New Analytical Method Development and Validation of an RP-HPLC for the Determination of REMOGLIFLOZIN ETABONATE in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Background and objectives: This research work make an attempt to establish sensitive and accurate methods for the development and validation of analytical methods for estimation of Remogliflozin Etabonate in bulk and pharmaceutical dosage form.
Methods: A mixture of 0.1% Triethylamine buffer pH 7.9 and methanol in the ratio of 70:30 (v/v%) was used as the mobile phase. A working standard solution of concentration 20 µg/ml was used. An XBridge™ C18 column 5µ (250 mm x 4.6 mm) was used for the analysis at a flow rate of 1 ml/min, injection volume of 20 µl, run time of 10 mins and detection wavelength of 227 nm. The repeatability (within-day in triplicates) and intermediate precision (for two days) were carried out by six injections and the obtained results within and between the days of trials were expressed as % RSD. The linearity of the method was determined by the analysis of analyte concentration across a range of 05µg/ml to 30µg/ml and area was plotted graphically as a function of analyte concentration. The accuracy of the method was determined by calculating recoveries of Remogliflozin Etabonate by the standard addition method. In a pre-quantified sample solution (20µg/ml), a known amount of standard solutions of Remogliflozin Etabonate (80%, 100% and 120%) were added. For robustness a change of ±0.1 ml/min and ±1 nm was done in flow rate and detection wavelength respectively. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.
Results: The %RSD values of precision studies were found to be below the accepted limit of 2%. The method was found to be linear with a correlation coefficient (r^2) of 0.9999. The method was also found to be accurate and robust with suitable values. The LOD and LOQ of the method was found to be 0.42 µg/ml and 1.41 µg/ml respectively.
Interpretation and conclusion: The results of analysis prove that this method can be used for the routine determination of Remogliflozin Etabonate in bulk drug and in pharmaceutical dosage forms.

Keywords: Remogliflozin Etabonate, RP-HPLC, method development, validation, LOD, LOQ.

1. INTRODUCTION:

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia (high blood sugar) and other signs, as distinct from a single disease or condition. It prevents the body from properly using the energy from the food. Diabetes mellitus occurs when
(a) Pancreas secretes little insulin or no insulin.
(b) Pancreas secretes enough insulin but the insulin does not work (insulin resistance).
Diabetes mellitus is a leading cause of death all over the world.[1,2,3] Hence, better glycemic control is essential to reduce diabetic complications such as kidney failure, retinopathy, neuropathy, cardiovascular complications, etc.[4] Recently, a new formulation containing Remogliflozin etabonate has been approved for the management of diabetes mellitus. It belongs to the gliflozin class of drugs. It is an anti-diabetic drug, primarily used to treat type2 diabetes mellitus and non-alcoholic steatohepatitis (non-alcoholic fatty liver disease). It is a recently developed insulin-independent oral hypoglycemic agent.[5,6] It acts by inhibiting sodium-glucose cotransporter-2 (SGLT2), an enzyme accountable for reabsorption of sugar (glucose) in the kidneys, thereby increase the elimination of sugar through the urine. A part from glycemic control, SGLT-2 inhibitors possess many beneficial effects that include lowering of body weight, reduction of systolic blood pressure and lowering hemoglobin A1c levels. Hence Remogliflozin Etabonate is more helpful when co-administered with metformin, particularly in patients with cardiac & renal diseases, who require further reduction of hemoglobin A1c level.[7,8,9,10,11,12]
Indication: As a monotherapy adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. Add on therapy with other glucose-lowering medicinal products. It can be taken with or without food.
Dosage and Administration: The recommended dose is 100 mg twice daily for monotherapy and add-on therapy with other glucose-lowering medicinal products. If diabetic control is inadequate a cautious increase in dosage to a maximum of 250 mg twice daily is justified.
Contraindications: Hypersensitivity to the active substance or to any of the excipients.
Over dosage: There is no specific antidote for an overdose of remogliflozin etabonate. Inhibition of SGLT2 is reversible.
Warnings & Precautions: It should not be initiated in patients with moderate to severe renal impairment (glomerular filtration rate [GFR] <60 mL/min). It is not recommended for use in patients with moderate to severe hepatic impairment. It can cause hypotension, hemoconcentration, or electrolyte abnormalities. Initiation of Remogliflozin in patients receiving concomitant diuretics should be undertaken cautiously. Rare cases of diabetic ketoacidosis (DKA), including life-threatening and fatal cases, have been reported in patients treated with sodium-glucose co-transporter 2 (SGLT2) inhibitors. No moderate to severe events of

DKA were reported in clinical studies with Remogliflozin. Urinary tract infections were reported for it. Patients on oral contraceptives should be advised to use alternative, non-hormonal methods of birth control during treatment with it.

