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Analytical Method Development and Validation of RP-HPLC For Estimation of Remogliflozin Etabonate in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A straightforward, creative, and selective reverse phase-high performance liquid chromatography (RP-HPLC) method that has been developed and optimized can be used to quantify remogliflozin etabonate (RMZ) in both bulk and dose forms. Phenomenex C18, 250 mm X 4.6 mm, 5 µm, was used as the stationary phase for the analysis at a flow rate of 1.2 mL/min, injection volume of 20 µl, run time of 8 min, and detection wavelength of 228 nm. The mobile phase was a mixture of 0.1% trifluoroacetic acid and methanol in a ratio of 30:70 v/v. Remogliflozin Etabonate Retention Time was evaluated by the analytical method at 3.7 minutes. To evaluate the linearity of the method, analyte concentration was measured throughout a range of ug/ml to 90 ug/ml, and area was plotted as a function of analyte concentration. For 50% to 150%, the individual recovery ranges from 95.0% to 105.0%, while the mean recovery ranges from 98.0% to 102.0%. The detection wavelength and flow rate were adjusted by ±2 nm and ±0.1 ml/min, respectively, for robustness. With a correlation coefficient (r2) of 0.9998, the approach was determined to be linear. With the right settings, the approach was also shown to be reliable and accurate.

Keywords: *Remogliflozin Etabonate, Diabetes mellitus, RP-HPLC, Method development, Validation.*

1. INTRODUCTION

Diabetes mellitus is a metabolic ailment characterized by symptoms and hyperglycaemia (high blood sugar), not a single illness. It stops the body from utilizing the food's energy in the right way. Diabetes mellitus is brought on by

(a) insufficient or absent insulin secretion by the pancreas.

(b) Insulin resistance occurs when the pancreas secretes adequate insulin yet it is ineffective. One of the main causes of death worldwide is diabetes mellitus (1, 2).

To lower diabetic consequences such as kidney failure, retinopathy, neuropathy, and cardiovascular issues, improved glycaemic management is crucial (3). Remogliflozin etabonate has recently been approved in a novel formulation for the treatment of diabetes mellitus. It is a member of the gliflozin drug class. This anti-diabetic medication is mostly used to treat non-alcoholic steatohepatitis and type 2 diabetes mellitus. It is a new oral hypoglycaemic medication that does not require insulin. (4, 5). Remogliflozin Etabonate, as ethyl (((2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-((4-(4-isopropoxybenzyl)-1-isopropyl-5-methyl-1H-pyrazol-3-yl) oxy) tetrahydro-2H-pyran-2-yl) methyl) carbonate. It blocks the sodium-glucose transport proteins (SGLT), which are in charge of the kidney's reabsorption of glucose. Blood glucose is eliminated through the urine when this transporter is inhibited (6–8). The structural formula is as shown in Fig. 1. (9).

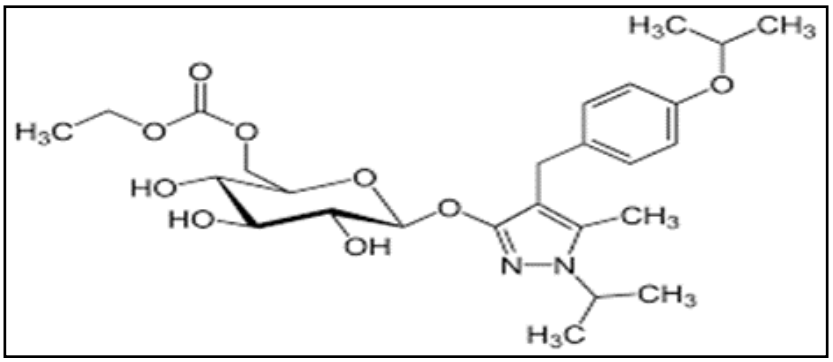

Fig.1: Remogliflozin Etabonate

The goal of this work is to create a simple, precise, and accurate RP-HPLC method for Remogliflozin Etabonate in pharmaceutical dosage form and bulk and to validate it in accordance with ICH recommendations (10-16).

2. MATERIALS AND METHODS:

Instruments and Software

The Agilent 1260 Infinity II HPLC was utilized. The separations were accomplished using the Phenomenex C18, 250 mm x 4.6 mm, 5 µm, and UV detection at 227 nm. An analytical weighing scale (Aczet CY224C) and a sonicator (Bio-technic 13.5 Liter) were used in the experiment. A double-beam UV-visible spectrophotometer (Jasco UV 550) was used to detect wavelengths. The OpenLab EZ Chrome workstation software was used to collect, analyze, and save chromatographic results.

