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NEW VALIDATED ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND ETRUGLIFLOZIN IN COMBINED PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC

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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 Metformin and Etrugliflozine in combined 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 BDS C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 45:55 v/v, at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 224 nm, where both drugs exhibited significant absorbance. Under the optimized chromatographic conditions, sharp and symmetrical peaks were obtained with retention times of 2.186 minutes for Metformin and 2.908 minutes for Etrugliflozine, 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 50–300 µg/mL for Metformin and 0.75–4.5 µg/mL for Etrugliflozine, 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 simultaneous analysis of Metformin and Etrugliflozine in combined dosage forms.

Keywords: *Metformin, Etrugliflozin, Method Validation.*

INTRODUCTION

Metformin is a biguanide antihyperglycemic agent and first-line pharmacotherapy used in the management of type II diabetes. Metformin is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. It is commonly described as an "insulin sensitizer", leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. Another well-known benefit of this drug is modest weight loss, making it an effective choice for obese patients type II diabetes. Metformin was first approved in Canada in 1972, and received subsequent FDA approval in the US in 1995 [1].

Ertugliflozin is an oral antidiabetic medication belonging to the sodium-glucose cotransporter-2 (SGLT2) inhibitor class, used to improve glycemic control in adults with type 2 diabetes mellitus, alongside diet and exercise. It works by blocking glucose reabsorption in the kidneys, thereby increasing urinary glucose excretion and lowering blood glucose levels. Ertugliflozin may also promote modest weight loss and reduce blood pressure. Common adverse effects include increased urination, genital fungal infections, urinary tract infections, and dehydration, while serious but less common risks include diabetic ketoacidosis, acute kidney injury, hypotension, and rare cases of Fournier's gangrene. It is not recommended for patients with type 1 diabetes or severe renal impairment, and kidney function should be assessed before and during treatment. Patients should maintain adequate hydration and be monitored for signs of infection, ketoacidosis, and changes in renal function during therapy. [2]

Detailed survey of literature revealed that analytical methods are reported by using RP-HPLCfor the estimation of Metformin and Ertugliflozin alone as well as in combination with other drugs using pharmaceutical formulations [3-8].
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 acetonitrile, 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) [9] 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 Metformin and Ertugliflozin 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.

MATERIALS AND METHODS

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 Metformin and Ertugliflozin 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. Metformin and Ertugliflozin pure drug samples were obtained as gift samples from a reputed pharmaceutical manufacturer. Acetonitrile (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 BDS C18 column (150 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. During method development, different mobile phase compositions, 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 acetonitrile in the ratio of 45:55 v/v, and a flow rate of 1.0 mL/min. The detection wavelength was set at 224 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 2.186 minutes for Metformin and 2.908 minutes for Etrugliflozin. Optimized Chromatographic Conditions were shown in Table 01.

Table 01: Optimized Chromatographic Conditions

Parameter	Optimized Condition
Column	BDS C18 (150 × 4.6 mm, 5 µm)
Mobile Phase	Phosphate Buffer: Acetonitrile [45:55 v/v]
Flow Rate	1.0 mL/min
Detection Wavelength	224 nm
Injection Volume	10 µL
Run Time	10 minutes
Column Temperature	Ambient
Mode	Isocratic Elution

Preparation of Standard Solutions

Accurately Weighed 1.5 Mg of Ertugliflozin 100mg Of Metformin And Transferred To 50ml Volumetric Flask. 3/4 th Of Diluents Was Added To The Flask And Sonicated For 10 Minutes. Flask Was Made Up With Diluents and Labeled As Standard Stock Solution. (2000µg/ml of Metformin and 30µg/ml Ertugliflozin)

Preparation of Sample Solution

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 500 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (5000µg/ml of Metformin and 75µg/ml of Ertugliflozin)

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 02).

Table 02: System Suitability Parameters

Parameter	Acceptance Criteria	Observation
Retention Time (min)	Consistent	2.186 (Metformin), 2.908 (Etrugliflozin)
Theoretical Plates (N)	≥ 2000	2822 (Metformin), 3571 (Etrugliflozin)
Resolution (Rs)	≥ 2.0	4.0
Tailing Factor	≤ 2.0	1.20 (Metformin), 1.15 (Etrugliflozin)
%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 Metformin and Ertugliflozin. It can be effectively applied for routine quality control, stability testing, and assay determination of pharmaceutical formulations containing these drugs.