Use in specific population:

Safety and effectiveness of Remogliflozin in children less than 18 years have not been established. There are no adequate and well-controlled studies in pregnant and lactating women.

Adverse Reactions:

Urinary tract infection, pyrexia, headache, bacteriuria, constipation, diarrhea, glomerular filtration rate decreased, ketonuria, cough, dyslipidaemia, asthenia, viral upper respiratory tract infection, hypoglycaemia, and orthostatic hypotension.[10]

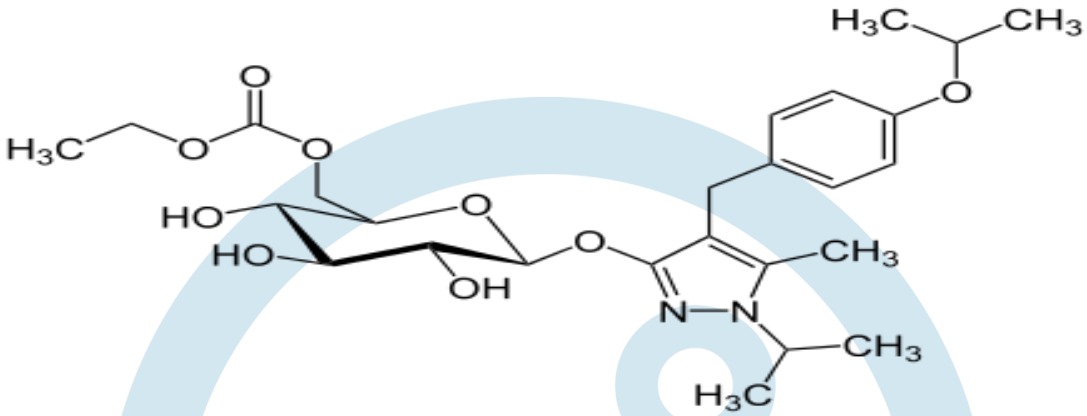

Fig17: Structure of Remogliflozin Etabonate

IUPAC Name: [5-methyl-4-(4-(1-methylethoxy) benzyl)-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O- (ethoxy carbonyl)- β-D-glucopyranoside]

Molecular Formula: $C_{26}H_{38}N_2O_9$

Molar Mass: 522.59g/mol

pKa value: 12.20

Melting Point: 661.3˚C

CAS: 442201-24-3

Color: White to off white powder

Route of Administration: Oral

Half Life: Plasma half-life 120min

Storage Temperature: Room temperature (25±2˚C)

Drug Category: Non-alcoholic steatohepatitis and type-2 diabetes

Solubility: soluble in organic solvents, sparingly soluble in aqueous buffers.

2. MATERIAL AND METHOD

2.1 Instrument and Software

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT VP pump) (4MPa or 40bar), Rheodyne injector, UV variable wavelength detector, Standard cell and Agilent syringe was used. The separations were achieved on XBridge™ C18 column 5µm (250mm x4.6mm), column length is 25 cm with UV detection at 227nm. Analytical weighing balance. (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON 230V AC, 50Hz), vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam UV-Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-dual channel was used for acquisition, evaluation and storage of chromatographic data.

2.2 Chemical and Reagents

Remogliflozin Etabonate was obtained as gift sample from actis pharma, Andhra Pradesh, India. It is a tablet dosage form each contains 100mg of Remogliflozin Etabonate were procured from local pharmacy, manufactured by Glenmark pharmaceuticals Ltd. HPLC grade methanol (Merck), Analytical grade triethyl amine and orthophosphoric acid buffer was used as the solvents throughout the experiment. Pharmaceutical formulation Remogliflozin Etabonate (label claim contain 100 mg) was used in HPLC analysis. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

2.3 Chromatography

After several trials with the different combination and ratio of solvents, the mobile phase 0.1%triethyl amine buffer pH 7.9: methanol

(70:30 v/v) and Stationary phase XBridge™ C18 column 5µ (250 mm x 4.6 mm) was used for the analysis at a flow rate of 1 ml/min, Column temperature 25±1°C, injection volume of 20 µl, run time of 10 mins and detection wavelength of 227 nm and Retention time (R_t) 2.48 min for Remogliflozin Etabonate.