Chemicals and Reagents:

The Indian state of Maharashtra's Vidisha Analytical was the supplier of remogliflozin etabonate. 100 mg of Remogliflozin Etabonate is contained in each tablet, which is manufactured by Glenmark Pharmaceuticals Ltd. It was bought at a local pharmacy. Methanol, an analytical 0.1% trifluoroacetic acid, and HPLC-grade methanol (Merck) were used as the mobile phase for the experiment. The pharmaceutical formulation Remogliflozin Etabonate (label claim includes 100 mg) was used for HPLC analysis. The HPLC study made use of Rankem's HPLC-grade water.

3. EXPERIMENTAL WORK

- **Chromatography**

Following a number of experiments with various solvent combinations and ratios, the stationary phase Phenomenex C18, 250 mm X 4.6 mm, 5 µm and the mobile phase 0.1% trifluoroacetic acid and methanol in a 30:70 v/v ratio were used for the analysis at a flow rate of 1.2 ml/min, column temperature of 40ºC, injection volume of 20 µl, run time of 8 min, detection wavelength of 228 nm, and retention time (Rt) of 3.70 minutes for Remogliflozin Etabonate.

- **Preparation of Mobile Phase**

Preparation of 0.1 % Trifluoroacetic acid: Carefully measure and transfer approximately 1 millilitre of Trifluoroacetic Acid into 1000 millilitres of water. Stir thoroughly. Degas after passing through a 0.45 µ nylon membrane disc filter.

Preparation of Mobile Phase: The mobile phase is made by mixing 0.1% trifluoroacetic acid with methanol in a 30:70 v/v ratio. well combined.

- **Selection of Detector and Detection Wavelength:**

The Agilent 1260 Infinity II HPLC with detector was selected, as it is reliable and easy to set at the correct wavelength, and 228 nm wavelengths were selected as the detection wavelength.

- **Preparation of standard stock solution for chromatographic development**

Accurately weigh 30 mg of the working standard for remogliflozin etabonate, then put the contents into a 50 mL volumetric flask that has been cleaned and dried. About 30 mL of diluent should be added. Sonicate to dissolve it, then add more diluent to make up the difference. Use diluent to further dilute 4 mL of each Remogliflozin Etabonate stock solution to 25 ml (Remogliflozin, 96 ppm).

- **Preparation of Sample Solution:**

Weighed and transferred 5 Remo Zen 100 mg tablets (Remogliflozin Etabonate 100 mg) into a 250 mL clean and dry volumetric flask. Add about 200 mL of diluent, sonicate for 60 minutes with intermittent shaking at control room temperature,

and make the volume up to the mark with diluent and mix. Further dilute 3 mL of sample stock solution to a 100 mL volumetric flask, make up with diluent up to the mark, and mix well. Filter the sample solution through a 0.45µ membrane PVDF filter. Discard the first 4.0 mL of filtrate and then collect the sample. **(Concentration of Sample Solution: 60 ppm)**

4. RESULT AND DISCUSSION

❖ Analytical Method Development

Reverse Phase High Performance Liquid Chromatography Method Development for estimation of Remogliflozin Etabonate.

Table.1. Chromatographic Conditions:

Mobile Phase	**:**	0.1% Trifluoroacetic acid and Methanol (30:70 v/v)
Column	**:**	Phenomenex C18, 250 mm X 4.6 mm, 5 µm
Flow Rate	**:**	1.2 mL/min
Injection Volume	**:**	20 µL
Wavelength	**:**	228 nm
Column oven Temp	**:**	40ºC
Auto Sampler Temp	**:**	10ºC
Run time	**:**	8 minutes
Seal wash	**:**	Water: Methanol, 90:10 v/v
Needle wash	**:**	Water: Methanol, 10:90 v/v