RESULTS AND DISCUSSION

The developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Metformin and Ertugliflozin in 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, and detection wavelength to obtain sharp, symmetrical, and well-separated peaks for both analytes. Various combinations of phosphate buffer and acetonitrile were studied in different ratios (45:55, 60:40, and 70:30 v/v) under isocratic conditions. The optimized chromatographic conditions were achieved using a BDS C18 column (150 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer and methanol (45:55 v/v), and a flow rate of 1.0 mL/min. The detection wavelength was set at 224 nm, which provided optimum response for both drugs. Under these optimized conditions, Metformin and Etrugliflozin were well resolved with retention times of 2.186 minutes and 2.908 minutes, respectively. The chromatogram showed sharp, symmetrical peaks with good baseline separation, confirming the suitability of the method for routine analysis (Figure 01).

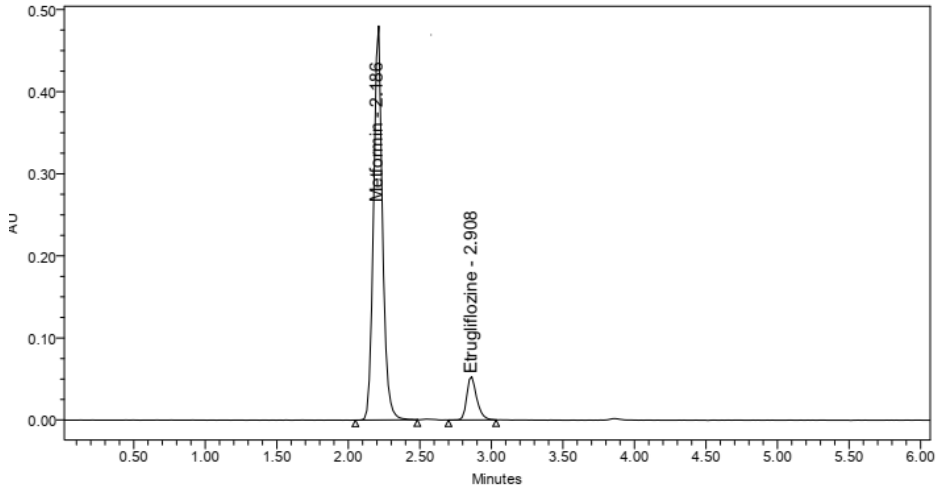


Figure 01: Optimized Chromatogram for Metformin and Etrugliflozin

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 4.0, indicating sufficient separation, while the theoretical plates for Metformin and Etrugliflozin were 2822 and 3571, respectively, which demonstrated excellent column efficiency. The tailing factors were 1.20 and 1.15, 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 simultaneous analysis of Metformin and Etrugliflozin.

Linearity and Calibration Curve: The linearity of the method was evaluated by preparing a series of standard solutions within the concentration range of 50–300 µg/mL for Metformin and 0.75–4.5 µg/mL for Etrugliflozin. 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 = 20438x + 92764 for Metformin and y = 25768x + 1835.7 for Ertugliflozin. The results are shown in Table 03 and figure. 02-09.

Table 03: Linearity table for Metformin and Ertugliflozin

Metformin		Ertugliflozin	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
0	0	0	0
50	1165981	0.75	22845
100	2177701	1.50	40609
150	3208800	2.25	60672
200	4165394	3.00	78744
250	5216094	3.75	98788
300	6174942	4.50	117033

