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Original Article

Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Quantification of Remogliflozin Etabonate and Metformin HCl

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ABSTRACT

Aim: This Study aimed to develop a stable, efficient, and reproducible RP-HPLC method that indicates stability, for the simultaneous determination of Remogliflozin Etabonate and Metformin HCl. **Materials and Methods:** The Separation Process By using Methanol: 0.05 M KH_2PO_4 (75:25%v/v) as the mobile phase and a linear gradient protocol with a detection wavelength of 240 nm, the chromatographic separation was accomplished on (Anachrom) Cosmosil C18 (250×4.6 mm, 5 µm) Column. 35°C was the column temperature, and the flow rate was 1 mL per min. For Remogliflozin Etabonate and Metformin HCl, the retention times were determined to be 8.51 and 2.63 min, respectively. Studies on forced deterioration were conducted in thermal, photolytic, oxidative, acidic, basic, oxidizing environments. According to ICH guidelines, the method's robustness, accuracy, precision, linearity, LOD, and specificity were all verified. **Results:** The regression analysis indicated a strong correlation with a linear curve in the concentration ranges 1-5 µg mL^{-1} for Remogliflozin Etabonate and 5-25 µg mL^{-1} for Metformin HCl, demonstrating the linearity of the developed method. Moreover, the approach used in the study was distinctive in that it successfully avoided degradants even after subjecting the dugs to forced degradation. The percentage recovery of REMO and MET from the pharmaceutical dosage form was in the range of 99.33%-99.73% and 99.64%-99.93% respectively. The method was characterized by high accuracy, precision, and robustness, with LOD and LOQ values of 2.218 µg mL^{-1} and 6.724 µg mL^{-1} for Remogliflozin Etabonate and 0.582 µg/mL and 1.764 µg/mL for Metformin HCl, respectively. **Conclusion:** The developed method can be used in routine analysis of bulk and dosage forms due to its adaptability, accuracy, and high precision.

Keywords: Remogliflozin, Etabonate, Metformin HCl, RP-HPLC Method, Validation, Stability, Degradation.

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INTRODUCTION

The third phase of the regulatory submission procedure can include forced degradation, also referred to as stress studies, forced decomposition studies, or stress decomposition studies. For the creation of new drug substances and new drug products, especially when they are subjected to harsh conditions, it is crucial. To find degradants produced and present in the finished drug product, early forced degradation studies are carried out. Additionally, it would enable the manufacturer to choose the Product's expiration life. The main factor that contributes to the degradation of drug compounds and products are hydrolysis, oxidation, heat, and photolysis. This stability method is essential for assessing sample stability and reliability in the pharmaceutical industry.

Figure 1 depicts Remogliflozin Etabonate with an empirical formula $C_{26}H_{38}N_2O_9$. The gliflozin drug family includes it. Type 2 diabetes and non-alcoholic steatohepatitis are the main conditions for which this medication is used. The kidney's ability to reabsorb glucose is mediated by sodium-glucose transport proteins, which are inhibited by remogliflozin.[1] With a molecular weight of 522.6 g/mol, it is a solid powder that melts between 135°-138°C. Solubility of Remogliflozin Etabonate is found in methanol, soluble in water and Acetonitrile, sparingly soluble in Chloroform, and only slightly soluble in acetone.[2]

The molecular name for Metformin HCl is (N,N-dimethylimido dicarbonimidicdiamide), and its empirical formula is $C_{14}H_{11}N_5$ in Figure 1. Metformin HCl reduced blood sugar in type 2 diabetes patients. Metformin, a dimethyl biguanide, lower high blood sugar level by decreasing hepatic glucose production and enhancing the sensitivity of peripheral tissues to insuline[1]. Melting point

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of Metformin HCl showed 220°C-225°C, a molecular weight of 165.5 g/mol. Metformin HCl is soluble in methanol, water, acetone, and only slightly soluble in chloroform.[2]

Remogliflozin Etabonate works by blocking the reabsorption of glucose in the kidneys, while Metformin HCl is used to control high blood sugar levels. This combination is prescribed as an additional treatment to improve glycemic control in patients.[3]

According to a thoroughly review of the literature, analysis of this combination using a variety of techniques, including Spectroscopic techniques, such as UV and Mass spectroscopy, and Chromatographic techniques, such as HPLC and HPTLC, has been reported individually and combined with another drugs.[4-14] Since none study has been found for combined effects of MET and REMO in method development. The goal of the current research is to create an RP-HPLC method that is easy to use, precise, and accurate for the simultaneous estimation of REMO AND MET.

