RESEARCH PAPER

Bio-Analytical Method Development and Validation for Estimation of Ertugliflozin by RP-HPLC in Dosage Form.

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ABSTRACT

Background: Ertugliflozin is an antidiabetic medication that belongs to the class of Sodium-Glucose Cotransporter-2 inhibitors. Ertugliflozin is mainly employed to reduce elevated blood glucose levels. **Objective:** The aim of the current study is to establish a reliable, precise, and validated bioanalytical method for quantifying Ertugliflozin in human plasma using reverse-phase high-performance liquid chromatography**. Materials & Methods:** Chromatographic analysis was performed at 239 nm on a Thermo C18 column (150 mm × 4.6 mm, 5 µm) using an optimized method. The flow rate was 0.9 mL/min, using a methanol:0.1 % GAA (75:25% v/v) mixture at pH 3, with 1 mL of plasma as the mobile phase. The method underwent validation for its linearity, accuracy, precision, and robustness, following the standards outlined in the ICH guidelines. The validated method was then used to determine the marketed formulation in plasma. **Results:** Among six trials, the optimized method showed a sharp peak. Chromatographic parameters included a retention time of 3.872 minutes, 5931 theoretical plates, a tailing factor of 0.94, and a resolution of 2.5. A linear response was observed over the concentration range of 2 to 10 µg/ml, with a correlation coefficient of 0.9992, indicating excellent linearity. Recovery studies at three concentration levels showed 99.39% at 80%, 99.25% at 100%, and 99.58% at 120%, all with RSD less than 2%. This indicated good accuracy. LOD and LOQ were 2.1782 µg/mL and 0.7188 µg/mL, respectively. The estimation of EFG in the marketed formulation was 99.25%. **Conclusion:** The developed RP-HPLC method was validated and is suitable for routine quantification of drugs in biological samples

Keywords: Ertugliflozin, RP-HPLC, Bioanalytical Method Validation, Human Plasma.

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Conflict of interest: Nil

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder identified by raised blood glucose levels due to insulin resistance and impaired insulin secretion (Galicia-Garcia U *et al*., 2020; Liu D *et al*., 2021). With its increasing global prevalence, there is a continuous need for effective therapeutic agents and precise analytical techniques to monitor their pharmacokinetics. Among the newer antidiabetic agents, Sodium-Glucose Cotransporter-2 (SGLT2) inhibitors have gained significant clinical importance due to their unique mechanism of action that promotes renal glucose excretion independent of insulin (Sen T *et al*., 2020; Scheen AJ, 2020; Salvatore T *et al*., 2022).

Ertugliflozin (EFG) is a specific inhibitor of SGLT2, used to effectively lower blood glucose levels in individuals with T2DM. Its use has been approved in several countries, both as monotherapy and in combination with other antidiabetic drugs (Fediuk DJ *et al*., 2020; Siddique I *et al*., 2023). To support pharmacokinetic studies, therapeutic drug monitoring, and bioequivalence evaluations, reliable and validated

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analytical methods for the quantification of EFG in biological matrices are essential.

High-performance liquid chromatography (HPLC) remains one of the most widely used techniques in bioanalytical research due to its robustness, sensitivity, and accuracy (Pushpa LE and Sailaja B, 2014; Deshmukh S *et al*., 2019). However, there is a continual demand to refine and validate new methods that are simpler, more precise, and capable of detecting low concentrations of drugs in complex biological matrices such as human plasma (Tuzimski T *et al*., 2020; Moein MM *et al*., 2017).

A literature review reveals that various analytical methods have been documented for the validation estimation of EFG, either independently or in combination with other drugs (Murugesan A *et al*., 2021; Mankar SD *et al*., 2023). The simultaneous determination of EFG and other drugs in plasma has been achieved using the LC-MS method (Rao PV *et al*., 2021; Rao PV *et al.,* 2021; Kedar P *et al*., 2025). However, at present, there is no validated and quantification method of EFG in human plasma using RP-HPLC.

The present study aims to develop and validate a simple, accurate, and precise RP-HPLC method for estimating EFG in human plasma. The developed method was systematically optimized and validated following the International Council for Harmonization (ICH) guidelines to ensure its accuracy, reliability, and reproducibility. Upon validation, the method was successfully employed to estimate EFG in a commercially available formulation, highlighting its suitability for routine quality control and clinical research applications.

MATERIALS & METHODS

Chemicals and Reagents

The API sample of EFG was obtained from Swapnroop Drug and Pharmaceutical, Shendraban, Maharashtra. Tablets of EFG (Steglatro 15mg) were purchased from Vital Overseas Pvt Ltd. HPLC Grade methanol, Acetonitrile, Potassium Phosphate Buffer were procured from Merck Ltd., India. Human plasma samples were procured from RD Labs, Jalgaon.

Instrumentation

The analysis of EFG was performed using an Agilent Technologies HPLC Gradient System equipped with an auto-injector, diode-array detector (DAD), and UV730D absorbance detector, operated via Chemstation 10.1 software. A reverse-phase C18 column (Agilent, 5 µm, 4.6 mm × 250 mm) was used for chromatographic studies. Additional instruments employed during method development included a Thermo C18 column (150 mm × 4.6 mm, 5 µm), a UV-spectrophotometer (Analytical Technology), a VSI pH meter (Model VSI 1-B), a precision balance from A&D Company, Japan, and a sonicator from ENERTECH Electronic Instruments.