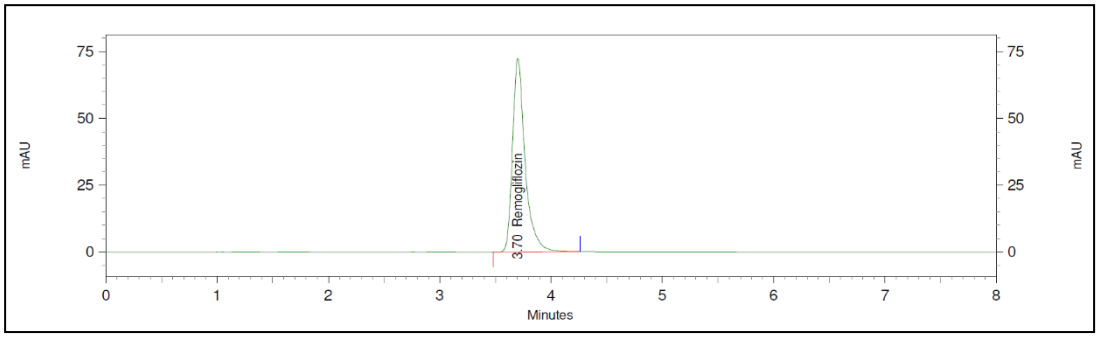

Fig.2: Typical chromatogram for Remogliflozin Etabonate

Observation: Remogliflozin Etabonate eluted at 3.70 minutes with acceptable chromatography. (Asymmetry: 1.30 and Theoretical plates 6165).

Conclusion: Method can be used for further analysis.

❖ Analytical method validation

According to ICH Q2 (R1) guidelines, the developed method was validated to assure the reliability of results of the analysis for different parameters like System Suitability, linearity, Specificity, accuracy, precision, robustness.

1. System Suitability

System suitability test is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph.

Table.2. System Suitability Test of Remogliflozin Etabonate

Tailing Factor	**1.32**
Theoretical plates	**6125**
Injection No.	**Area**
1	9163529
2	9165415
3	9156859
4	9163529
5	9160294
Mean	9161925
%RSD	**0.5**

Conclusion:

The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

2. SPECIFICITY: (Identification, Interference & Peak Purity)

The retention time for the remogliflozin peak is the same in the standard and sample. At the Remogliflozin peak retention time, there is no interference from the blank or placebo. The chromatograms from the Standard and Sample solutions show the same peak purity.

Table.3. Specificity (Identification and Interference)

Solution	Specificity Data		
	Retention Time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard Solution	3.70	**Purity angle**	**Purity threshold**
		1.52	3.84
Sample Solution	3.71	1.39	3.02

