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A New RP-HPLC Method Development And Validation For The Simultaneous Estimation Of Remogliflozin Etabonate And Metformin In Pure Form And Marketed Dosage Form

NJR Hepsebah[1], Shankar Cheruku[2]*, R Nazemoon[1], Fatima Sultana[1]

[1]Department of Pharmacy, Vijaya College of Pharmacy, Munaganoor, Hyderabad, Telangana-501511, India.
[2]Department of Pharmaceutical Analysis, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Telangana-500097, India.

Corresponding author*,
SHANKAR CHERUKU,
Assistant Professor, Department of Pharmaceutical Analysis, Teegala Ram Reddy College of Pharmacy, Meerpet, Saroornagar (M), R.R.Dist**,** Hyderabad-500097. Telangana, India.

Abstract

A new, precise, accurate, specific, rugged and sensitive, isocratic RP-HPLC stability indicating method has been developed and subsequently validated for the determination of Remogliflozin etabonate and Metformin in API and pharmaceutical dosage forms as per ICH guidelines. The separation achieved on a reversed phase Zorbax C18 (250 mm x 4.6 mm) 5µm Particle size Column as a stationary phase and Mobile phase, Methanol: Phosphate Buffer pH-4.2 (80:20 v/v) and other conditions optimized were: flow rate (1.0 ml/minute), wavelength (250 nm), Run time was maintained at seven minutes. The retention time for remogliflozin etabonate and metformin was found to be 2.46 min and 4.32 min respectively. The stability of the drug was determined by studying the degradation of the drug under acidic, alkaline, peroxide, neutral, heat and UV conditions. The developed method was found to be linear in the range of 20-100µg/ml for of Remogliflozin etabonat and 40-120µg/ml for of Metformin with a correlation coefficient (r^2) of 0.999. Recovery of Remogliflozin etabonate and Metformin was found to be in the range of 98-102% which confirms the accuracy of the method. The percentage purity of Remogliflozin etabonate and Metformin in pharmaceutical dosage form was found to be 99.87%. The limit of detection and the limit of quantification were found to be 0.75µg/ml and 3.30µg/ml respectively for of Remogliflozin etabonate and 1.56µg/ml and 6.28µg/ml respectively for Metformin. The sensitivity, accuracy, range, precision, robustness, ruggedness, stability, specificity, limit of detection, limit of quantification and system suitability parameters were validated for the developed method as per ICH Guidelines.

Keywords: Remogliflozin etabonate, Metformin, RP-HPLC, sensitivity, linearity, ICH Guidelines.

Introduction

Remogliflozin etabonate (ethyl[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6[5-methyl1--propan-2- yl-4- [(4-propan-2- yloxyphenyl)methyl]pyrazol-3-yl]oxyoxan-2-yl]methyl carbonate) (Fig. 1) (Mudaliar et al., 2012;

Dobbins et al., 2012) is an antidiabetic agent that resulting from complete or relative in insulin excretion and or insulin action. It is prodrug of Remogliflozin, with benzylpyrazole glucoside based inhibitor of renal SGLT2 with antihyperglycemic activity (Sykes et al., 2015).

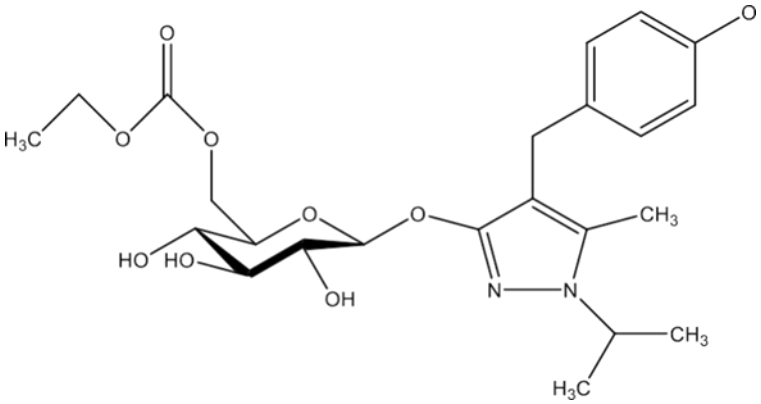

Figure 1. Structure of Remogliflozin etabonate.

Metformin is a first line agent for the treatment of type 2 diabetes that can be used alone or in combination with sulfonylureas, thiazolidinediones, incretin-based drugs, sodium glucose cotransporter-2 inhibitors, or other hypoglycemic agents (Marchesini et al., 2001; Nair et al., 2004; Rena et al., 2017). Metformin has not been linked to serumenzyme elevations duringtherapy and is an exceeding rare cause of idiosyncratic clinically apparent acute liver injury (Madiraju et al., 2018; Lucis, 1983).