MATERIALS AND METHODS

Chemicals and Reagents

Torrent Pharmaceutical provided the bulk drug Remogliflozin Etabonate, while S. G. Healthcare Pvt. Ltd., provided Metformin HCl as a gift sample. Solvents were procured Finar chemicals, Ahmedabad, while Astron Chemical Ltd., India provided AR grade potassium dihydrogen phosphate. Fresh solutions were prepared every day. The marketed formulation Remogliflozin Etabonate and Metformin HCl Combination tablets (100/500 mg) brand name of Remo-M 500 was procured from the local drug store.

Equipment and Chromatographic Condition

The present assay was carried out on a Shimadzu LC-2010 CHT, Photodiode array detector, autosampler injector and column (Anachrom) Cosmosil C_{18} (250×4.6 mm, 5 µm), respectively.[15] The Lab Solution software was utilized to monitor and integrate the output data. Mobile phase (isocratic) like 0.05 M KH_2PO_4: Methanol (25:75%v/v) was used. Analyte detected at a wavelength of 240 nm (10 min run time).

It was determined that Remogliflozin Etabonate and Metformin HCl had retention times of 8.51 and 2.63 min, respectively, under the ideal chromatographic circumstances.

Method for preparation of analytical solutions

Stock and Standard Solution

Remogliflozin Etabonate and Metformin HCl should be accurately weighed (10 mg). Methanol should be added, and the mixture should be sonicated for 30 min to obtained 100 µg mL^{-1}. The Solution should be labelled as a Standard stock solution. For further dilutions, pipetted out Remogliflozin Etabonate (0.2 mL) and Metformin HCl (1 mL) in flask (10 mL) and obtained concentration 2 µg/mL and 10 µg/mL by adding mobile phase, respectively.

Preparation of Sample Solution

Equivalent amounts of MET (500 mg) and REMO (100 mg) were transferred into a volumetric flask (100 mL) and half the mark was made with Methanol. The solution was sonicated until the drug dissolved and then made up to the mark with Methanol. Thus, the obtained concentration of MET (5000 µg mL^{-1}) and REMO (1000 µg mL^{-1}). 0.02 mL was pipetted out from the sample solution and transferred into a volumetric flask (10 mL), and the volume was made up with mobile phase to obtain a Concentration of 10 µg mL^{-1} for MET and 2 µg mL^{-1} for REMO.

Preparation of Buffer (0.05 M KH_2PO_4)

The Procedure for preparing a 0.05 M KH_2PO_4 Solution is followed as per Indian Pharmacopoeia 2020.[16]

Preparation of Mobile phase

A mixture of 0.05 M KH_2PO_4: Methanol (250:750%v/v) was thoroughly mixed, followed by filtration under vacuum through a 0.45 µ filter.

Selection of Suitable Analytical Wavelength

The blank solution was then tested for absorbance in the 200-400 nm range. Detection of the analytes occurred using a wavelength 240 nm. Both drugs yielded satisfactory results.

Forced Degradation Study

Stock Solution for Metformin HCl (100 µg mL^{-1}): MET (10 mg) were precisely weighed and then moved into volumetric flask (100 mL), followed by dilution with Methanol.

Stock solution I for Remogliflozin Etabonate (100 µg mL^{-1}): REMO (10 mg) were precisely weighed and then moved into a volumetric flask (100 mL), followed by dilution with Methanol.

Standard stock solution II for Remogliflozin Etabonate (20 µg mL^{-1}): 20 mL of Standard Solution I were extracted using a pipette and then transferred into a volumetric flask (100 mL) where it was subsequently diluted with Methanol.

