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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF REMOGLIFLOZIN ETABONATE BY RP-HPLC TECHNIQUE

Ayushi Jain*, Akhlesh Kumar Singhai

School of Pharmacy, LNCT University, Bhopal (Madhya Pradesh)

ayushijain131195@gmail.com

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Abstract

A simple, precise, accurate, and reproducible RP-HPLC method was developed and validated for the estimation of Remogliflozin Etabonate in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved using a Thermo C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of Methanol: Acetonitrile (50:50 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 238 nm using methanol as diluent. The retention time of Remogliflozin Etabonate was found to be 5.558 ± 0.03 min. The method exhibited good linearity in the concentration range of 1–5 µg/mL with a correlation coefficient (R^2) of 0.9999. The developed method was validated according to ICH guidelines for parameters including accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The percentage recovery values were found within acceptable limits, indicating good accuracy of the method. Precision studies showed low %RSD values, confirming reproducibility and reliability. The LOD and LOQ values were found to be 0.15 µg/mL and 0.45 µg/mL respectively, demonstrating adequate sensitivity of the method. The developed RP-HPLC method was found to be suitable for routine quantitative analysis of Remogliflozin Etabonate in pharmaceutical formulations.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and is associated with serious complications such as cardiovascular disorders, nephropathy, neuropathy, and retinopathy [1-2]. The increasing prevalence of type 2 diabetes mellitus has created a significant demand for effective and safe antidiabetic agents for long-term management of the disease [3].

Remogliflozin Etabonate is a novel sodium-glucose co-transporter-2 (SGLT2) inhibitor used in the treatment of type 2 diabetes mellitus. It acts by inhibiting glucose reabsorption in the renal proximal tubules,

thereby increasing urinary glucose excretion and reducing blood glucose levels [4-5]. Due to its therapeutic importance and increasing clinical use, accurate and reliable analytical methods are essential for its quantitative estimation in bulk drugs and pharmaceutical dosage forms [6]. High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis because of its high sensitivity, specificity, precision, and reproducibility [7]. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is particularly preferred for routine quality control analysis owing to its simplicity and efficiency in separating compounds with varying polarity [8].

Analytical method development and validation play a crucial role in ensuring the identity, purity, potency, and safety of pharmaceutical products. According to International Council for Harmonisation (ICH) guidelines, validated analytical methods are necessary for the quality assessment of drug substances and formulations. Parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ) are evaluated during validation to establish the reliability of the method [9].

The present study was undertaken to develop and validate a simple, rapid, precise, and accurate RP-HPLC method for the estimation of Remogliflozin Etabonate in bulk and pharmaceutical dosage forms. The developed method was optimized and validated as per ICH guidelines to ensure its suitability for routine pharmaceutical analysis and quality control applications.

Material and Methods

Selection of mobile phase

Initially, a variety of mobile phase ratios were tested in order to estimate Remogliflozin etabonate in a fixed dosage form. Acetonitrile: Methanol at a 50:50 v/v ratio was determined to be the most appropriate mobile phase for analysis after accounting for system suitability parameters such as RT, Tailing factor, number of theoretical plates, and HETP. After removing particulate matter using 0.45m filter paper, the mobile phase was sonicated to degas it. 1.0 ml/min was the flow rate used for analysis.

Selection of diluents

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of

analyte. After various trials methanol was used as diluents.

Preparation of standard stock solution

After 10 mg of Remogliflozin etabonate was precisely weighed and put into 50 ml volumetric flasks, it was dissolved in 10 ml of methanol. Acetonitrile was then added to get the volume up to 50 ml, and the mixture was vortexed to ensure the drug was completely dissolved. Set away for a few minutes. Remogliflozin etabonate's concentration was 200 µg/ml (Stock-A).

Preparation of sub stock solution

Remogliflozin etabonate stock-A yielded 5 ml of solution, which was then placed into a second 10 ml volumetric flask and diluted with 10 ml of diluent (methanol) to achieve a concentration of 100µg/ml (Stock-B).

Preparation of different solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml for drug.

Linearity and calibration graph

A series of dilutions ranging from 1 to 5µg/ml was created in order to demonstrate the linearity of the analytical technique. Chromatograms were acquired at 238 nm after all of the fluid had been filtered through a 0.2 nm membrane filter and injected three times. The regression equation was obtained by plotting a calibration graph between the mean peak area and the corresponding concentration.

System suitability parameters

After setting the separation parameters, the mobile phase was allowed to fill the column at a rate of 1 millilitre per minute. Three consecutive injections of the working standard of Remogliflozin etabonate (5µg/ml) were made once the column had fully saturated. Every chromatogram has a peak report and a column performance report.