Chromatographic Conditions

Chromatographic separation was done by a C18 column using a binary gradient solvent system. Wavelength used for EGF detection was 239 nm. Ambient temperature conditions were used throughout the experiment. The degassing of the mobile was done for 15 minutes. The 20 µL injection volume with a flow rate of 0.9 mL/min and solvent, methanol: 0.1 % GAA 75:25 % v/v was used after optimizing conditions.

Standard Stock Solution

An accurately weighed 5 mg of EFG was transferred into a 25 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes. The volume was then adjusted to 25 mL with methanol to obtain a working stock solution with a concentration of 200 µg/mL.

Preparation of Blank Plasma

Collected blood samples were centrifuged at 5000 rpm for 1 hr. to separate the plasma from the blood. Acetonitrile (4 mL) was added to 1 mL of the collected plasma as an extraction solvent. The separated plasma was then used for further HPLC analysis.

Preparation of Calibration curve standard:

The above standard stock solution (200 µg/ml + 1 ml plasma) of EGF was diluted with the mobile phase to yield five calibration curve standards with concentrations of 2, 4, 6, 8, and 10 µg/ml. The mobile phase was equilibrated with the stationary phase until a stable baseline was achieved. EFG samples were then injected, and chromatographic peaks were monitored at a wavelength of 239 nm.

Validation of Parameters

Linearity

The linearity of the developed method was assessed by plotting a calibration curve using plasma spiked with a known quantity of EGF, ranging from 2 to 10 µg/mL. From the calibration curve regression equation was found of peak area versus EGF concentration. The correlation coefficient should not be less than 0.99.

Precision and Accuracy

Spiking EGF in human plasma, precision and accuracy studies were performed. For assessment of intra-day and inter-day precision studies, sample solutions of EFG (4,

6, and 8 µg/mL) were examined on the same day at three concentration levels: low, medium, and high. Accuracy was determined through recovery studies conducted at three concentration levels 80%, 100%, and 120%. Known amounts of the standard drug at these levels were added to previously analyzed plasma samples. The percentage recovery and %RSD were then calculated to assess the method's accuracy.

LOD and LOQ

According to ICH Q2(R1) guidelines, the Limit of Detection (LOD) and Limit of Quantification (LOQ) based on signal-to-noise (S/N) ratios, using an S/N ratio of 3:1 for LOD and 10:1 for LOQ.

Robustness:

Robustness was assessed by introducing deliberate, minor variations to the chromatographic conditions to evaluate the method's reliability under varied analytical settings. The mobile phase composition was changed in the proportion of methanol: 0.1 % GAA (74:26 & 76:24 % v/v). The change in detection wavelength and the effect of the results was examined.

Analysis of Marketed Formulation

To estimate the EFG content in marketed tablets (label claim: 5 mg per tablet), 20 tablets were accurately weighed to determine the average tablet weight. The tablets were finely powdered, and a quantity equivalent to 15 mg of EFG was weighed. The drug was extracted using 10 mL of methanol along with the addition of human plasma. To facilitate complete extraction, the mixture was sonicated for 15 minutes. The supernatant (0.4 mL) was then diluted to 10 mL with the mobile phase. The prepared solution was injected into the HPLC system, and the chromatographic peak area corresponding to EFG was recorded. A calibration curve was constructed from the peak areas of standard solutions, and the regression equation derived from this curve was used to calculate the amount of EFG present in the tablet sample based on the sample's peak area.

RESULTS

Melting point: The procured reference standard of EFG was found to melt in the range of 152-156 °C.

The λ_{max} in the UV spectrum of a 10 µg/mL solution of EFG was found to be 239 nm.

System Suitability Test

Trial run 6 was selected based on system suitability parameters including resolution, tailing factor, retention time, and the number of theoretical plates, all of which met the predefined acceptance criteria. These results confirmed the reproducibility and suitability of the chromatographic system for the analysis of EFG.

Validation Parameters

Calibration Curve & Linearity

The calibration data for EFG (Table 3 and Figure 4) demonstrated a linear correlation between peak area and concentration over the range of 2–10 µg/mL, as confirmed by linear regression analysis. The respective linear equation for EFG was y = 35.90x + 0.483, where x is the concentration and y is the area of the peak. The correlation coefficient was 0.9992. The calibration curve of EFG is depicted in (Figure 3).

Accuracy

Recovery studies were conducted to confirm the accuracy of the developed method. Known concentrations of the standard drug at 80%, 100%, and 120% levels were spiked into the pre-analyzed tablet solution, and the percentage recovery was evaluated (Figure 5, Table 4).

Repeatability

Repeatability studies on RP-HPLC for EFG were found to be 99.5 % (Table 5). The %RSD was less than 2%, indicating a high percentage range of 98% to 102%.

Precision

The method was established by analyzing various replicate standards of EFG using biological fluid. All the solutions were analyzed three times to record any intra-day & inter-day variation in the results that were concluded. The results obtained for intraday & interday are shown in (Figure 6 & Table 6) respectively.

Robustness

For robustness studies small but deliberate variations in the optimized method parameters were made (Table 7, Figure 7) and found in limits.