Acid Degradation

In order to achieve a solution containing MET (10 µg/mL) and REMO (2 µg/mL), a 1 mL aliquot was taken from the standard stock solution, which contained MET (100 µg/mL) and REMO II (20 µg/mL). The sample was placed in a volumetric flask (10 mL) and treated with a 2 mL of 0.1 N HCl, after which it was heated at 60°C for 1 hr. After cooling the solution, 2 mL of 0.1 N NaOH was added to neutralize it. Finally, the solution was brought up to a volume of 10 mL using Mobile Phase. The Same Process Repeated

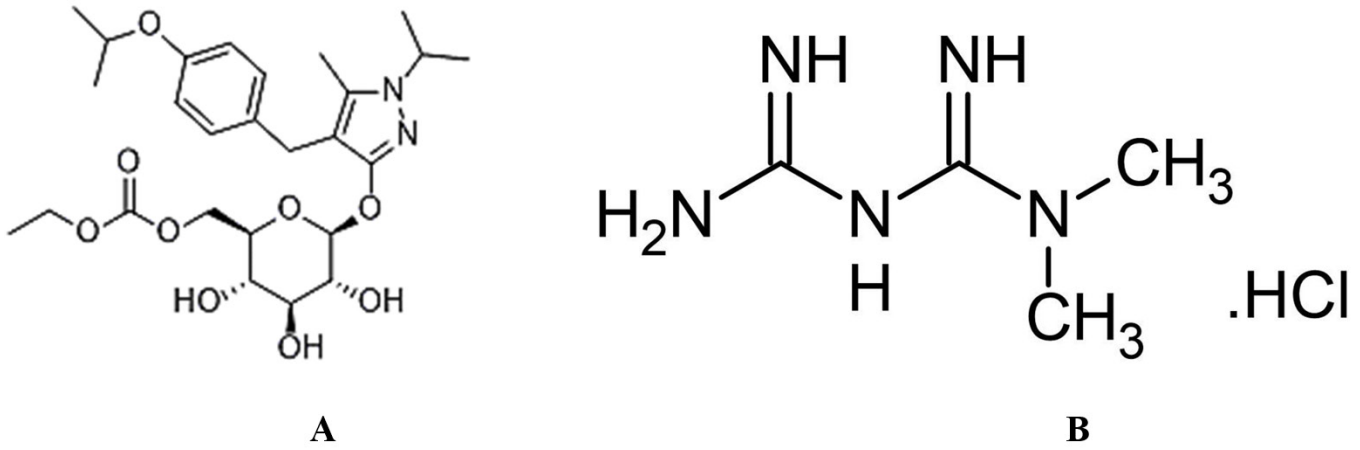

Figure 1: Structure of Remogliflozin Etabonate (A) and Metformin HCl (B).