Validation of developed method [10]

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 1 to 5µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug was plotted. The response ratio, also known as the response factor, was calculated

by dividing the measured AUC by the corresponding concentration value.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

To confirm the accuracy of the established approach, recovery tests were conducted. A certain concentration of the standard medication (80%, 100%, and 120%) was added to the preanalyzed sample solution, and the recovery was then examined.

Precision

The precision are established in three differences:

Repeatability

The precision under the same operating circumstances for a brief period of time was demonstrated by the repeatability test, which was conducted for five replicates at five concentrations in the linearity range of 1, 2, 3, 4, and 5µg/ml for Remogliflozin etabonate.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations.

Robustness

To test the method's ability to stay unaffected, slight but intentional changes in the mobile phase's concentration were done in accordance with ICH guidelines. The mobile phase's methanol:acetonitrile ratio (50:50% v/v) was switched to 45:55% v/v.

Detection Limit and Quantitation Limit

The slope of the linearity curve and the response standard deviation were used to compute the LOD and LOQ of the devised approach.

Results and Discussion

The developed RP-HPLC method for the estimation of Remogliflozin Etabonate was found to be simple, rapid, accurate, and reproducible. The chromatogram of blank diluent shown in Figure 1 indicated the absence of interfering peaks at the retention time of the drug, confirming the specificity of the method. The chromatogram of pure Remogliflozin Etabonate presented in Figure 2 exhibited a sharp and symmetric peak with a retention time of 5.558 ± 0.03 min, indicating proper separation and good peak characteristics.

The optimized chromatographic conditions are summarized in Table 1. A Thermo C18 column (250 mm × 4.6 mm, 5 µm) with Methanol: Acetonitrile (50:50 v/v) as the

mobile phase at a flow rate of 1.0 mL/min produced satisfactory peak resolution and acceptable system suitability parameters. Detection at 238 nm provided adequate sensitivity for quantitative estimation of the drug.

Linearity studies demonstrated that the method obeyed Beer-Lambert's law over the concentration range of 1–5 µg/mL, as presented in Table 2. The regression equation obtained was $y = 1004.3x + 17.875$ with a correlation coefficient (R^2) of 0.9999, indicating excellent linearity between concentration and peak area. The low values of standard deviation and %RSD confirmed the consistency and reliability of the analytical response.

Validation results summarized in Table 3 revealed that the developed method complied with ICH guidelines for analytical method validation. System suitability parameters such as retention time, theoretical plates, and tailing factor were within acceptable limits, indicating efficient chromatographic performance. Accuracy studies at 80%, 100%, and 120% levels showed recovery values close to 100%, confirming the accuracy of the method. Precision studies including repeatability and day-to-day precision exhibited very low %RSD values, demonstrating excellent precision and reproducibility. Robustness evaluation indicated that minor deliberate changes in chromatographic conditions did not significantly affect the analytical performance.

The sensitivity of the method was confirmed by the low values of Limit of Detection (LOD) and Limit of Quantification (LOQ), which were found to be 0.15µg/mL and 0.45µg/mL respectively, as shown in Table 4. These results indicate that the method is sufficiently sensitive for routine quantitative analysis of Remogliflozin Etabonate in pharmaceutical formulations.