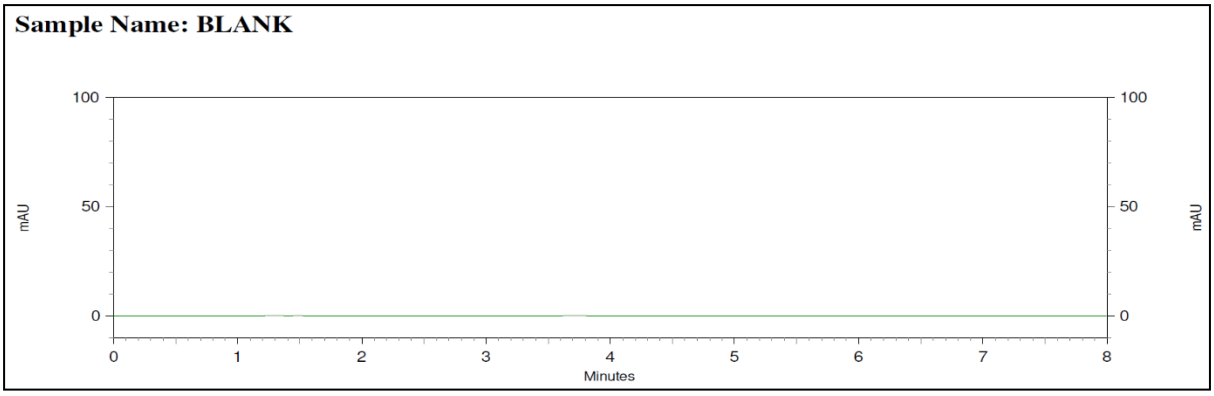

Fig.3. Chromatogram of Blank

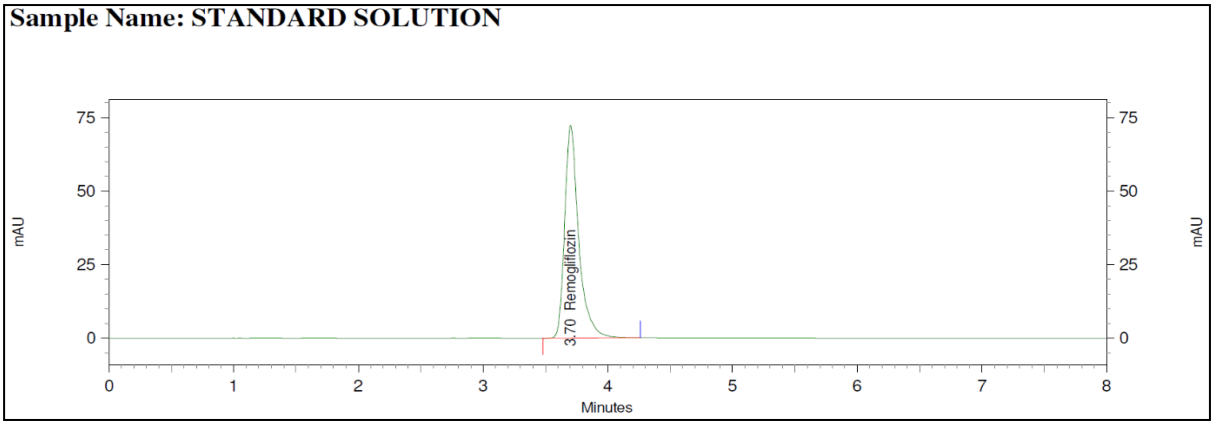

Fig.4: Chromatogram of Standard

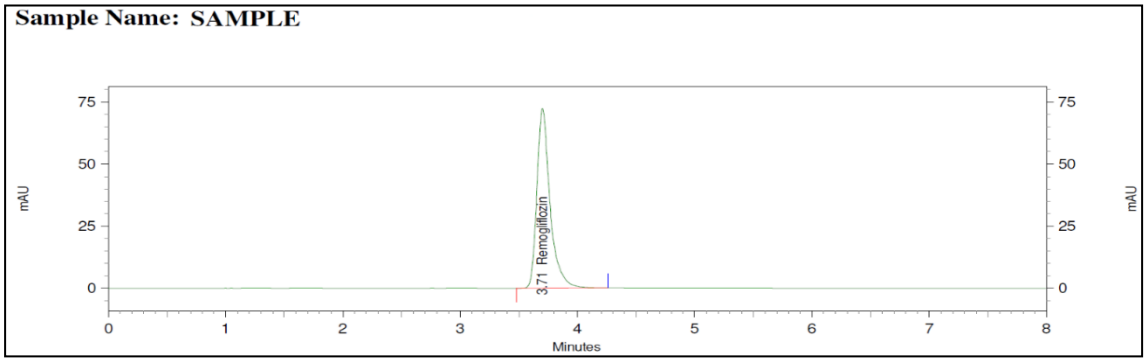

Fig.5: Chromatogram of Sample

3. LINEARITY

Linearity was evaluated in the range of 50 % to 150 % of Remogliflozin for working concentration. The working concentration of Remogliflozin in solution is 60 µg/ml.

Table.4. Linearity of Remogliflozin Etabonate

Level	Conc. (µg/mL)	Area	Mean
50%	30	4502632	4507021
		4513201	
		4505229	
75%	45	6926524	6915165
		6913529	
		6905441	
100%	60	9168521	9157128
		9156524	
		9146339	
125%	75	11380524	11393987
		11396142	

		11405296	
150%	90	13695026	13650884
		13652198	
		13605429	
Corr. Coeff			0.9998
Intercept			18218
Slope			151777
% Y-intercept			0.20