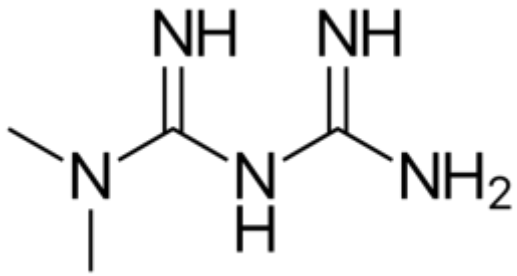

Figure 2. Structure of Metformin

Literature review reveals that few methods are reported for determination of Remogliflozin etabonate and metformin hcl by UV spectroscopy, LC-MS/MS. But.no RP-HPLC method has been reported for stability indicating analytical method and validation for the Estimation Remogliflozin Etabonate and metformin in its API or pharmaceutical dosage form. Therefore, The aim of the present work was to develop stability indicating RP-HPLC Method for the Estimation of Remogliflozin Etabonate and metformin in its dosage forms. Because analytical methods must be validate before use by the pharmaceutical industry, the proposed RP- HPLC detection method was validated in accordance with International conference in Harmonization (ICH) guidelines, (ICH Q2R1, 2005; ICH Q2B, 1996) by assessing its selectivity, linearity, accuracy, and precision, limit of detection and limit of quantification in this method.

Materials and Methods

Chemicals and reagents

Remogliflozin Etabonate and metformin hcl were procured from Glyra Healthcare, Ahmedabad., Gujarat, India. HPLC grade reagents methanol, acetonitrile (Finar, Ahmedabad) were used for study. The entire reagent prepared by carbon dioxide free water and whereas the sample solution prepared in double distilled water for HPLC purpose.

Table 1. Instruments used

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Method development

Preparation of standard solution

Accurately weigh and transfer 10 mg of remogliflozin etabonate and metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol (Swathi et al., 2017).

Further pipette 0.6ml of Remogliflozin etabonate and 0.8ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions (Shweta et al., 2017) of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH-4.2 (80:20 v/v).

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Zorbax C18 (250 mm x 4.6 mm) 5µm Particle size Column was found to be ideal as it gave good peak shape and resolution (Prasanthi et al., 2019) at 1ml/min flow.

Preparation of Buffer and Mobile Phase

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.2 with diluted orthophosphoric acid solution. Filter and sonicate the solution by vacuum filtration and ultra-sonication. Accurately measured 800 ml (80%) of Methanol and 200 ml of Phosphate Buffer (20%) a were mixed and degassed in digital ultrasonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent preparation

The Mobile phase was used as the diluent.

Method validation parameters

System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits (Kafiya et al., 2019).

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Remogliflozin etabonate and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.6ml of Remogliflozin etabonate and 0.8ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Remogliflozin etabonate and Metformin sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.6ml of Sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

Accurately weigh and transfer 10 mg of Remogliflozin etabonate and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) (Ramesh et al., 2016).

From the stock solution prepare the further dilutions to get the concentration levels of 20-100 ppm and 40-120ppm of Remogliflozin etabonate and Metformin respectively. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Accurately weigh and transfer 10 mg of Remogliflozin etabonate and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) (Charde et al., 2014; Balaswami et al., 2018). Further pipette 0.6ml of Remogliflozin etabonate and 0.8ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions (Estella et al., 2011).

Procedure

Day 1, the standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2, the standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

To determine the accuracy, prepare the standard stock solutions at 50%, 100% and 150% levels for the both drugs in combination. Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions (Kishore et al., 2017; Ngwa et al., 2010). Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Remogliflozin etabonate and Metformin and calculate the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results (Sharon et al., 2018).

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected twice and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 75:25, 85:15 instead (80:20), remaining conditions are same. 20µl of the above sample was injected twice and chromatograms were recorded (Madhavi et al., 2018).