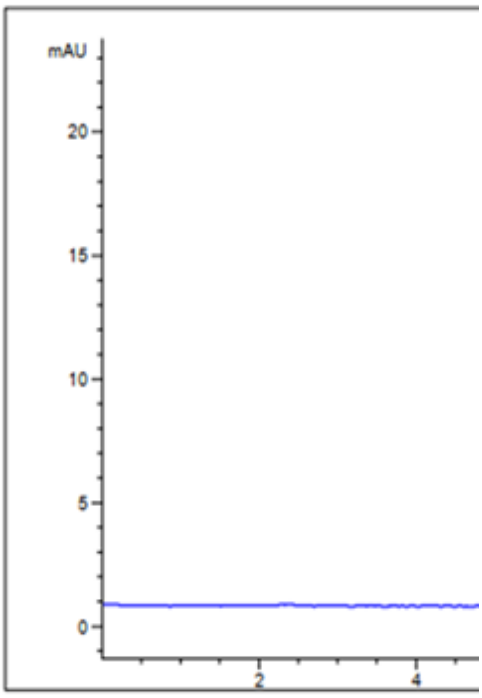

Figure 1: Chromatogram of blank diluent

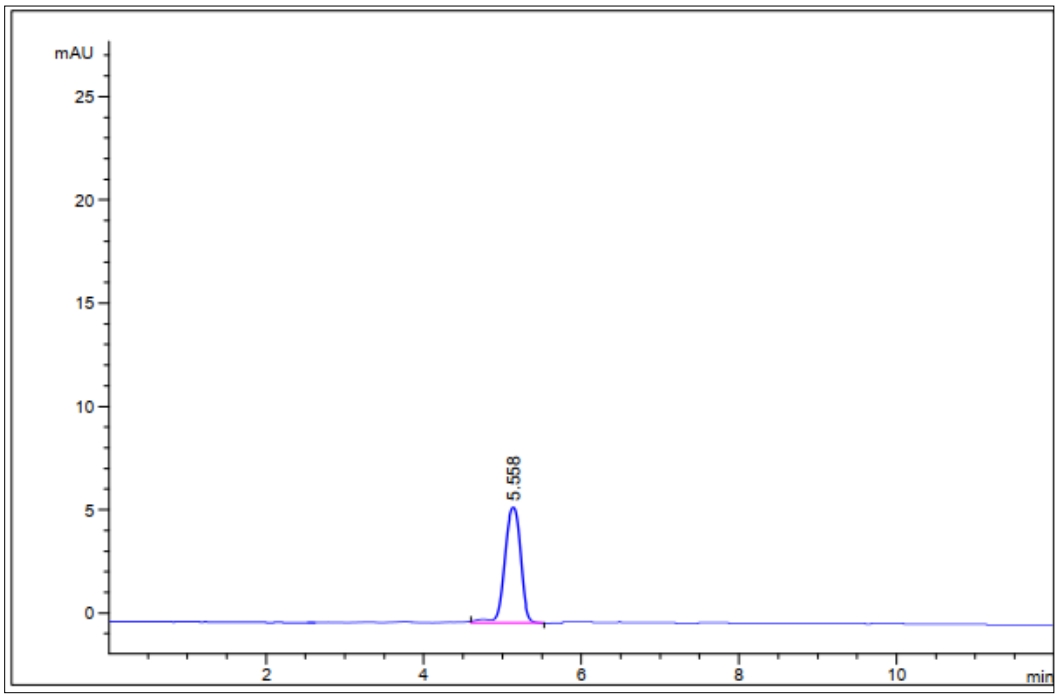

Figure 2: Chromatogram of pure drug

Table 1: Optimized

Chromatographic Conditions for Remogliflozin Etabonate

Parameter	Condition
Column	Thermo C18 Column
Column Dimension	250 mm × 4.6 mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C18)
Mobile Phase	Methanol : Acetonitrile (50:50 v/v)
Diluent	Methanol
Flow Rate	1.0 mL/min
Column Temperature	Ambient
Injection Volume	20 µL
Detection Wavelength	238 nm
Retention Time of Remogliflozin Etabonate	5.558 ± 0.03 min

Table 2: Statistical Data for Linearity of Remogliflozin Etabonate

S. No.	Parameter	Result
1	Linearity	1–5 µg/mL
2	Regression Equation	y = 1004.3x + 17.875
3	Slope (m)	1004.3
4	Intercept (c)	17.875
5	Correlation Coefficient (R^2)	0.9999
6	Mean AUC Range	1026.412 – 5034.673
7	Standard Deviation Range	5.86 – 10.12
8	% RSD Range	0.19 – 0.38

Table 3: Validation Parameters of Remogliflozin Etabonate

Validation Parameter	Mean (%)	SD	% RSD
System Suitability (RT)	5.8545	0.003	0.044
System Suitability (AUC)	2566.291	5.605	0.218

System Suitability (Theoretical Plates)	2676.500	8.216	0.307
System Suitability (Tailing Factor)	1.153	0.016	1.416
Linearity Response Ratio	1014.552	12.076	1.190
Accuracy (80% Level)	98.56	0.401	0.407
Accuracy (100% Level)	97.39	1.084	1.113
Accuracy (120% Level)	98.61	0.556	0.563
Repeatability	97.20	0.038	0.040
Day-to-Day Precision	97.685	0.040	0.041
Robustness	98.001	0.037	0.038

Table 4: LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Remogliflozin etabonate	0.15	0.45

Conclusion

The present study successfully developed and validated a simple, rapid, accurate, precise, and reproducible RP-HPLC method for the estimation of Remogliflozin Etabonate in bulk drug and pharmaceutical dosage forms. The optimized chromatographic conditions using a Thermo C18 column with Methanol: Acetonitrile (50:50 v/v) as the mobile phase provided satisfactory separation with a retention time of 5.558 min and good peak symmetry.

The developed method exhibited excellent linearity over the concentration range of 1–5 µg/mL with a correlation coefficient (R^2) of 0.9999. Validation studies performed according to ICH guidelines demonstrated acceptable accuracy, precision, robustness, and system suitability parameters with low %RSD values, indicating the reliability and reproducibility of the method. The low LOD and LOQ values confirmed the sensitivity of the analytical procedure. The validated RP-HPLC method was found to be suitable for routine quantitative analysis and quality control of Remogliflozin Etabonate in pharmaceutical formulations due to its simplicity, sensitivity, accuracy, and cost-effectiveness.

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