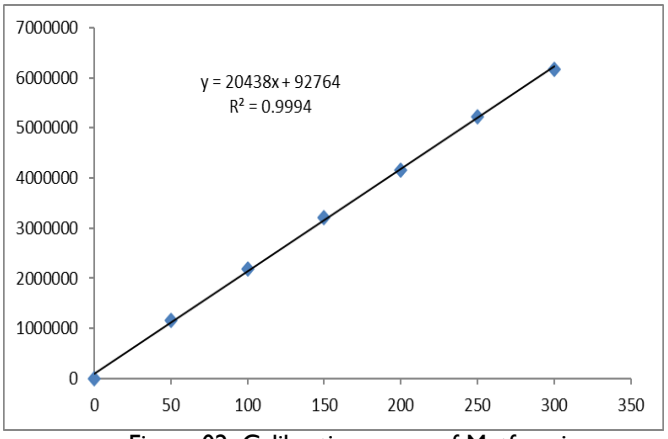


Figure 02: Calibration curve of Metformin

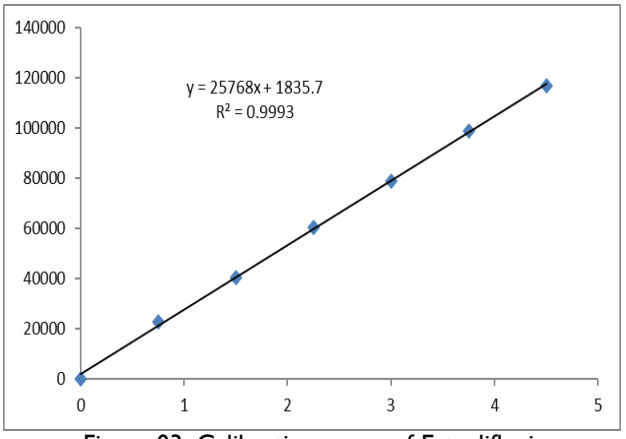


Figure 03: Calibration curve of Ertugliflozin

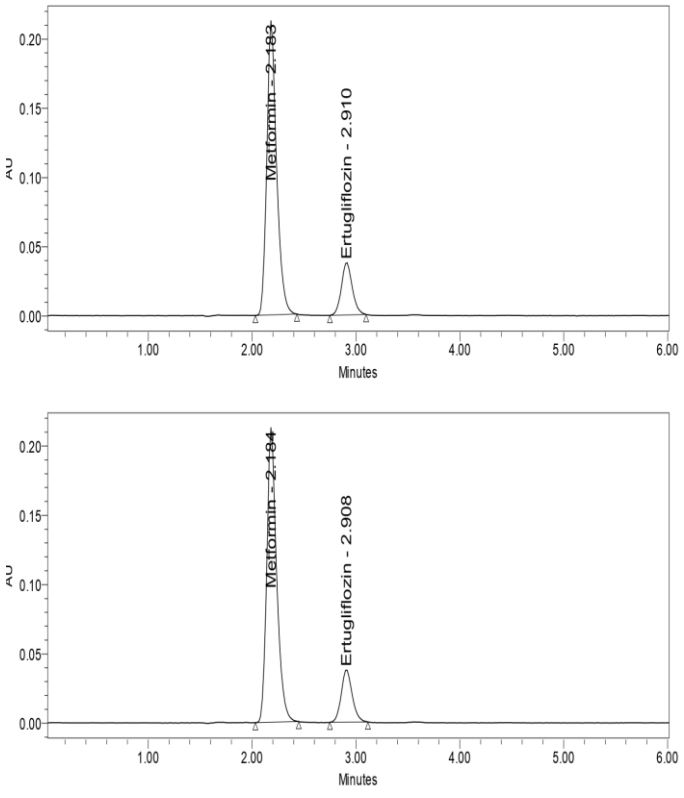


Figure 04: Linearity 25% Chromatogram of Metformin and Ertugliflozin of

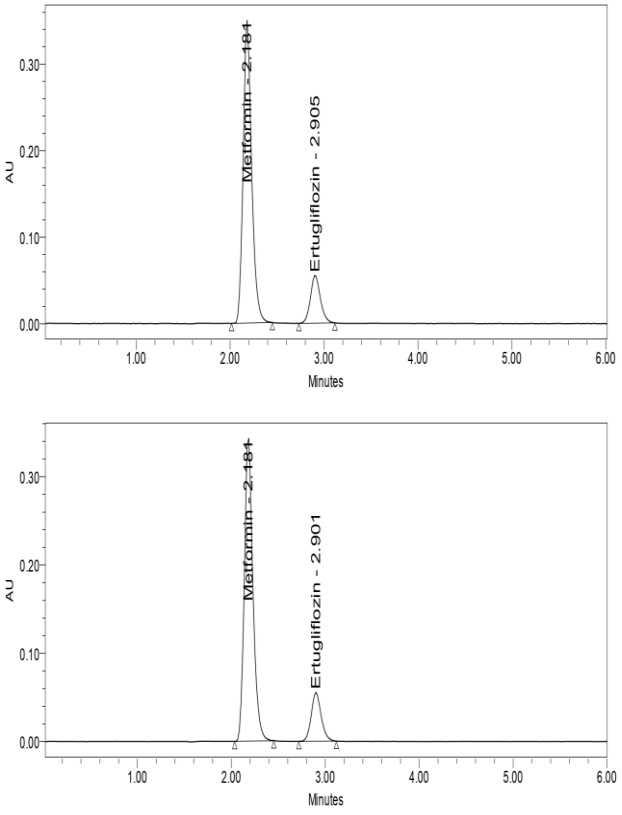