LOD and LOQ

The detection limit for EFG was found to be 2.1782 µg/mL, while the quantification limit was found to be 0.7188 µg/mL.

Analysis of Tablet Formulation

Analysis of the marketed formulation of EFG showed 99.25 % (Table 8, Figure 8). The label claim was 99-101%.

DISCUSSION

The present study successfully developed and validated a simple, rapid, and precise RP-HPLC method for the quantification of EFG in human plasma. The method was optimized using a mobile phase of methanol: 0.1% glacial acetic acid (75:25 v/v) and demonstrated clear chromatographic separation with a retention time of 3.872 minutes. The optimized chromatographic conditions yielded excellent system suitability parameters, including a tailing factor of 0.94 and a theoretical plate count of 5931, confirming the efficiency and sharpness of the eluted peak. Linearity was seen in range of 2–10 µg/mL concentration, with a correlation coefficient of 0.9992, indicating a strong linear relationship between EFG concentration and peak area. The method displayed great precision and accuracy, with %RSD values below 2% across different concentrations, both in intra- and inter-day studies. The recovery rates across three concentration levels (80%, 100%, and 120%) were within the acceptable range of 99.25% to 99.58%, confirming the method's accuracy in detecting the analyte from spiked plasma samples. The method's robustness was validated by introducing small deliberate variations in chromatographic conditions, such as mobile phase ratio and detection wavelength. The resulting %RSD values remained below 1%, indicating that the method is highly reliable under varied conditions. Furthermore, the LOD and LOQ values (2.1782 µg/mL and 0.7188 µg/mL, respectively) demonstrate the method's sensitivity and suitability for detecting low concentrations in biological matrices.

The developed method was successfully applied to estimate EFG in plasma of a marketed tablet formulation, yielding a recovery of 99.25%. This confirms the method's suitability for routine investigations in biological sample.

CONCLUSION

A sensitive, accurate, and reproducible RP-HPLC method was successfully developed and validated for the estimation of EFG in human plasma. The method complies with ICH guidelines and exhibits excellent linearity, accuracy, precision, robustness, and sensitivity. Its successful application to the analysis of a marketed formulation further supports its utility in both clinical and quality control settings. This method can be reliably employed for routine bioanalytical studies, including therapeutic drug monitoring and pharmacokinetic evaluations of EFG.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study

Table 1: Chromatographic behavior of EFG mobile phase of various compositions.

Run	Mobile Phase	Flow rate	Remarks
1	Methanol: 0.1% GAA 80:20% v/v + PH-3 + 1 ml Plasma	0.7 ml/min	No sharp peak
2	Methanol: 0.1% GAA 80:20% v/v + PH-3 + 1 ml plasma	0.7 ml/min	No Sharp peak
3	Methanol: 0.1% GAA 75:25% v/v + PH-3 + 1 ml Plasma	0.7 ml/min	No Sharp peak
4	Methanol: 0.1% GAA 75:25% v/v + PH-3 + 1 ml Plasma	0.8 ml/min	No sharp peak
5	Methanol: 0.1% GAA 70:30 % v/v + PH-3 + 1 ml Plasma	0.9 ml/min	No sharp peak
6	Methanol: 0.1% GAA 75:25% v/v + PH-3 + 1 ml Plasma	0.9 ml/min	Sharp peak

Table 2: System Sutability Parameters

No.	RT [min]	Area[mV*s]	TP	TF	Resolution
1	3.872	70.80663	5931	0.94	2.5

Table 3: Calibration Curve & Linerarity of EFG (n=3)

Method	Conc µg/ml	Mean±SD	% RSD
RP-HPLC Method	2	70.42±0.54	0.76
	4	148.67±1.68	1.13
	6	212.13±0.53	0.25
	8	288.95±0.36	0.12
	10	359.32±0.42	0.12

Table 4: Results of Accuracy studies of EGF (*n*=3).

Level (%)	Amt. Taken (µg/mL)	Amt. Added (µg/mL)	RT [min]	Area[mV*s]	TP	TF	%Recovery Mean	% RSD
80%	2	1.6	3.898	128.76559	10017	0.98	99.39	0.09
100%	2	2	3.903	142.9457	5860	0.98	99.25	0.23
120%	2	2.4	3.902	157.18024	5857	0.98	99.58	0.06

Table 5: Results of Repeatability studies of EFG (*n*=3).

Replicate (6 µg/ml)	**Peak Area (mV*s)**	**Amount Found (µg/ml)**
1	211.866	5.98
2	211.836	5.97
3	211.789	5.96
	Mean	**5.97**
	SD	**0.01**
	%RSD	**0.17%**
	%Recovery	**99.5%**

Table 6: Result of Intraday and Interday Precision Studies of EFG *(n=3)*

Conc (µg/ml)	**Intraday Precision**		**Interday Precision**	
	Mean± SD	**%Amt Found**	**Mean± SD**	**%Amt Found**
4	141.53±1.00	99.22	143.12±1.38	99.33
6	212±0.04	99.62	214.06±0.33	99.15
8	286.70±1.75	99.66	284.79±2.15	99.54

