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Development And Validation Of An Rp – Hplc Method For The Simultaneous Estimation Of Esomeprazole And Domperidone

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Abstract: The present research focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical dosage forms. The primary objective of this study was to establish a simple, accurate, precise, and cost-effective analytical method suitable for routine quality control and stability analysis. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer and methanol in the ratio of 60:40 v/v, adjusted to a pH of 7.0 with orthophosphoric acid, at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 285 nm, where both drugs exhibited significant absorbance. Under the optimized chromatographic conditions, sharp and symmetrical peaks were obtained with retention times of 3.42 minutes for Esomeprazole and 5.18 minutes for Domperidone, showing excellent resolution and separation. The developed method was validated as per the International Council for Harmonisation (ICH Q2 R1) guidelines. The method demonstrated good linearity over the concentration range of 5–25 µg/mL for Esomeprazole and 10–50 µg/mL for Domperidone, with correlation coefficients (R^2) greater than 0.999. Accuracy studies showed mean recoveries between 98% and 102%, while precision results confirmed reproducibility with %RSD values below 2. The method also proved to be robust and specific, showing no interference from excipients or degradation products. Hence, the developed RP-HPLC method is rapid, reliable, and suitable for routine analysis of Esomeprazole and Domperidone in combined dosage forms.

Index Terms - Esomeprazole, Domperidone, Method Validation.

I. INTRODUCTION

Analytical chemistry forms the foundation of pharmaceutical analysis, playing a vital role in ensuring the quality, efficacy, and safety of drug substances and formulations. Among the available analytical techniques, High-Performance Liquid Chromatography (HPLC) has become one of the most powerful, reliable, and widely used tools in both qualitative and quantitative pharmaceutical analysis. Its versatility, precision, and reproducibility make it indispensable for the analysis of single as well as multiple drug components in complex formulations [1-3]. In particular, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has gained widespread application because of its simplicity, speed, and ability to separate compounds with diverse polarities efficiently. Fixed-dose combination (FDC) formulations have become increasingly popular in modern therapeutics, especially in the management of chronic and multifactorial disorders such as gastrointestinal diseases, cardiovascular conditions, and metabolic syndromes. These formulations enhance patient compliance, reduce pill burden, and provide synergistic therapeutic benefits. However, simultaneous estimation of multiple active ingredients in such combinations poses analytical challenges due to differences in their chemical structures, solubility, pKa, and UV absorption characteristics. Thus, the development of an accurate, precise, and validated analytical method capable of estimating multiple drugs simultaneously is essential for maintaining quality standards and ensuring therapeutic efficacy. The present study focuses on the

development and validation of a simple, rapid, and accurate RP-HPLC method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical dosage forms. Esomeprazole, the S-isomer of omeprazole, is a proton pump inhibitor (PPI) used for the treatment of gastroesophageal reflux disease (GERD), peptic ulcers, and Zollinger–Ellison syndrome [4-9]. It acts by irreversibly inhibiting the H^+/K^+ ATPase enzyme system in the gastric parietal cells, thereby reducing gastric acid secretion. Domperidone, on the other hand, is a dopamine D_2 receptor antagonist that exhibits both prokinetic and antiemetic activities. It increases gastrointestinal motility and facilitates gastric emptying without affecting gastric acid secretion. The combination of these two drugs provides a synergistic therapeutic effect in treating acid-related disorders accompanied by nausea, vomiting, or delayed gastric emptying. Although several analytical methods have been reported for the individual estimation of Esomeprazole and Domperidone, very few methods are available for their simultaneous determination in combined dosage forms. Some existing methods are time-consuming, use costly reagents, or lack sensitivity and reproducibility. Therefore, there is a need to develop a simple, economical, and precise RP-HPLC method that can accurately quantify both drugs in a single run, ensuring shorter analysis time and better resolution [10].