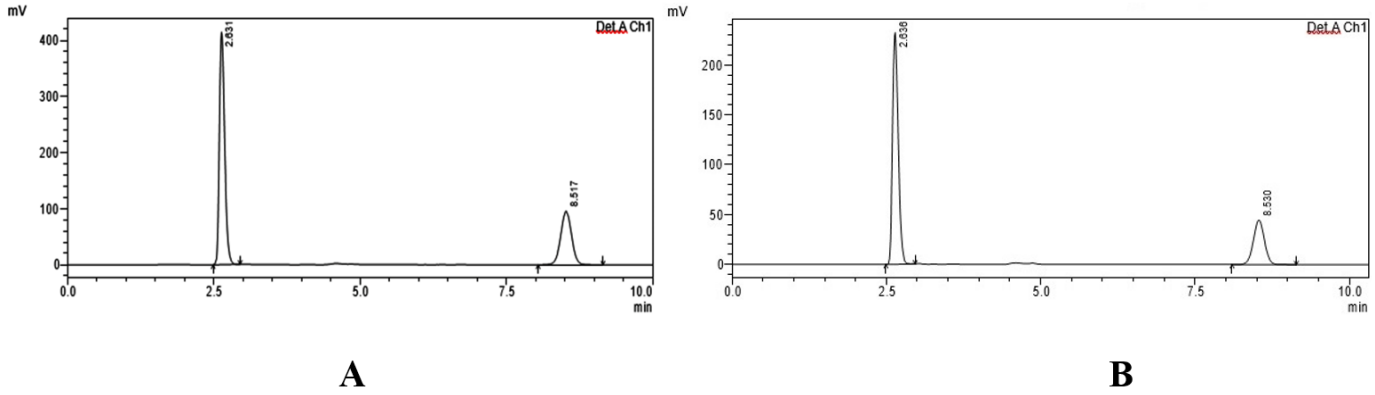

Figure 2: RP- HPLC Chromatogram of Remogliflozin Etabonate (2 µg/mL) and Metformin HCl (10 µg/mL) (A) and Pharmaceutical dosage form (B) in Methanol: Phosphate Buffer (75:25%v/v) Flow rate: 1 mL/min 240 nm.

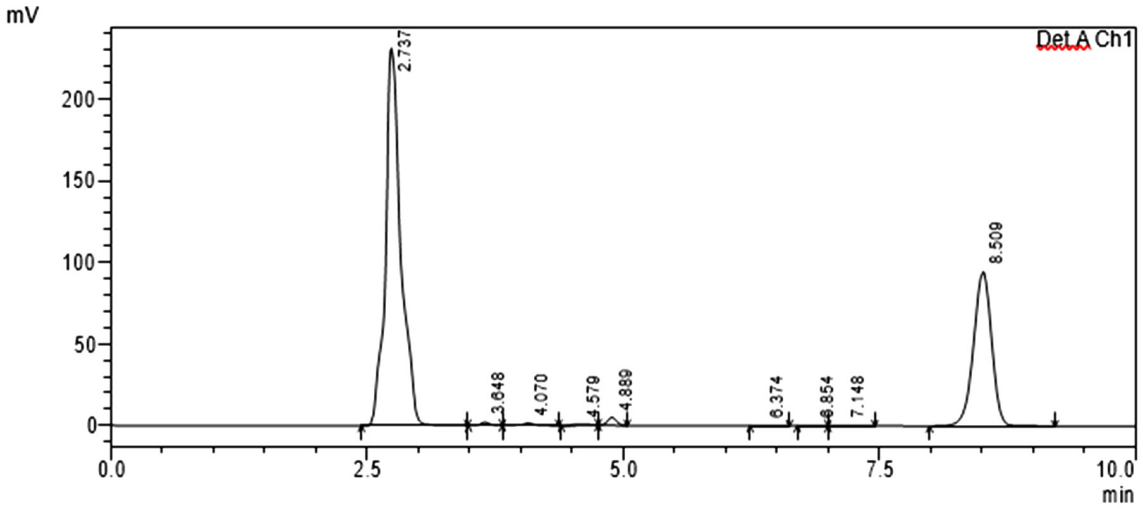

Figure 3: RP-HPLC Chromatogram of Remogliflozin Etabonate (2 µg/mL) and Metformin HCl (10 µg/mL) after in Methanol: Phosphate Buffer (75:25%v/v) Flow rate: 1 mL/min 240 nm 3 hr heating with 0.5 N HCl (Acid Degradation Study).

for 3 hr in 0.1 N HCl, 1 hr and 3 hr in 0.5 N HCl for appropriate degradation.[17]

Base Degradation

In order to achieve a solution containing MET (10 µg/mL) and REMO (2 µg/mL), a 1 mL aliquot was taken from the standard stock solution, which contained MET (100 µg/mL) and REMO II (20 µg/mL). The sample was placed in a volumetric flask (10 mL) and treated with 2 mL of 0.1 N NaOH, then heated at 60°C for 1 hr. After cooling the solution, 2 mL of 0.1 N HCl was added to neutralize it. Finally, the solution was brought up to a volume of