Table 7: Result of Robustness of EFG *(n=3)*

Parameters		**RT [min]**	**TP**	**TF**	**Mean ±SD**	**%RSD**
Mobile Phase Ratio (%v/v) MeOH: 0.1 % GAA	74:26	4.689	6365	1.02	174.51±1.07	0.61
	76:24	4.185	6210	0.98	171.69±1.13	0.66
Wavelength change	238 nm	3.923	5922	0.99	171.9±1.58	0.92
	240 nm	3.923	5922	0.99	171.21±0.89	0.69

Table 8: Result of EFG Marketed Formulation

RT [min]	**Area[mV*s]**	**TP**	**TF**	**% Found**	**SD**	**%RSD**
3.904	142.56436	5995	1.00	99.25	0.013	0.320

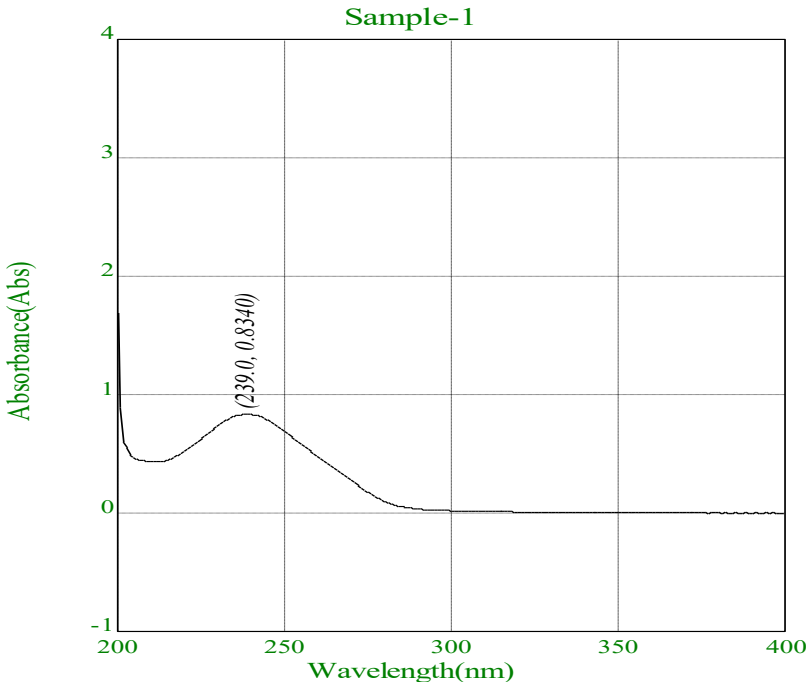
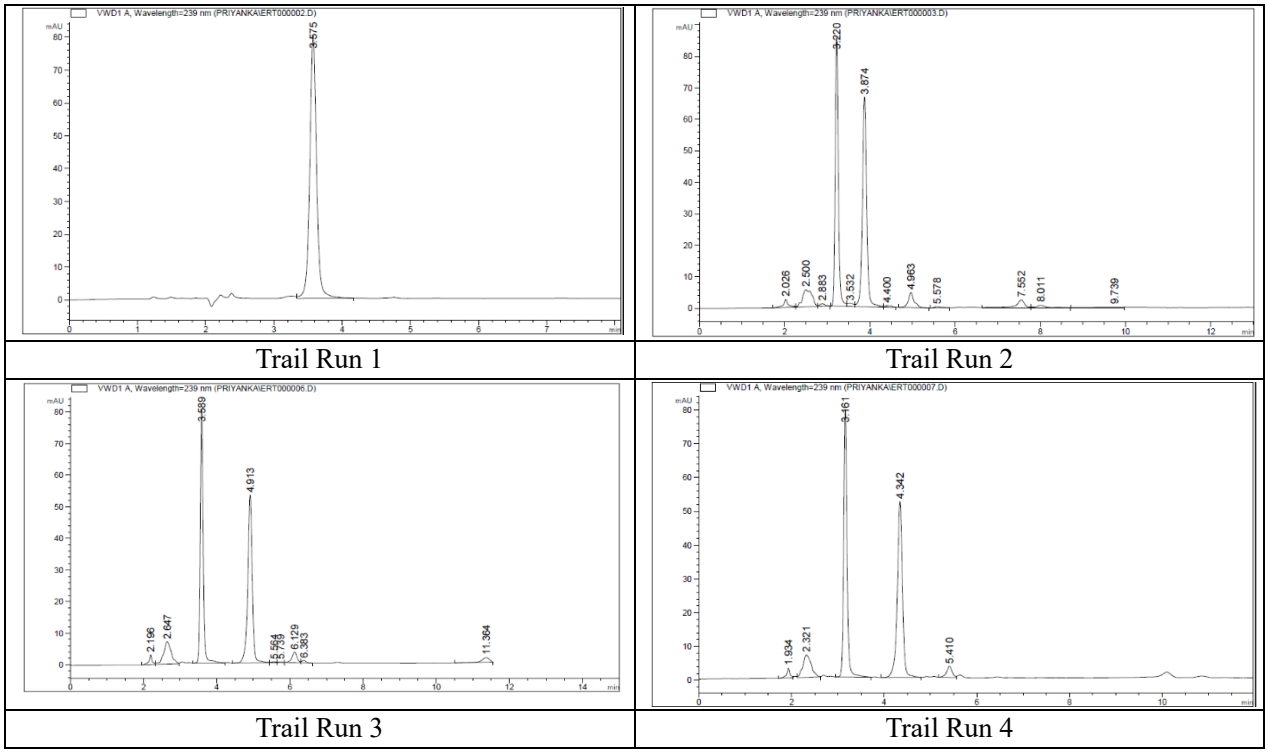
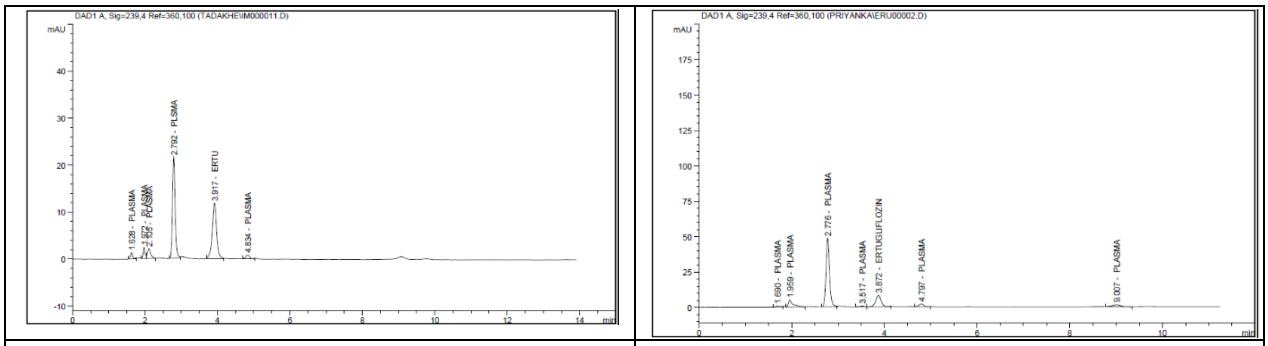