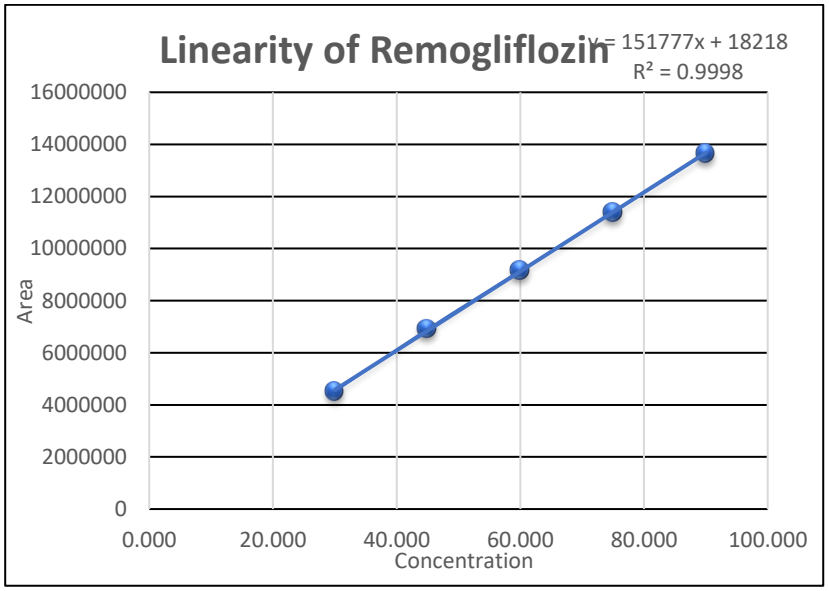

Fig.6: Linearity plot of Remogliflozin Etabonate

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient ($r^{2)}$ was found 0.9998.

4. ACCURACY (Recovery):

Evaluated accuracy from 50% to 150% of Remogliflozin tablet, working concentration level. Each level prepared in triplicates.

Table.5. % Recovery for Remogliflozin Etabonate

Level (%)	Remogliflozin etabonate Added Conc. (µg/mL)	Remogliflozin Recovered conc.	Area	% Recovery	Mean % Recovery
50	30.04	30.06	4639529	100.48	99.99
	30.08	29.95	4582146	98.45	
	29.87	30.23	4609631	101.03	
100	60.04	60.00	9176524	99.76	100.18

	60.06	59.98	9206283	99.88	
	60.08	60.15	9316529	100.88	
150	90.08	89.93	13726009	99.35	99.77
	89.95	90.09	13836421	100.81	
	90.02	89.85	13642586	99.14	

Conclusion:

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0%.

5. Precision

Method Precision:

Single injection of blank (Diluent), Standard solution (Five replicates) and sample solution (six preparations) was injected on the system.

Table.6: Method precision

Sample	**Area**	**% Assay**
Sample 1	8925413	97.10
Sample 2	9065429	98.99
Sample 3	9126051	99.20
Sample 4	9062459	97.96
Sample 5	8953601	97.95
Sample 6	9023509	98.35
Mean		**98.26**
STD DEV		**0.7667**
% RSD		**0.780**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

Intermediate Precision:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table.7. Intermediate Precision

Sample	**Area**	**% Assay**
Sample 1	8960565	97.85
Sample 2	9012534	98.69
Sample 3	9120135	98.59
Sample 4	9082541	98.81
Sample 5	8842634	96.12

Sample 6	8965260	98.35
Mean		**98.07**
STD DEV		**1.0154**
% RSD		**1.035**

Table.8. Intermediate Precision Pool Data

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-017**	**HPLC-012**
Sample No.	**%Assay**	
1	97.10	97.85
2	98.99	98.69
3	99.20	98.59
4	97.96	98.81
5	97.95	96.12
6	98.35	98.35
Mean	**98.26**	**98.07**
Mean of Precision % Assay	**98.16**	
Absolute Mean difference % assay	**0.9**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6. Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table.10. Robustness for Remogliflozin Etabonate

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	8925413	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	9086294	0.9
	0.9 ml/min	8793107	-0.7

Change in wavelength (±2 nm)	230 nm	8842168	-1.9
	226 nm	9083851	0.2

Conclusion:

- System suitability criteria were fulfilled.
- The difference of Area value in each modified condition is within acceptance criteria.

5. CONCLUSION

Apart from the favourable prerequisites for analytical techniques, the most notable benefit of all created methods is their affordability, accuracy, and economy. For the determination of Remogliflozin Etabonate, the suggested RP-HPLC method was an appropriate methodology. According to the ICH guidelines for Method Validation, every parameter used to analyze remogliflozin etabonate satisfied the requirements. In this study, we have created a simple, sensitive, accurate, and exact RP-HPLC method for quantifying remogliflozin etabonate in pharmaceutical formulations and bulk. The procedure determined that the recoveries obtained were satisfactory. When compared to spectrophotometric approaches, the HPLC process is more accurate, sensitive, and precise. For the routine measurement of Remogliflozin Etabonate in pharmaceutical formulations and bulk drugs, the established HPLC method can be suggested.

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