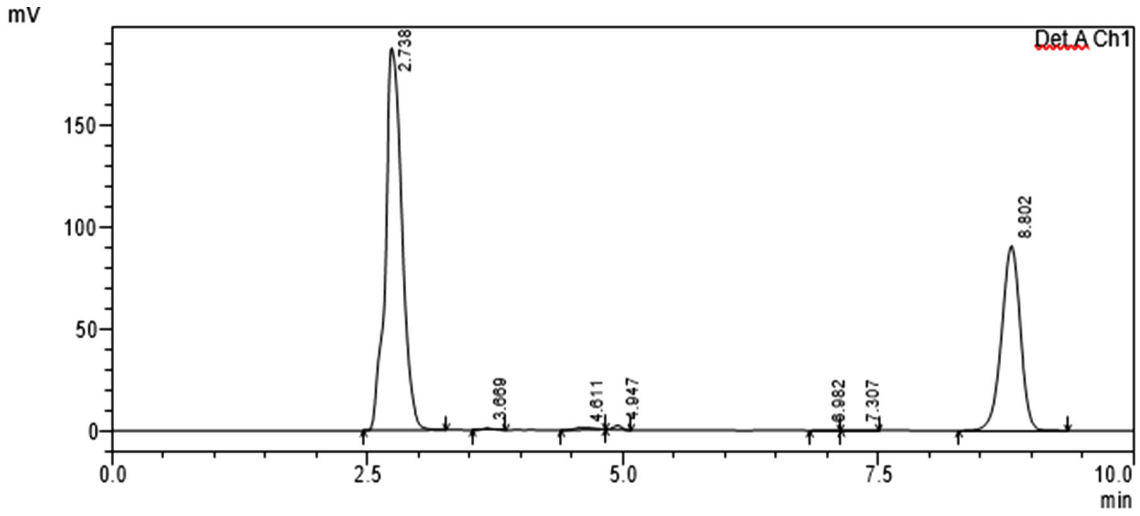

Figure 4: RP-HPLC Chromatogram of Remogliflozin Etabonate (2 µg/mL) and Metformin HCl (10 µg/mL) after in Methanol: Phosphate Buffer (75: 25%v/v) Flow rate: 1 mL/min 240 nm 1 hr heating with 0.5 N NaOH (Alkaline Degradation Study).

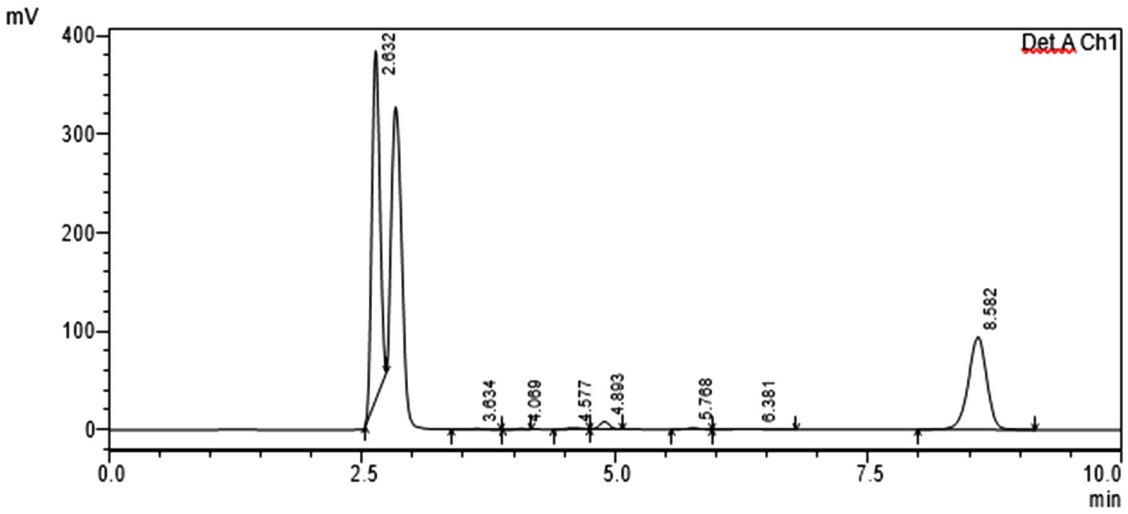

Figure 5: RP-HPLC Chromatogram of Remogliflozin Etabonate (2 µg/mL) and Metformin HCl (10 µg/mL) after in Methanol: Phosphate Buffer (75: 25%v/v) Flow rate: 1 mL/min 240 nm 3 hr 3% H_2O_2 (Oxidative Degradation Study).