Figure 1: UV Spectrum of Ertugliflozin



Trail Run 1	Trail Run 2
Trail Run 3	Trail Run 4



Trail Run 5	Trail Run 6

Figure 2: Trail Chromatograms of EFG in different chromatographic conditions.

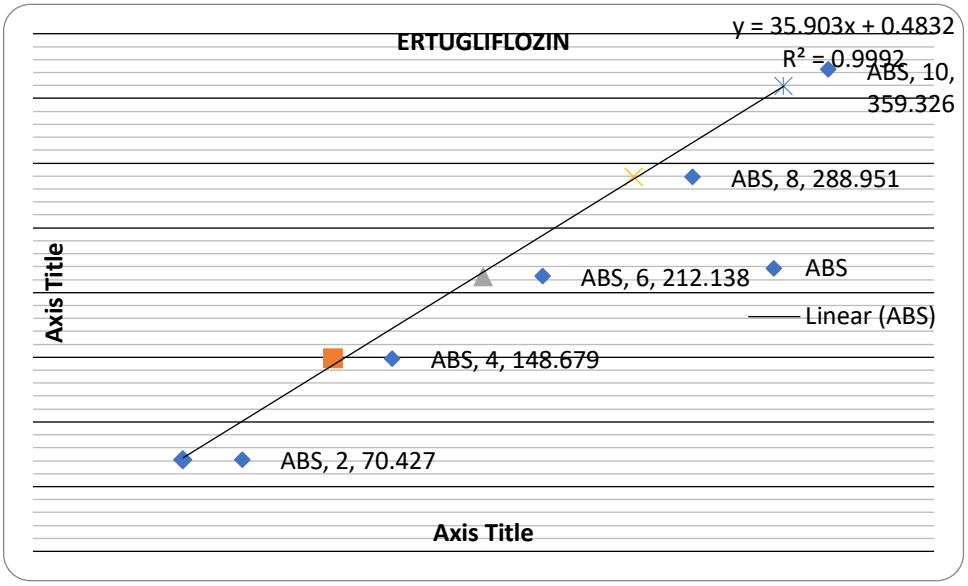
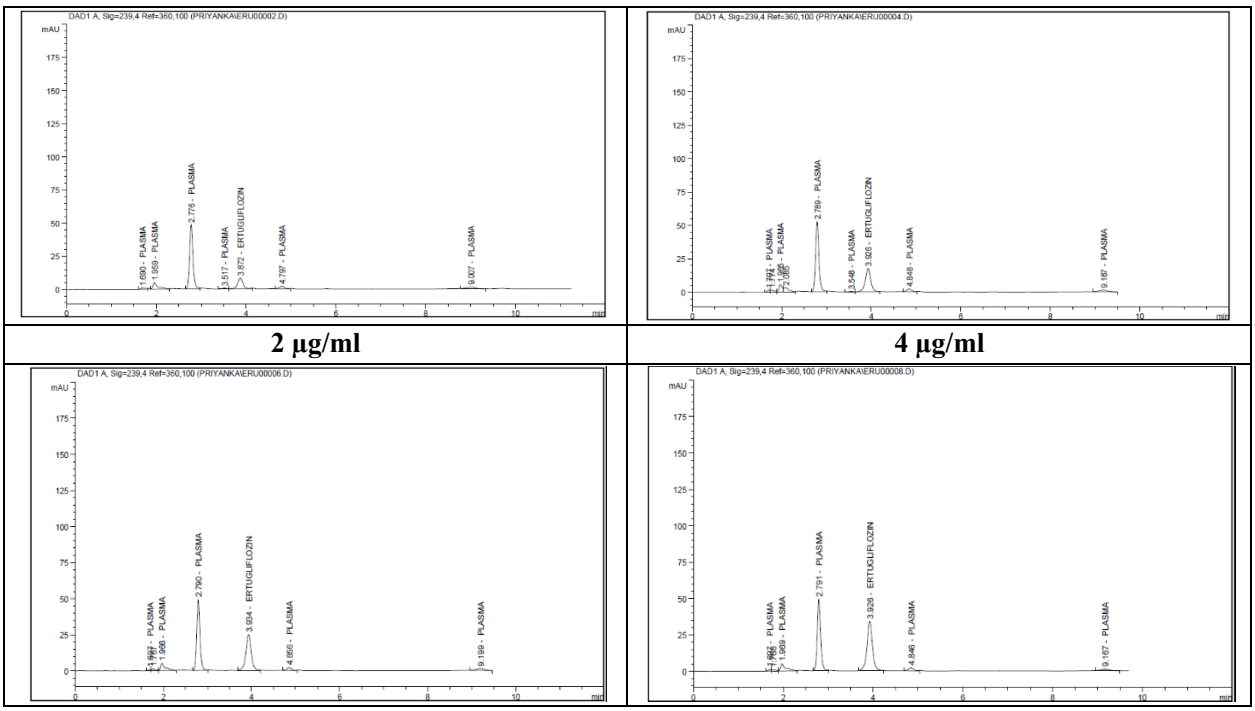