In the current research, chromatographic conditions such as mobile phase composition, pH, flow rate, and detection wavelength were optimized to achieve sharp, symmetrical, and well-resolved peaks for both drugs. The analytical method was developed using a C18 column under isocratic conditions with a mobile phase comprising buffer and methanol, adjusted to the required pH to ensure effective separation. Detection was carried out at an appropriate wavelength where both drugs exhibited sufficient absorbance. The developed method was validated as per the International Council for Harmonisation (ICH) Q2 (R1) guidelines to confirm its reliability and suitability for routine analysis. Parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability were thoroughly evaluated. Linearity studies were performed across a defined concentration range for both drugs, and recovery studies were conducted to assess accuracy. System suitability parameters such as theoretical plates, tailing factor, and resolution were determined to ensure the method's performance. The proposed RP-HPLC method offers several advantages, including reduced analysis time, excellent reproducibility, and high accuracy. It can be effectively used for routine quality control, stability testing, and assay determination of Esomeprazole and Domperidone in pharmaceutical industries. Overall, this validated analytical approach ensures reliable quantification of both drugs in combined dosage forms, supporting regulatory compliance and product consistency in pharmaceutical formulations [11-14].

II. MATERIALS AND METHODS

Analytical The present study was carried out to develop and validate a simple, precise, accurate, and economical Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical dosage forms. The work was performed according to International Council for Harmonisation (ICH Q2 R1) guidelines to ensure reliability and reproducibility for routine quality control analysis. All reagents and chemicals used were of analytical and HPLC grade. Esomeprazole and Domperidone pure drug samples were obtained as gift samples from a reputed pharmaceutical manufacturer. Methanol (HPLC grade), phosphate buffer, and orthophosphoric acid were procured from Merck India Ltd., while distilled water was used throughout the analysis. The chromatographic analysis was performed on a Waters HPLC system equipped with an auto-sampler, PDA detector, and Empower 2.0 software for data processing. The separation was achieved using a C18 column (250 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. During method development, different mobile phase compositions, pH values, and flow rates were evaluated to obtain sharp and symmetrical peaks with optimal resolution between both analytes. The final optimized chromatographic conditions consisted of a mobile phase composed of phosphate buffer and methanol in the ratio of 60:40 v/v, adjusted to pH 7.0 with orthophosphoric acid, and a flow rate of 1.0 mL/min. The detection wavelength was set at 285 nm, and the injection volume was 10 µL. The total run time was about 10 minutes, providing well-resolved peaks for both drugs with retention times of 3.42 minutes for Esomeprazole and 5.18 minutes for Domperidone.

Table 1: Chromatographic Conditions

Parameter	Optimized Condition
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Ammonium Acetate Buffer: Acetonitrile: Methanol [55:35:10]
pH	7.0 (adjusted with orthophosphoric acid)
Flow Rate	1.0 mL/min
Detection Wavelength	288 nm
Injection Volume	20 µL
Run Time	25 minutes
Column Temperature	Ambient
Mode	Isocratic Elution

Preparation of Standard Solutions

Accurately weighed 10 mg each of Esomeprazole and Domperidone were transferred into two separate 100 mL volumetric flasks. Both drugs were dissolved in a small amount of methanol and then diluted to the mark with the same solvent to obtain a concentration of 100 µg/mL (stock solutions). Working standard solutions were prepared by appropriate dilution of the stock solutions with the mobile phase to obtain concentrations in the range of 5–25 µg/mL for Esomeprazole and 10–50 µg/mL for Domperidone.

Preparation of Sample Solution

Twenty tablets containing Esomeprazole and Domperidone were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 10 mg of Esomeprazole and 30 mg of Domperidone was transferred to a 100 mL volumetric flask. About 50 mL of methanol was added, and the mixture was sonicated for 10 minutes to ensure complete dissolution. The solution was filtered through a 0.45 µm membrane filter, and the final volume was made up with methanol. Suitable dilutions of this stock were made using the mobile phase to bring the concentration within the linear range.

System Suitability Parameters

Prior to analysis, the system was evaluated for performance consistency. Parameters such as retention time, theoretical plates, tailing factor, resolution, and %RSD were calculated using six replicate injections of the standard solution.

Table 2: System Suitability Parameters

Parameter	Acceptance Criteria	Observation
Retention Time (min)	Consistent	3.42 (Esomeprazole), 5.18 (Domperidone)
Theoretical Plates (N)	≥ 2000	5890 (Esomeprazole), 6215 (Domperidone)
Resolution (Rs)	≥ 2.0	2.8
Tailing Factor	≤ 2.0	1.09 (Esomeprazole), 1.10 (Domperidone)
%RSD (Peak Area)	≤ 2.0%	0.54%

The system suitability results confirmed the efficiency and reproducibility of the chromatographic system. Linearity studies demonstrated an excellent correlation between concentration and peak area within the tested ranges for both drugs, with correlation coefficients greater than **0.999**. The method exhibited satisfactory accuracy, with recovery results between **98% and 102%**, and high precision, with %RSD values below **2.0%** for both intra-day and inter-day studies. Minor variations in chromatographic parameters such as pH, wavelength, and flow rate did not significantly affect the results, confirming the robustness of the method. In conclusion, the developed RP-HPLC method was proven to be simple, rapid, precise, accurate, and cost-effective for the simultaneous estimation of Esomeprazole and Domperidone. It can be effectively applied for routine quality control, stability testing, and assay determination of pharmaceutical formulations containing these drugs.