Figure 05: Linearity 50% Chromatogram Metformin and Ertugliflozin

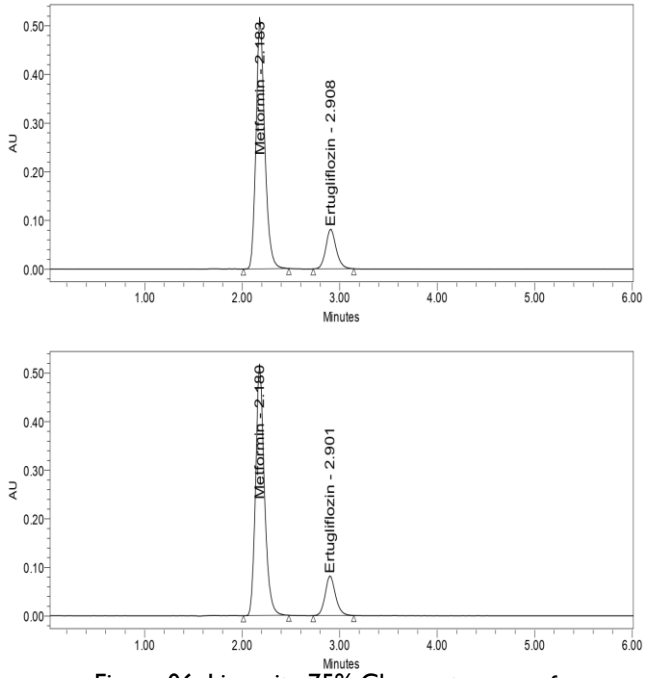
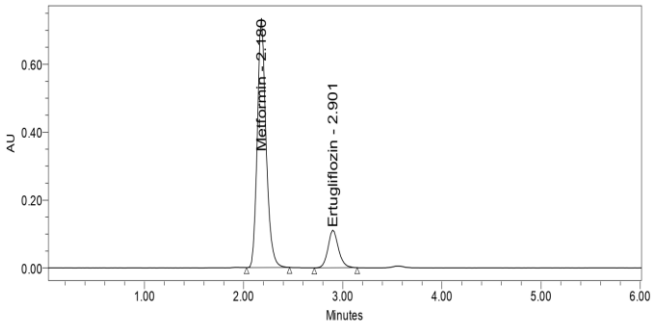


Figure 06: Linearity 75% Chromatogram of Metformin and Ertugliflozin



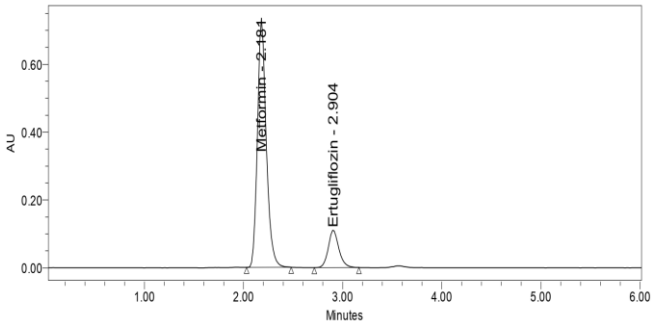
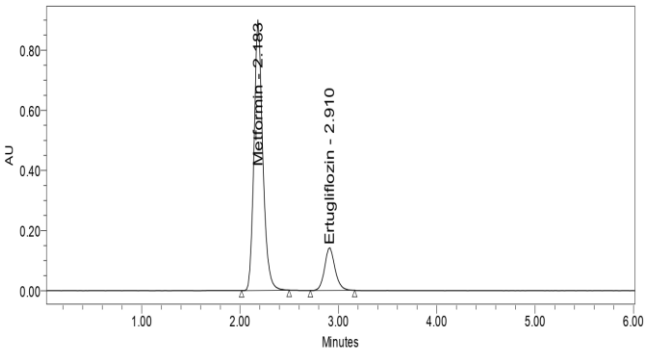