Figure 3: Calibration curve of Ertugliflozin



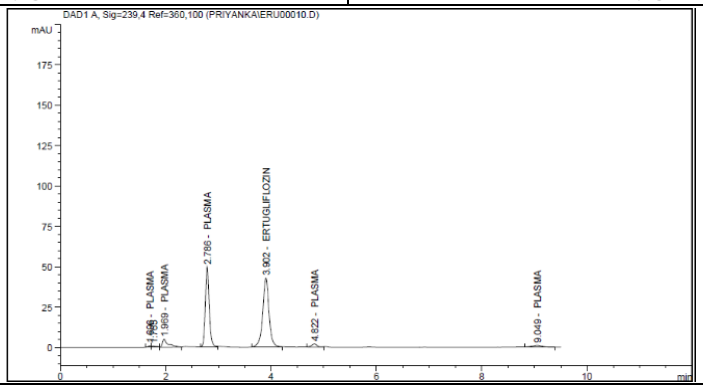
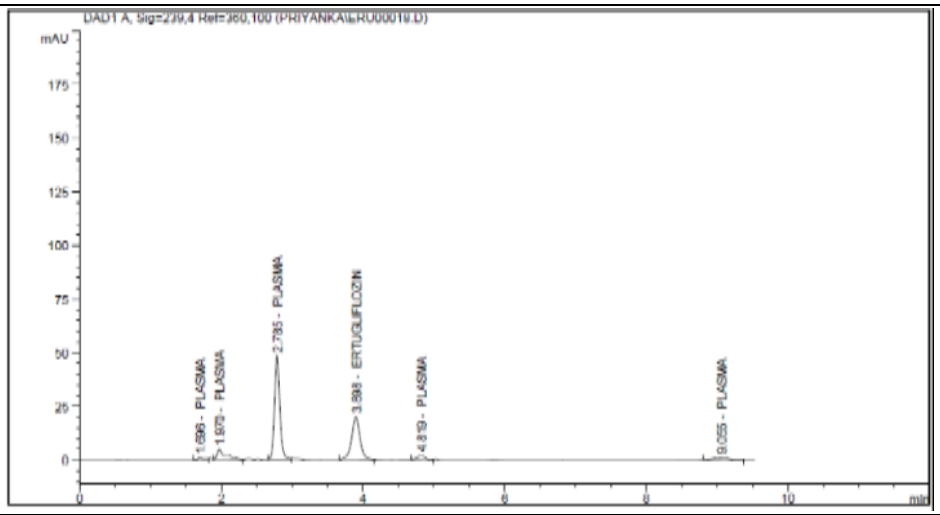
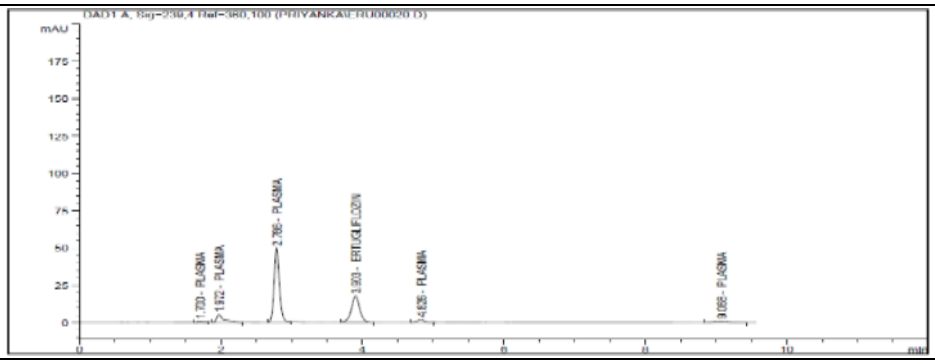
6 µg/ml	8 µg/ml
	