III. RESULTS AND DISCUSSION

The developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical formulations was successfully optimized and validated according to the International Council for Harmonisation (ICH Q2 R1) guidelines. The objective was to establish a simple, accurate, and reproducible analytical method capable of determining both drugs simultaneously with high sensitivity and resolution. During method development, several trials were conducted by varying the mobile phase composition, pH, and detection wavelength to obtain sharp, symmetrical, and well-separated peaks for both analytes. Various combinations of methanol and phosphate buffer were studied in different ratios (50:50, 60:40, and 70:30 v/v) under isocratic conditions. The optimized chromatographic conditions were achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer and methanol (60:40 v/v), adjusted to pH 7.0 with orthophosphoric acid, and a flow rate of 1.0 mL/min. The detection wavelength was set at 285 nm, which provided optimum response for both drugs. Under these optimized conditions, Esomeprazole and Domperidone were well resolved with retention times of 3.42 minutes and 5.18 minutes, respectively. The chromatogram showed sharp, symmetrical peaks with good baseline separation, confirming the suitability of the method for routine analysis.

Figure 1: Optimized Chromatogram for Esomeprazole and Domperidone

The optimized chromatogram confirmed that both drugs were completely resolved with excellent peak symmetry and without interference from formulation excipients. The resolution (Rs) between the two peaks was 2.8, indicating sufficient separation, while the theoretical plates for Esomeprazole and Domperidone were 5890 and 6215, respectively, which demonstrated excellent column efficiency. The tailing factors were 1.09 and 1.10, within acceptable limits (⩽2.0), confirming symmetrical peak shapes. The %RSD of peak area for six replicate injections was found to be 0.54%, satisfying the precision criteria for system suitability.

The system suitability results confirmed that the developed method was precise, efficient, and suitable for the analysis of Esomeprazole and Domperidone.

Linearity and Calibration Curve: The linearity of the method was evaluated by preparing a series of standard solutions within the concentration range of 5–25 µg/mL for Esomeprazole and 10–50 µg/mL for Domperidone. The calibration curves were plotted between concentration and peak area, and both drugs exhibited excellent linearity with correlation coefficients (R^2) greater than 0.999, confirming the proportional relationship between concentration and response. The regression equations were found to be y = 41235x + 15327 for Esomeprazole and y = 53214x + 17294 for Domperidone.

Accuracy: Accuracy of the method was evaluated through recovery studies conducted at three levels: 50%, 100%, and 150% of the target concentrations. The percentage recoveries for Esomeprazole and Domperidone ranged between **98.4% and 101.6%**, indicating that the method is highly accurate and free from interference by excipients in the formulation.

Precision: Precision studies, including intra-day and inter-day variations, were performed to assess the repeatability and intermediate precision of the method. The %RSD values for peak areas were found to be less than **2.0%** for both drugs, demonstrating the excellent precision and reproducibility of the method.

LOD and LOQ: The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The LOD values were 0.14 µg/mL for Esomeprazole and 0.45 µg/mL for Domperidone, while the LOQ values were 0.42 µg/mL and 1.35 µg/mL, respectively. These low values indicate the high sensitivity of the method for detecting and quantifying both analytes.

Robustness: The robustness of the method was tested by introducing small, deliberate variations in method parameters such as flow rate (±0.1 mL/min), pH (±0.2), and detection wavelength (±2 nm). These changes did not significantly affect the chromatographic performance, as the retention times and peak areas remained consistent with %RSD values below 2.0%, confirming that the method is robust.

Discussion: The developed RP-HPLC method proved to be simple, rapid, and reliable for the simultaneous determination of Esomeprazole and Domperidone. The results demonstrated that the method is accurate, precise, and sensitive, with excellent resolution and reproducibility. All validation parameters complied with ICH guidelines, confirming that the method is suitable for the routine quality control, assay, and stability testing of pharmaceutical formulations containing Esomeprazole and Domperidone. The sharp and symmetrical peaks with distinct retention times reflect the efficiency of the optimized chromatographic conditions. Overall, this

method offers a robust analytical tool for simultaneous estimation in combined dosage forms, ensuring consistent pharmaceutical quality and regulatory compliance.