Figure 07: Linearity 100% Chromatogram of Metformin and Ertugliflozin



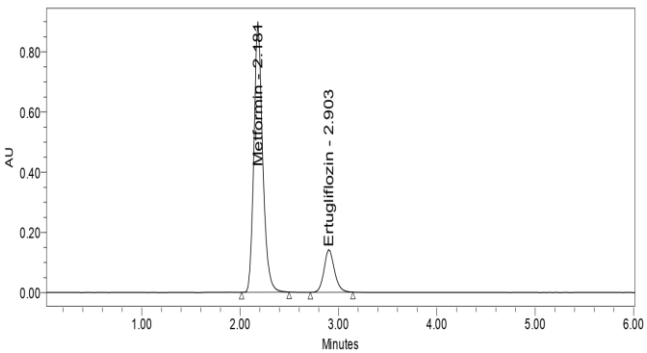
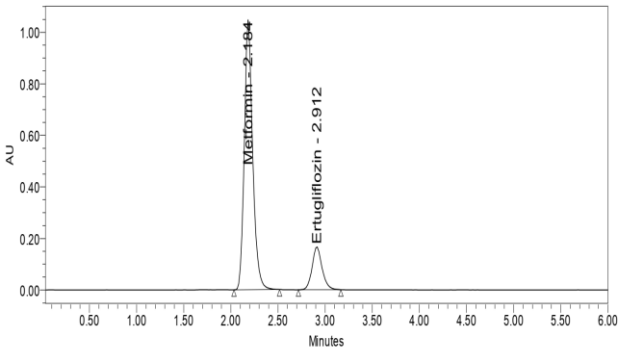


Figure 08: Linearity 125% Chromatogram of Metformin and Ertugliflozin



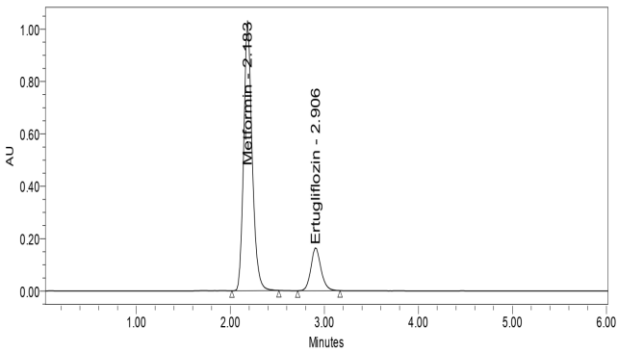


Figure 09: Linearity 150% Chromatogram of Metformin and Ertugliflozin

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 Metformin and for Ertugliflozin ranged between **98.86% and 100.25%**, 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 0.39 µg/mL for

Metformin and 0.02 µg/mL for Ertugliflozin, while the LOQ values were 1.17 µg/mL and 0.06 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, mobile phase composition, and temperature. 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 Metformin and Ertugliflozin. 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 Metformin and Ertugliflozin. 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.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Ertugliflozin in Tablet dosage form. Metformin and Ertugliflozin were eluted at 2.186 min and 2.908 min respectively %RSD of the Metformin and Ertugliflozin were and found to be 0.1 and 0.4 respectively. %Recovery was obtained as 99.60% and 99.48% for Metformin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Ertugliflozin were 0.39, 1.17 and 0.02, 0.06 respectively. Regression equation of Metformin is y = 20438x + 92764., and y = 25768x + 1835.7 of Ertugliflozin . Retention times were 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.

AUTHOR CONTRIBUTIONS

All authors are contributed equally.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

No external funding was received for this study.

INFORM CONSENT AND ETHICAL STATEMNET

Not Applicable.

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