10 µg/ml	

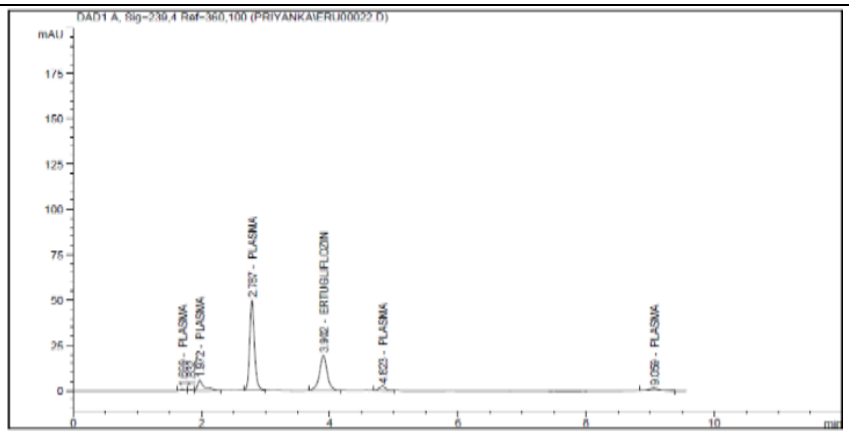
Figure 4: Lineraity Chromatograms



Chromatogram of Accuracy 80%

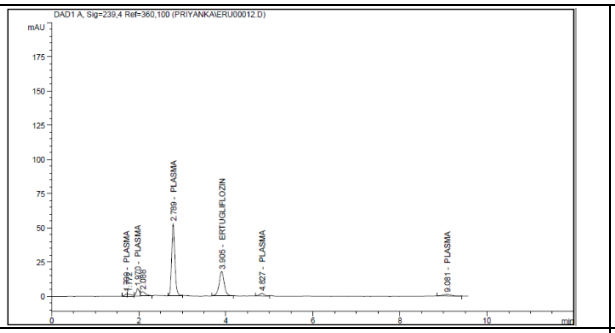


Chromatogram of Accuracy 100 %

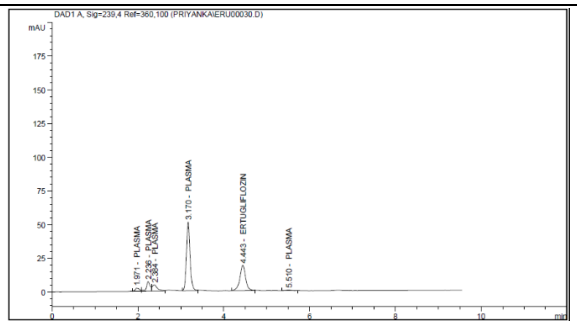


Chromatogram of Accuracy 120 %

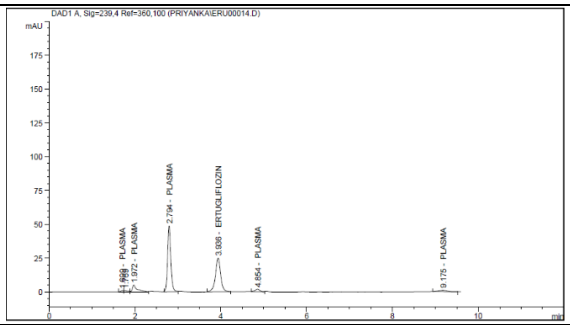
Figure 5: Chromatogram of Accuracy



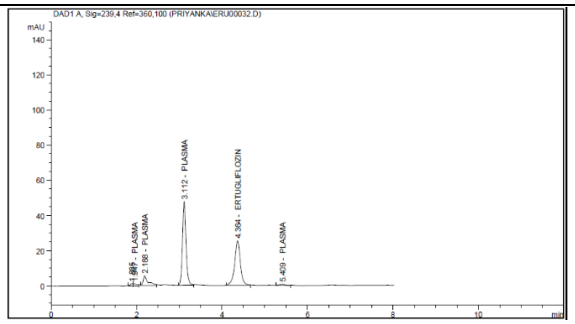
Intraday 4 µg/ml Chromatogram



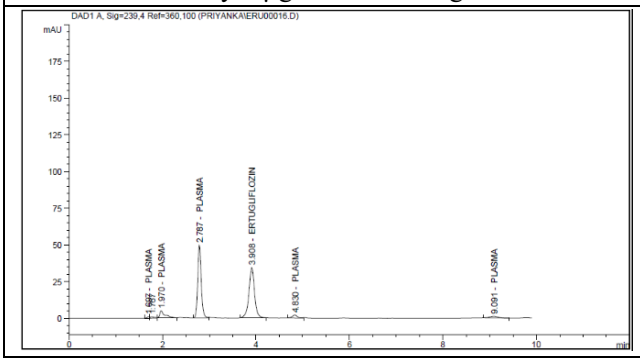