Results and Discussion

Method development

Optimized chromatogram condition

Mobile phase : Methanol: Phosphate Buffer pH-4.2 (80:20v/v)

Column : Zorbax C18 (250 mm x 4.6 mm) 5µm Particle size Column

Flow rate : 1 ml/min

Wavelength : 250 nm

Column temp : 32ºC

Injection Volume : 20 µl

Run time : 7 minutes

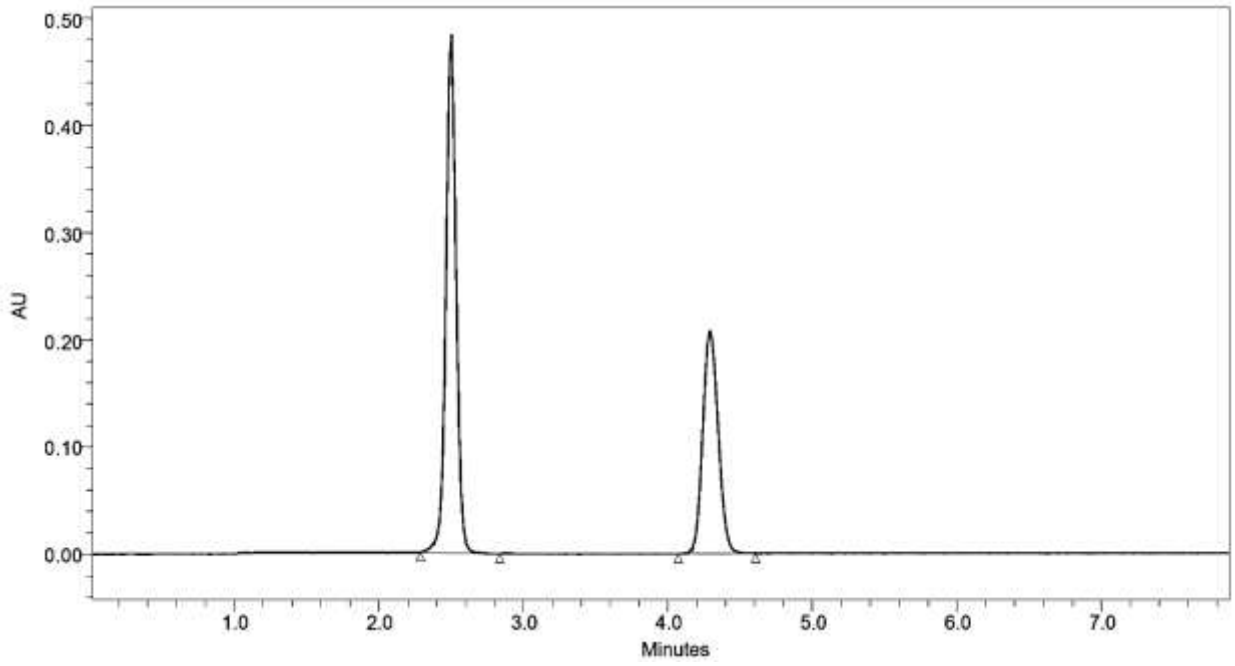

Figure 3. Optimized Chromatogram Condition

Validation of method

All the method validation parameters such as accuracy, linearity, precision, detection limit, quantification limit and robustness were validated as per the International Conference on Harmonization (ICH) guidelines.

System Suitability

To evaluate system suitability parameters such as theoretical plates, tailing factor and retention time of five replicate injections of standard solution of Remogliflozin etabonate and Metformin concentration 60µg/ml and 80µg/ml was used and the % RSD values were calculated (Table 2, 3).

Table 2. Results of system suitability for Remogliflozin etabonate

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin etabonate	2.459	8659784	126654	7542	1.35
2	Remogliflozin etabonate	2.466	8659845	126915	8654	1.35
3	Remogliflozin etabonate	2.472	8652701	125894	9542	1.34
4	Remogliflozin etabonate	2.452	8653682	126602	5632	1.35

5	Remogliflozin etabonate	2.450	8659901	126546	6321	1.34
Mean			**8657259**			
Std. Dev			**3648.01**			
% RSD			**0.042127**			

Table 3. Results of system suitability for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	4.322	422594	50988	7845	1.5	3.2
2	Metformin	4.323	424662	49813	6854	1.5	3.3
3	Metformin	4.342	421841	49826	7521	1.4	3.2
4	Metformin	4.300	415621	51804	6395	1.50	3.2
5	Metformin	4.295	416841	51274	7845	1.49	3.2
Mean			**420029**				
Std. Dev			**724.72**				
% RSD			**0.19**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Remogliflozin etabonate and Metformin in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Remogliflozin etabonate and Metformin in pharmaceutical dosage form was found to be 99.98%.