10 mL using Mobile Phase. Same Process Repeated for 3 hr in 0.1 N NaOH and 1 hr in 0.5 N NaOH for appropriate degradation.[18]

Oxidative Degradation

A 1 mL solution was obtained from a Stock solution containing MET (100 µg/mL) and REMO II (20 µg mL^{-1}). The 1 mL solution was placed in a volumetric flask (10 mL), followed by the addition of 2 mL 3% H_2O_2. The Solution was sonicated for 5 min, left to keep at room temperature for an hour. After an hour had passed, the solution was made up to 10 mL with Mobile phase. This final solution had concentration MET (10 µg/mL) and REMO (2 µg/mL). Same Process Repeated for 3 hr.[18]

Thermal Degradation (Dry Heat Degradation)

Prepared solution for Thermal degradation study of Metformin HCl

MET was exposed to heat for 1,3 and 5 hr at 80 °C. Precisely measured MET (10 mg) and added into volumetric flask (100 mL) that had been cleaned and dried. Around 75 mL of diluent was added and the solution was sonicated. Then, diluent up to mark on the flask, resulting in a concentration of 100 µg mL^{-1} (Stock I).

Prepared solution for Thermal degradation study of REMO

Precisely measured of REMO (100 mg) and added into volumetric flask (100 mL) that had been cleaned and dried. Approximately 75 mL of diluent was added to the flask, and the solution was

sonicated until the REMO had completely dissolved. The volume was then increased with diluent up to the mark on the flask to get a concentration of 100 µg mL^{-1} (Stock I). Pipette out 2 mL of Stock I was moved into a volumetric flask (10 mL) and then diluted with diluent, resulting in a concentration of 20 µg/mL (Stock II).

Prepared solution for Thermal degradation study of Metformin HCl and Remogliflozin Etabonate

A 1 mL portion of the Stock solution of MET and REMO II was taken and added into volumetric flask (10 mL). The flask was the filled up to mark with diluent to obtain concentrations of MET (10 µg/mL) and REMO (2 µg/mL).[19]

Photolytic Degradation

Prepared solution for Photolytic degradation study of MET

Around MET (10 mg) was placed in a clean and dry petri dish. The dish was then kept in a UV cabinet for 3 and 5 hr continuously. Afterward MET was transferred in Volumetric flask (100 mL) and dilute with diluent, then it was sonicated with intermittent shaking at a controlled temperature until the MET was dissolved. Then used diluents to dilute the volume until it reached the desired 100 µg/mL.

Prepared solution for Photolytic degradation study of REMO

Around Remogliflozin Etabonate (10 mg) was placed in clean and dry petri dish. The dish was then kept in a UV cabinet for 3 and 5 hr continuously. Afterward Remogliflozin Etabonate was transferred in Volumetric flask (100 mL) and dilute with diluent, it was sonicated with intermittent shaking at a controlled temperature until the Remogliflozin Etabonate was dissolved. To obtain a concentration of 100 µg mL^{-1} (Stock I). A pipetted out 2 mL Solution from the above stock solution and moved into volumetric flask (10 mL) and diluted up to the mark to obtained concentration of 20 µg/mL (Stock II).

Prepared solution for Photolytic degradation study of MET and REMO

A 1 mL portion of the Stock solution containing MET and REMO II was added to volumetric flask (10 mL), and the flask was diluted with diluent. This resulted in a concentration Metformin HCl (10 µg mL^{-1}) and Remogliflozin Etabonate 2 µg mL^{-1}.[20]

RP-HPLC method development and validation

The Purpose of this research was to develop new, trustworthy, practical, and affordable technique for the simultaneous estimation of both Drugs using RP-HPLC in Combined dosage form. The method that was established underwent validation for various factors, including system suitability, linearity, precision, detection and quantitation limit, accuracy, assay and robustness.

