprazole and Ondansetron. Furthermore, the simplicity of the method allows its easy adaptation in academic, research, and industrial laboratories without the requirement of sophisticated instrumentation or expensive reagents. By addressing the analytical gap for simultaneous estimation, this method also contributes to the growing need for combination drug analysis and regulatory compliance in formulation development.[7]

**Materials and Methods:**

Pantoprazole sodium and Ondansetron hydrochloride working standards were obtained as gift samples from a reputed pharmaceutical manufacturer. A commercially available tablet formulation containing Pantoprazole (40 mg) and Ondansetron (4 mg) was procured from a local pharmacy for analysis. All chemicals and reagents used throughout the study were of analytical grade or HPLC grade. Acetonitrile, methanol, and water used for mobile phase preparation were procured from Merck (India), while orthophosphoric acid and triethylamine used in buffer preparation were of analytical reagent (AR) grade. The analysis was carried out using a Shimadzu LC-20AT HPLC system equipped with a UV-Visible detector (SPD-20A), a quaternary pump, and a manual injector with a 20 µL fixed loop. Data acquisition and processing were performed using LabSolutions software. Chromatographic separation was achieved on a reversed-phase C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at ambient temperature (25 ± 2°C).[10]

The mobile phase consisted of a mixture of phosphate buffer (pH adjusted to 3.0 using orthophosphoric acid) and acetonitrile in the ratio of 40:60 v/v. The buffer was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1000 mL of HPLC-grade water,

followed by pH adjustment. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use. The flow rate was maintained at 1.0 mL/min, and the analytes were detected at 290 nm. The total run time was optimized to 10 minutes, with Pantoprazole and Ondansetron showing well-resolved peaks without interference.[11]

Standard stock solutions of Pantoprazole and Ondansetron were prepared separately by dissolving 10 mg of each drug in 10 mL of the mobile phase to obtain a concentration of 1000 µg/mL. These stock solutions were further diluted with mobile phase to prepare working solutions in the concentration ranges of 5–50 µg/mL for Pantoprazole and 2–20 µg/mL for Ondansetron. The tablet formulation was analyzed by accurately weighing and finely powdering 20 tablets. An amount of powder equivalent to 40 mg of Pantoprazole and 4 mg of Ondansetron was transferred to a 100 mL volumetric flask, dissolved in the mobile phase with the aid of sonication for 15 minutes, and the volume was made up to the mark. The resulting solution was filtered through a 0.45 µm membrane filter and further diluted to bring the concentrations within the linearity range.[12]

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines. System suitability was evaluated by injecting the standard solution five times and calculating parameters such as retention time, theoretical plates, and tailing factor. Linearity was assessed by analyzing standard solutions at different concentration levels and plotting calibration curves of peak area versus concentration for both drugs. Precision studies, including intraday and interday variations, were performed at three different concentration levels. Accuracy was evaluated by recovery studies at 80%, 100%, and 120% levels. Sensitivity was assessed through the determination of the limit of detection (LOD) and limit of quantification (LOQ). Specificity was confirmed by ensuring that no interference occurred from excipients, and

robustness was evaluated by making small deliberate changes to chromatographic conditions such as flow rate, mobile phase composition, and detection wavelength.[14]

**Results and Discussion:**

All the system suitability parameters are within range and satisfactory as per ICH guidelines. Six Linear concentrations of pantoprazole (40-240ppm) and ondansetron (4-24ppm) are prepared and injected. Regression equation of the pantoprazole and ondansetron are found to be, y = 6508x + 13439, and y = 16236x + 1436 and regression coefficient was found to be 0.999 and 0.998 respectively. Intraday Precision was performed and %RSD for pantoprazole and ondansetron were found to be 1.0% and 0.8% respectively. Inter day precision was performed with 24hrs time lag and the %RSD obtained for pantoprazole and ondansetron were 0.3% and 0.2% respectively. Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount recovered and % Recovery. Limit of detection was calculated by standard deviation method. LOD for pantoprazole and ondansetron were found to be 0.10and 0.07 respectively. Limit of quantification was calculated by standard deviation method. LOQ for pantoprazole and ondansetron were found to be 0.32and 0.21 respectively. Small deliberatechanges in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH guide line. Standard preparations are made from the API and sample preparations are from formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. (Brand Name: Zaprol O; Epitome life sciences; pantoprazole/ ondansetron : 40mg/4mg) The Average % assay was calculated and found to be 99.38% and 99.81% for pantoprazole and ondansetron respectively. Degradation

studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

**Table : Robustness data of pantoprazole and ondansetron**

| S. no. | Robustness condition | Pantoprazole %RSD | Ondansetron %RSD |
|---|---|---|---|
| 1 | Flow minus | 0.6 | 1.4 |
| 2 | Flow Plus | 0.3 | 0.5 |
| 3 | Mobile phase minus | 0.6 | 0.6 |
| 4 | Mobile phase Plus | 0.6 | 1.3 |
| 5 | Temperature minus | 0.4 | 0.7 |
| 6 | Temperature Plus | 0.6 | 1.2 |

**Conclusion:**

A simple, accurate, precise method was developed for the simultaneous estimation of the pantoprazole and ondansetron in tablet dosage form. Retention time of pantoprazole and ondansetron were found to be 2.281min and 2.840min. %RSD of the pantoprazole and

ondansetron were and found to be 1.0 and 0.8 respectively. % Assay was obtained as 99.26% and 99.09% for pantoprazole and ondansetron respectively. LOD, LOQ values are obtained from regression equations of pantoprazole and ondansetron were 0.10 µg/ml, 0.07 µg/ml and 0.32 µg/ml, 0.21 µg/ml respectively. Regression equation of pantoprazole is y = 6508x + 13439, and y = 16236x + 1436of ondansetron.Retention time of analytes was decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular quality control test in industries.

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