Linearity

The linearity was analyzed through the standard curves ranging from 20µg/ml to 100µg/ml and 40µg/ml to 120µg/ml respectively (Table 4, 5). The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

Remogliflozin etabonate

Table 4. Chromatographic Data for Linearity

Concentration (µg/ml)	Peak Area
20	2869624
40	5685395
60	8459452
80	11265906
100	13858846

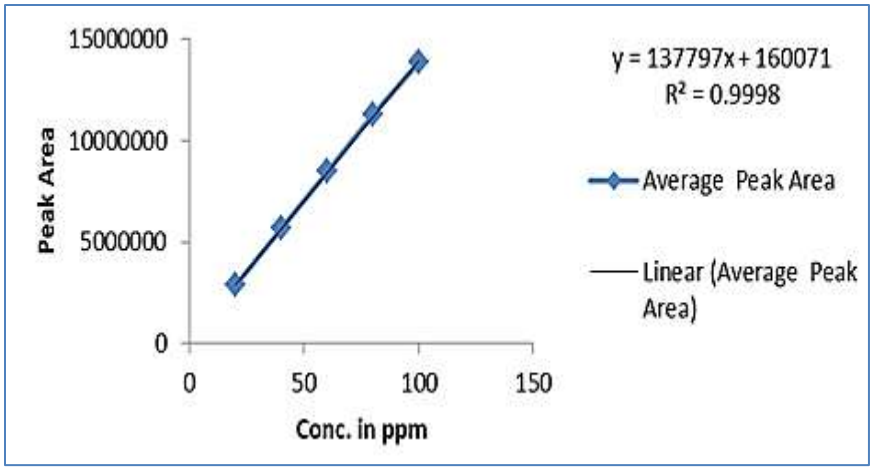

Figure 4. Calibration graph for Remogliflozin etabonate

Metformin

Table 5. Chromatographic Data for Linearity Study

Concentration (μg/ml)	Peak Area
40	265867
60	405698
80	536985
100	685685
120	822568

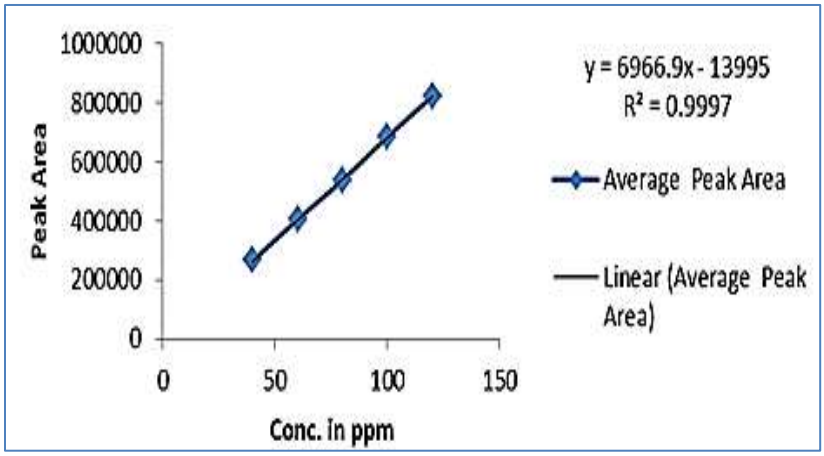

Figure 5. Calibration graph for Metformin

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) of standard solutions. Precision was determined in five replicates of standard solutions. The results were expressed as % RSD of the measurements.

Repeatability

Obtained five replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD (Table 6, 7).

Table 6. Results of Repeatability for Remogliflozin etabonate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin etabonate	2.453	8658785	125698	6359	1.36
2	Remogliflozin etabonate	2.455	8652474	126985	6485	1.35
3	Remogliflozin etabonate	2.453	8659865	126587	6459	1.36
4	Remogliflozin etabonate	2.452	8659328	125498	6359	1.35
5	Remogliflozin etabonate	2.450	8657487	126525	6375	1.36
Mean			**8657588**			
Std. Dev			**2992.003**			
% RSD			**0.034559**			

Table 7. Results of Method Precision for Metformin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	4.289	536985	46985	1.29	8548	5.38
2	Metformin	4.309	534887	46536	1.28	8498	5.39
3	Metformin	4.306	536588	46365	1.29	8426	5.38
4	Metformin	4.300	532642	46359	1.28	8425	5.36
5	Metformin	4.295	536985	46825	1.29	8457	5.38

Mean	**535617.4**
Std. Dev	**1875.447**
% RSD	**0.350147**

Intermediate Precision

Day 1

Table 8. Results of Intermediate precision for Remogliflozin etabonate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin etabonate	2.465	8758685	136528	6452	1.36
2	Remogliflozin etabonate	2.472	8756846	136598	6435	1.38
3	Remogliflozin etabonate	2.467	8769852	135264	6435	1.38
4	Remogliflozin etabonate	2.466	8745985	136582	6582	1.37
5	Remogliflozin etabonate	2.472	8758472	136598	6529	1.36
6	Remogliflozin etabonate	3.424	8759864	136582	6547	1.38