IV. CONCLUSION

The present research successfully developed and validated a simple, precise, accurate, and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical dosage forms. The primary objective of the study was to establish an analytical method that is reliable, cost-effective, and suitable for routine quality control analysis, ensuring the quality and efficacy of fixed-dose combination formulations. The chromatographic conditions were carefully optimized to achieve maximum separation, peak symmetry, and reproducibility, while minimizing analysis time and solvent consumption. The final optimized method employed a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer and methanol (60:40 v/v) adjusted to pH 7.0 with orthophosphoric acid, at a flow rate of 1.0 mL/min, and detection at 285 nm. Under these conditions, sharp, symmetrical, and well-resolved peaks were obtained with retention times of 3.42 minutes for Esomeprazole and 5.18 minutes for Domperidone, confirming the suitability of the chromatographic system. The developed method was validated according to the ICH Q2 (R1) guidelines, and all parameters met the specified acceptance criteria. The results demonstrated excellent linearity, with correlation coefficients (R^2) greater than 0.999, indicating a strong relationship between peak area and concentration across the selected range. Accuracy was confirmed by recovery studies, where the mean recovery percentages for both drugs were found within 98–102%, proving the method's reliability and the absence of interference from excipients. The precision results, both intra-day and inter-day, showed %RSD values below 2.0%, confirming repeatability and reproducibility. The low values of LOD and LOQ indicated the method's high sensitivity and ability to detect and quantify trace amounts of both analytes. Furthermore, robustness and ruggedness studies demonstrated that minor changes in analytical conditions such as flow rate, pH, or detection wavelength did not significantly affect the results, confirming the stability and reliability of the method. Developed RP-HPLC method proved to be efficient, accurate, and reproducible for the simultaneous quantification of Esomeprazole and Domperidone. Its simplicity, reduced run time, and high sensitivity make it ideal for routine quality control analysis, assay determination, and stability studies in pharmaceutical industries. The successful validation of this method ensures compliance with regulatory standards and provides a dependable analytical tool for maintaining consistency and quality in combined dosage formulations containing Esomeprazole and Domperidone.

V. REFERENCES

1. Lacy C, Armstrong LL, Lipsy RJ. Drug information handbook 1993. Lexi-comp; 1993.
2. Al-Worafi YM. Quality indicators for medications safety. InDrug safety in developing countries 2020 Jan 1 (pp. 229-242).
3. Academic Press.
4. Hansen S, Hansen SH, Pedersen-Bjergaard S, Rasmussen K. Introduction to pharmaceutical chemical analysis. John Wiley & Sons; 2011 Dec 12.
5. Christian GD, Dasgupta PK, Schug KA. Analytical chemistry. John Wiley & Sons; 2013 Oct 7.
6. Lopez-Avila V, Hill HH. Field analytical chemistry. Analytical Chemistry. 1997 Jun 15;69(12):289-306.
7. Smith I, editor. Chromatography. Elsevier; 2013 Oct 22.
8. Liu Y, Vailaya A. Normal-Phase HPLC. HPLC for Pharmaceutical Scientists. 2007 Jan 22:241-61.
9. LoBrutto R, Kazakevich Y. Reversed-Phase HPLC. HPLC for Pharmaceutical Scientists. 2007 Jan 22:139-239.
10. Yamamoto S, Nomura M, Sano Y. Adsorption chromatography of proteins: Determination of optimum conditions. AIChE Journal. 1987 Sep;33(9):1426-34.
11. Jungbauer A, Hahn R. Ion-exchange chromatography. Methods in enzymology. 2009 Jan 1;463:349-71.
12. Cecchi T. Ion-pair chromatography and related techniques. CRC Press; 2009 Oct 15.
13. Barth HG, Jackson C, Boyes BE. Size exclusion chromatography. Analytical chemistry. 1994 Jun 1;66(12):595-620.
14. Hage DS. Affinity chromatography: a review of clinical applications. Clinical chemistry. 1999 May 1;45(5):593-615.
15. Beesley TE, Scott RP. Chiral chromatography. John Wiley & Sons; 1999 Jan 26.