2.4 Preparation of mobile phase:

The buffer solution was prepared by dissolving 1ml of triethyl amine buffer in 1000ml HPLC grade water (10 mM). The pH of the resulting solution was adjusted 7.9 by using HPLC grade Orthophosphoric acid, HPLC experiments were carried out using binary pump A containing Methanol and pump B containing triethyl amine buffer.

2.5 Standard solutions and Working Solutions:

Remogliflozin Etabonate powder 100mg is transferred into 100 ml of volumetric flask and is dissolved in Methanol (HPLC grade) volume were made up to the mark with same solvent. This gave the concentration of 1000 µg/ml of Remogliflozin Etabonate (Stock-1). further dilution 1 ml in 10ml methanol in volumetric flask gives 100 µg ml-1(stock-2) From stock solution 2, 6 dilution was prepared between 05-30µg/ml which is working concentration. Before injecting solutions, the column was equilibrated for at least 40 min with the mobile phase flowing through the system. Six determinations were carried out for each solution, peak areas were recorded for all the solutions. All stock and working solutions were sonicated for 15 min then filtered through the nylon membrane filter (0.22µ) prior to use.

3. 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 linearity, Range, Specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness.

3.1 Linearity and Range

By using the working standard, aliquots of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml, were prepared with Methanol. Six dilutions of each of the above-mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration were injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

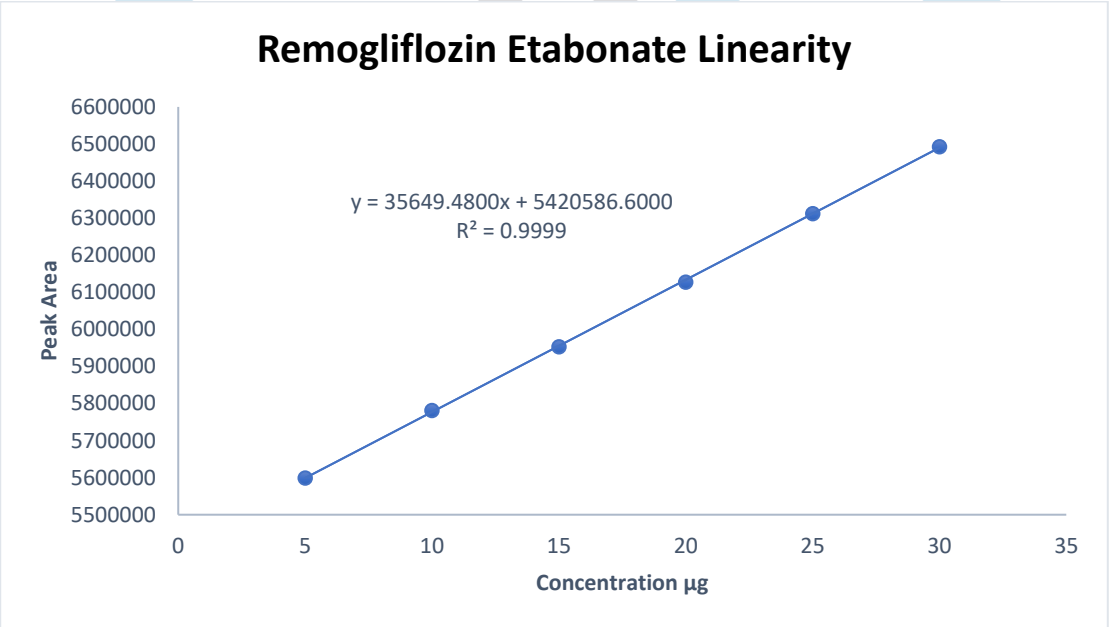

Figure 2: Linearity graph of Remogliflozin Etabonate

3.2 Precision

The precision of the assay was determined in terms of intra and inter day variation in the peak area for a set of drug solution 20µg/ml, assayed six times on the same day and on different 2 days. The Intra and Inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean by100. (CV=SD/MEAN × 100)

3.3 Accuracy

The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the methanol. For 80% Accuracy for Remogliflozin Etabonate: 80mg of the pure drug was added to 100mg of formulation. For 100% Accuracy for Remogliflozin Etabonate:100mg of the pure drug is added to 100mg of formulation. For 120% Accuracy for Remogliflozin Etabonate:120mg of the pure drug is added to 100mg of formulation.