Table 9. Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	4.323	548568	47586	8587	1.29	5.30
2	Metformin	4.343	547854	47568	8569	1.30	5.31
3	Metformin	4.324	542578	47526	8547	1.29	5.31
4	Metformin	4.323	542365	47258	8692	1.29	5.30

5	Metformin	4.342	548752	47895	8567	1.30	5.31
6	Metformin	4.323	542689	47568	8693	1.31	5.30
Mean			**545467.7**				
Std. Dev			**3218.422**				
% RSD			**0.59003**				

Day 2

Table 10. Results of Intermediate precision Day 2 for Remogliflozin etabonate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin etabonate	2.456	8569853	136598	6298	1.38
2	Remogliflozin etabonate	2.457	8579869	135894	6235	1.39
3	Remogliflozin etabonate	2.456	8585865	135876	6198	1.38
4	Remogliflozin etabonate	2.459	8545852	136589	6258	1.39
5	Remogliflozin etabonate	2.467	8549585	135687	6285	1.38
6	Remogliflozin etabonate	2.459	8594872	135698	6295	1.39
Mean			**8570983**			
Std. Dev			**19808.27**			
% RSD			**0.231109**			

Table 11. Results of Intermediate Precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	4.312	526985	458655	8365	1.27	5.27
2	Metformin	4.308	524653	457892	8426	1.28	5.26
3	Metformin	4.312	526538	456825	8396	1.27	5.27
4	Metformin	4.322	526985	458624	8345	1.26	5.26
5	Metformin	4.324	528473	452658	8412	1.26	5.26
6	Metformin	4.322	524865	452315	8452	1.28	5.27
Mean			**526416.5**				
Std. Dev			**1442.735**				
% RSD			**0.274067**				

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated (Table 12, 13).

Table 12. The accuracy results for Remogliflozin etabonate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	493113.3	30	30.004	100.013%	
100%	912300.3	60	60.175	100.291%	100.20%
150%	1330473	90	90.272	100.302%	

Table 13. The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	281726	40	40.298	100.745%	
100%	554209.7	80	79.978	99.972%	100.25%
150%	829292	120	120.036	100.030%	

Limit of Detection and Limit of Quantification

The detection limit and the quantitation limits were processed and were determined. The limit of detection and the limit of quantification were found to be 0.75µg/ml and 3.30µg/ml respectively for of remogliflozin etabonate and 1.56µg/ml and 6.28µg/ml respectively for metformin.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Remogliflozin etabonate and Metformin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of Remogliflozin etabonate and Metformin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count (Table 14, 15).

Table 14. Robustness Test for Remogliflozin etabonate

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	8658972	2.466	6358	1.34
Less Flow rate of 0.9 mL/min	9122485	2.741	6587	1.39
More Flow rate of 1.1 mL/min	8587852	2.270	6152	1.35
Less organic phase	8326585	3.266	6258	1.36
More organic phase	8256854	2.147	6354	1.37

Table 15. Robustness Test for Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	536584	4.323	8476	1.28
Less Flow rate of 0.9 mL/min	612548	4.830	8859	1.30
More Flow rate of 1.1 mL/min	546584	3.979	8622	1.29
Less organic phase	526587	3.266	8854	1.31
More organic phase	512586	2.147	8726	1.28

Stability Studies

The results of the stress studies indicated the specificity of the method that has been developed. Remogliflozin etabonate and Metformin were stable only in photolytic stress conditions and little bit in thermal stress conditions. The results of forced degradation studies are given in the following Table 16.

Table 16. Results of forced degradation studies of Remogliflozin etabonate and Metformin

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid hydrolysis (0.1N HCl)	24Hrs.	93.013	6.987	100.00
Basic hydrolysis (0.IN NaOH)	24Hrs.	71.322	28.678	100.00
Thermal degradation (50 ^{0}C)	24Hrs.	92.104	7.896	100.00
UV (254nm)	24Hrs.	81.231	18.769	100.00
3% Hydrogen peroxide	24Hrs.	67.125	32.875	100.00

Conclusion

The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of Remogliflozin etabonate and Metformin

in bulk as well as in tablet formulation. The proposed study describes HPLC method for the identification and quantification of Remogliflozin etabonate and Metformin. The method was validated and found to be simple, sensitive, rapid, accurate and precise. The developed method was cost effective as compared to the reported methods. The high percentage of recovery shows that the method can be successfully used for routine analysis. Hence the present RP-HPLC method is suitable for the quality control analysis of raw materials, formulation and stability studies.

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