System Suitability

Six replicates of freshly prepared standard solutions of Remogliflozin Etabonate and Metformin HCl were injected to conduct system suitability tests. Parameters like the theoretical plate, resolution, retention time, and tailing factor were assessed by the standard chromatogram.

Specificity

To check for degradation and interferences, sample solutions of Remogliflozin Etabonate (2 µg/mL) and Metformin HCl (10 µg/mL) were prepared and injected. The analysis of the drugs was verified by checking for interference from Remogliflozin Etabonate and Metformin HCl using a blank chromatogram.[21]

Table 1: System Suitability Parameter.

Name of drugs	Area	Retention time	Tailing factor	No. of Theoretical Plates	Resolution
Metformin HCl	2593994	2.63	1.40	23733.47	3.48
Remogliflozin Etabonate	1227630	8.51	1.03	65180.78	

Table 2: Linearity of Remogliflozin Etabonate and Metformin HCl.

Concentration (µg/mL)		Area ± SD (*n*=6)		% RSD	
Remogliflozin Etabonate	Metformin HCl	Remogliflozin Etabonate	Metformin HCl	Remogliflozin Etabonate	Metformin HCl
1	5	555990.5 ± 8424.04	1514236 ± 18669.1	1.51	1.23
2	10	1222733.0 ± 17027.18	2559018 ± 29379.0	1.39	1.14
3	15	1768988.0 ± 21138.41	3866387 ± 42433.7	1.19	1.09
4	20	2427178.0 ± 24437.93	5054314 ± 44295.4	1.00	0.87
5	25	2971343.0 ± 25478.8	6450658 ± 45362.6	0.85	0.70

Linearity

Remogliflozin Etabonate (100 µg mL^{-1}) stock solution aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 as well as Metformin HCl (100 µg mL^{-1}) stock solution aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 were pipetted out in five separates volumetric flasks (10 mL). These were further diluted with mobile phase [0.05 M KH_2PO_4: Methanol (25:75%v/v)] to obtain different concentrations, such as 1, 2, 3, 4, and 5 µg mL^{-1} for Remogliflozin Etabonate and 5, 10, 15, 20, and 25 µg mL^{-1} for Metformin HCl. The RP-HPLC system's injecting column was used to inject 20 µL of each solution using a Hamilton syringe for analysis. Standard solution calibration curves were plotted against corresponding concentrations using their response ratio.[21,22]

Precision

Intraday, Interday, and Repeatability study has been performed. For Intraday, on same day, REMO solution (1, 2, and 3 µg mL^{-1}) and MET solution (5, 10, and 15 µg mL^{-1}) were analyzed in triplicate. For Interday, three different days, REMO solution (1, 2, and 3 µg mL^{-1}) and MET solution (5, 10, and 15 µg mL^{-1}) were analyzed. For Repeatability, REMO (2 µg mL^{-1}) and MET (10 µg mL^{-1}) were analyzed for six times. The results were expressed as % RSD.[22]

Accuracy

The pre-tested solution was supplemented with a known quantity of Remogliflozin Etabonate and Metformin HCl at three concentration levels, namely 50%, 100%, and 150%. These samples were injected in triplicate at each concentration level into the HPLC system. The average percentage recovery of Both Drugs at each level was computed.[23]

Detection Limit and Quantification Limit

The ICH guidelines provide an equation for calculating the Detection Limit and Quantification Limit.[24]

Robustness

Robustness was tested with the requirement that they should satisfy the system suitability criteria. Optimized method parameters such as Detection wavelength, and flow rate robustness was evaluated.[25]

The RP-HPLC method's durability was assessed thrice by examining sample under different conditions, including variations in flow rate and detection wavelength.