Interday 4 µg/ml Chromatogram



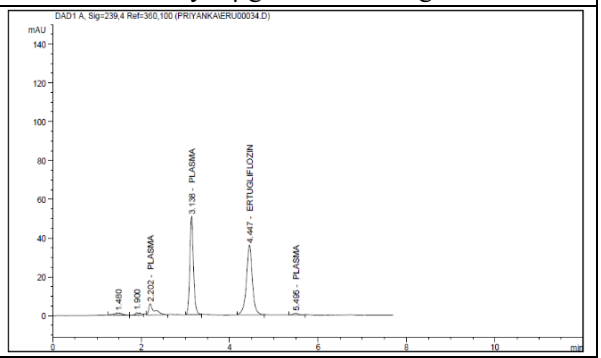
Intraday 6 µg/ml Chromatogram



Interday 6 µg/ml Chromatogram



Intraday 8 µg/ml Chromatogram



Interday 8 µg/ml Chromatogram

Figure 6: Chromatogram of Intraday & Interday

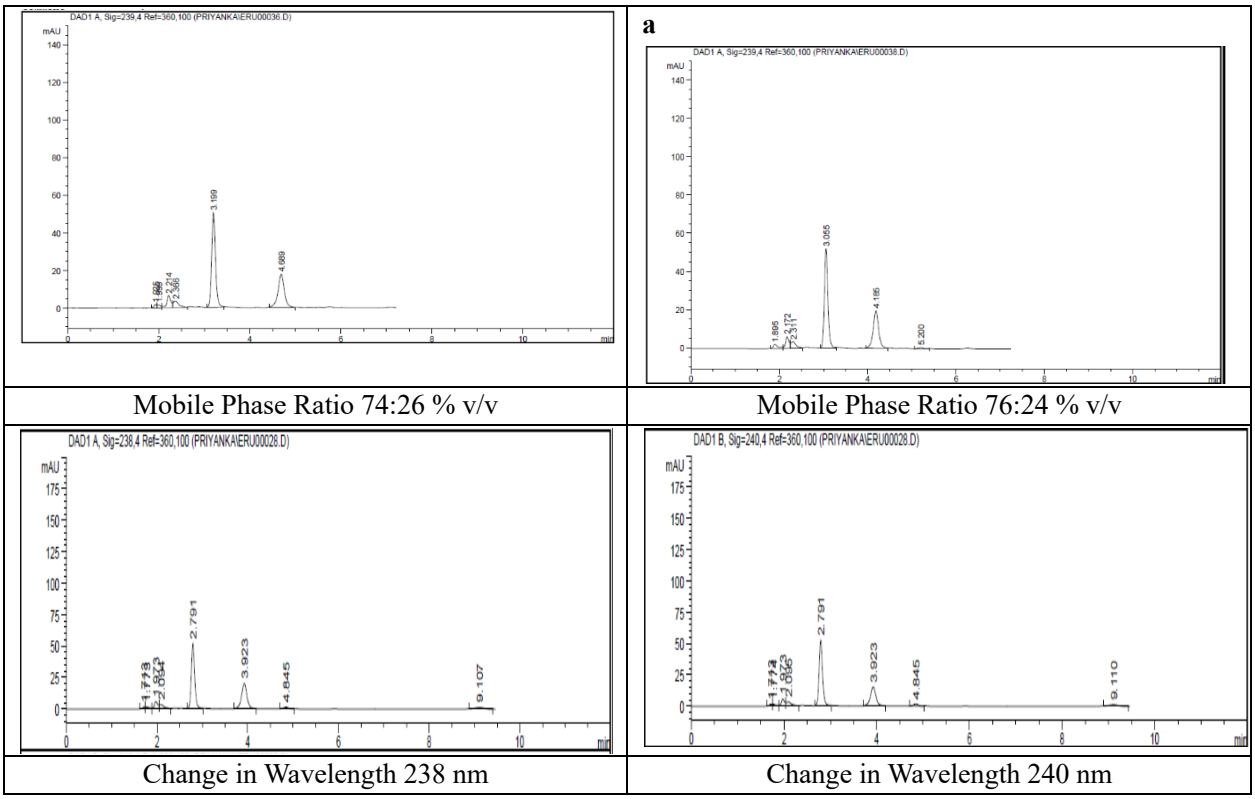


Figure 7: Robustness Chromatogram

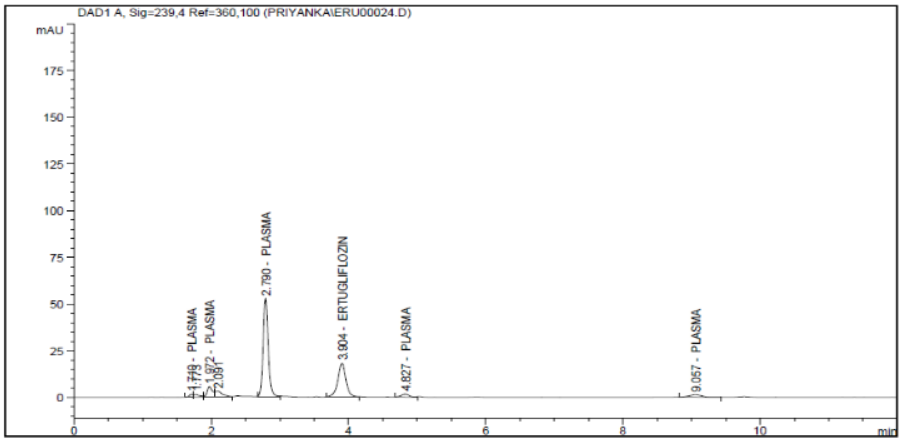


Figure 8: Chromatogram for Marketed Formulation

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