3.4 Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, and wavelength ±1nm.

3.5 Limit of Detection and limit of Quantification

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal–to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of the blank samples. A signal–to–noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

$$\mathbf{LOD = 3.3\ X\ S.D\ of\ y - intercepts\ /\ mean\ of\ slopes}$$

$$\mathbf{LOQ = 10\ X\ S.D\ of\ y - intercepts\ /\ mean\ of\ slopes}$$

3.6 System Suitability

The resolution, number of theoretical plates, Capacity Factor, S/N (6 Sigma)and peak asymmetry were calculated for the standard solutions.

3.7 Assay

Preparation of Sample Solutions:

Twenty tablets, each containing 100mg of Remogliflozin Etabonate were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Remogliflozin Etabonate was weighed and transferred to 50 ml volumetric flask containing 30 ml Methanol. The mixture was sonicated for 20 min. The volume was made up to 50 ml with Methanol. The contents were filtered through 0.45µ membranefilter. Further dilutions were made to get a concentration of 20 µg/ml. Twenty microliters of the test and standard solutions were injected separately and chromatograms were recorded up to 10 min. The proposed method was found to be specific and no interference from tablet excipients was observed.

4. RESULT

4.1 Linearity

For linearity analyte concentration for Remogliflozin Etabonate taken across 5µg/ml to 30µg/ml of Remogliflozin Etabonate. They were prepared using HPLC grade methanol as solvent. Then tested at 227 nm. Absorbance is plotted graphically as a function of analyte concentration.

Concentration ($\mu g/ml^{-1}$)	Area
5	5598819
10	5781388
15	5953379
20	6127612
25	6312450
30	6493067
Slope	35649.48
Y-intercept	5420586.6
correlation	0.9999

Table 1: Linearity data for Remogliflozin Etabonate

4.2 Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogenous sample. The inter day (between 2 days) and intraday (at the same days: morning and evening) precision were carried out. The variation of results was calculated and % RSD was determined.

Injection 20 µg/ml	Area
1	6164996
2	6148170
3	6189360
4	6111562
5	6144526
6	6128599
Average	6147868.83
Standard Deviation	27260.35
RSD (%)	0.44

Table 2: Intraday precision of Remogliflozin Etabonate (Morning)

Injection 20 µg/ml	Area
1	6103840
2	6118510
3	6141445
4	6162138
5	6100246
6	6121294
Average	6124578.83
Standard Deviation	23515.81
RSD (%)	0.38

Table 3: Intraday precision of Remogliflozin Etabonate (Afternoon)

Injection 20 µg/ml	Area
1	6165062
2	6172081
3	6140202
4	6117767
5	6132482
6	6112068
Average	6139943.67
Standard Deviation	24447.25
RSD (%)	0.40

Table 4: Intraday precision of Remogliflozin Etabonate (Day 1)

Injection 20 µg/ml	Area
1	6172642
2	6156136
3	6167642
4	6172392
5	6141142
6	6166656
Average	6162768.33
Standard Deviation	12170.92
RSD (%)	0.20

Table 5: Intraday precision of Remogliflozin Etabonate (Day 2)

4.3 Accuracy

The accuracy for estimation of Remogliflozin Etabonate using methanol was determined by adding known amount of the analyte. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay

Level of Percentage recovery	80%	100%	120%
Amount present (mg/tablet)	100	100	100
Amount of standard drug added(mg)	80	100	120
Area response	4913067	6136084	7375398
	4938082	6156314	7341250
	4927006	6127896	7367282
Mean	4926051.66	6140098.00	7361310
Standard Deviation	12534.78	14628.05	17840.13
RSD	0.25	0.24	0.24
Total amount recovery (mg)	180.88	200.4	220.24
% Recovery	100.49	100.20	100.11

Table 6: Accuracy for Remogliflozin Etabonate

4.4 Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Name of the drug	LOD µg/ml	LOQ µg/ml
Remogliflozin etabonate	0.846	2.820

Table 7: LOD and LOQ for estimation of Remogliflozin Etabonate

4.4 Robustness

The robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, and wavelength ±1nm.

Sl.no.	Parameter	Optimized	Used	Retention time (mins)
1	Flow rate	1 ml/min	0.9 ml/min	2.483
			1.1ml/min	2.477
2	Detection wavelength	227 nm	226nm	2.463
			228nm	2.487

Table 8: Robustness of Remogliflozin Etabonate

4.5 Assay

Assay was carried out by making dilutions to get a concentration of 20 µg/ml. Twenty microliters of the test and 5 standard solutions were injected separatelyand chromatograms were recorded up to 10 min. By using the formula, the assay was found to be 99.98%

$$\text{Assay} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \text{ X } 100$$

Injection 20 µg/ml	Peak Area
1	61673803
2	61769013
3	61685184
4	61728871
5	61702335
6	61640239
Average	61699907.50
Standard Deviation	44897.45
RSD (%)	0.07

Table 9: Assay of Remogliflozin Etabonate

5. Discussion

The system suitability test was applied to the chromatograms taken under optimum conditions to check various parameters such as theoretical plates (2068), capacity factor (0.00013), asymmetry (1.35378) and signal to noise ratio (70.9193). Suitable tests results were achieved for the proposed method. All these results indicate the suitability of the instrument for the developed method. For study of precision six replicates of the standard solution was injected into the HPLC system in inter day and intraday intervals. The %RSD values of day one and day two for inter day intervals were found to be 0.40 % and 0.20 % respectively. While the %RSD values of morning and afternoon sessions for intraday intervals were found to be 0.44% and 0.38% respectively. Therefore, the %RSD values for precision studies are within the accepted limits of 2%. Linearity was performed using standard solutions in the concentration range of 05- 30 µg/ml. Calibration curve was constructed for the standards by plotting the concentrations versus peak areas and evaluated by linear regression analysis. The correlation coefficient (R^2) was found to be 0.9999 which is within the accepted limits. Accuracy was performed by spiking a pre-quantified sample with standard at 80%, 100% and 120%. The solutions were prepared in triplicates and analyzed through the developed method. The mean recovery values of obtained for the three trials were 100.49%, 100.20% and 100.11% respectively, which indicates that there is an extremely less interference coming from matrix components. For robustness a change of ±0.1 ml/min in the optimized flow rate of 1 ml/min of the method was done, resulting in the change of retention time from 2.480mins to 2.483mins and 2.477mins respectively for each deliberate change in flow rate. Similarly, a change of ±1 nm in the optimized detection wavelength of 227 nm of the method was done, resulting in the change of retention time from 2.480mins to 2.487 mins and 2.463mins respectively for each deliberate change. Considering the accepted limits for signal to noise ratio of 3:1 and 10:1 for calculating LOD and LOQ respectively, the LOD and LOQ of the method was found to be 0.846µg/ml and 2.82µg/ml respectively.

6. Conclusion

In addition to positive requirements for analytical methods, the striking advantage of all the developed method is that they are economical, cheap, and precise. The proposed RP-HPLC method were suitable technique for the determination of Remogliflozin Etabonate. All the parameters analyzing Remogliflozin Etabonate met the criteria of ICH guidelines for Method Validation. In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Remogliflozin Etabonate in bulk and pharmaceutical formulations. The recoveries achieved were found good by the method. The HPLC method is more sensitive, precise and accurate compared to the spectrophotometric methods. The HPLC method developed may be recommended for the routine determination of Remogliflozin Etabonate in bulk drug and pharmaceutical formulations.

7. Summary

The method was developed and validated for system suitability, specificity, linearity, precision, accuracy, limit of detection and limit of quantification. The system suitability was found to be within the limits. The limit was not more than RSD <2%. This indicates that the method is precise.

Sl. No	Validation Parameters	Estimation of Remogliflozin Etabonate
1.	Column	**XBridge™ C18 column 5µ (250 mmx 4.6 mm)**
2.	Mobile phase	**0.1%Triethylamine buffer: Methanol 70:30 (v/v)**
3.	pH	**7.9**
4.	Flow rate	**1 ml/min**
5.	Absorption maxima	**227 nm**
6.	Run time	**10 min**
7.	No. of theoretical plates	**2068**
8.	Retention time	**2.48**
9.	Tailing factor	**1.35**
10.	Linearity range	**5-30 µg/ml**
11.	Correlation coefficient (r^2)	**0.9999**
12.	Precision (%RSD)	**0.35**
13.	Accuracy (%RSD)	**0.24**
14.	Limit of Detection	**0.846µg/ml**
15.	Limit of Quantification	**2.820µg/ml**
16.	% Recovery	**100.27**

Table 10: The summary of the experiment for the qualitative estimation of Remogliflozin Etabonate using RP-HPLC

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Conflict Of Interest

This is a non-funding research work. There were no conflicts of interest.

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