RESULTS AND DISCUSSION

A method using isocratic RP-HPLC has been developed and validated for the simultaneous estimation of Remogliflozin Etabonate and Metformin HCl in both pure form and formulation. The method is characterized by its simplicity, rapidity, accuracy, and precision, and the absorption maximum of both compounds was determined to be 240 nm utilized for the RP-HPLC system. An improved technique was created by adjusting chromatographic parameters like flow rate and detection wavelength. Metformin HCl and Remogliflozin Etabonate and were successfully separated, and a good peak was obtained, by utilizing a mobile phase composed of 0.05 M KH_2PO_4 and Methanol at a ratio of 25:75%v/v with a flow Rate of 1 mL/min. To enhance reproducibility and repeatability, (Anachrom) Cosmosil C_{18} (250×4.6 mm, 5 µm) was utilized as the stationary phase, and the ambient temperature was maintained, with an injection volume 20 µL.

The system suitability was assessed by calculating various parameters, which are presented in Table 1. In Figure 2 Remogliflozin Etabonate and Metformin HCl exhibited 23733.47 and 65180.78 theoretical plates, tailing factors of 1.032 and 1.407, and retention times of 8.51 and 2.63 min, respectively. Since the studied parameters fall within the range of acceptance criteria, the process was considered appropriate, indicating that it is pure

Table 3: Precision Study of Remogliflozin Etabonate.

Intraday Precision of Remogliflozin Etabonate		
Conc. (µg/mL)	**Mean Area ± SD (*n*=3)**	**% RSD**
1	554140 ± 7190.86	1.29
2	1235297 ±12423.1	1.00
3	1784163 ±15867.8	0.88
Interday Precision of Remogliflozin Etabonate		
Conc. (µg/mL)	Mean Area ± SD (***n***=3)	% RSD
1	553873.3 ± 7832.919	1.41
2	1233197 ± 14476.3	1.17
3	1780830 ± 17949.24	1.00
Repeatability of Remogliflozin Etabonate		
Conc. (µg/mL)	Mean Area ± SD (***n***=6)	% RSD
2	1221338 ± 11027.8	0.90

Table 4: Precision Study of Metformin HCl.

Intraday Precision of Metformin HCl		
Conc. (µg/mL)	**Mean Area ± SD (*n*=3)**	**% RSD**
5	1500230 ± 17698.1	1.17
10	2562498 ± 27291.35	1.06
15	3870554 ± 33732.6	0.87
Interday Precision of Metformin HCl		
Conc. (µg/mL)	Mean Area ± SD (***n***=3)	% RSD
5	1500863 ± 18451.01	1.22
10	2560831 ± 28763.97	1.12
15	3873554 ± 38867.36	1.00
Repeatability of Metformin HCl		
Conc. (µg/mL)	Mean Area ± SD (***n***=6)	% RSD
10	2555026 ± 23686.89	0.92

in nature. Therefore, the method is accurate and devoid of any hints of impurities.[26]

Linearity

At various concentration levels of 1-5 µg mL^{-1} and 5 -25 µg mL^{-1} for Remogliflozin Etabonate and Metformin HCl, respectively, the developed method exhibited a linear correlation. Correlation coefficients of REMO was 0.9987 and for MET was 0.998. Table 2 contains the tabulated results.

Precision

The Precision of a Method refers to how closely a series of measurements of the same sample agree with each other. In order to assess the precision, Interday, Intraday, and Repeatability tests were conducted. % RSD for system precision reported in Tables 3 and 4 for Remogliflozin Etabonate and Metformin HCl, respectively. The outcome showed method is reproducible, precise and repeatable as the RSD was less than 2%.

Accuracy

Recovery studies were conducted at 50%, 100%, and 150%. Concentration levels, which Three samples from each concentration level were injected, and the mean percentage recoveries were calculated. The results, which are presented in Table 5, showed that the mean percentage recoveries for Remogliflozin Etabonate and Metformin HCl were in the ranges of 99.33%-99.73% and 99.64%-99.93%, respectively.

The method was deemed accurate as the obtained results fell within the accepted range of 98.0% to 102%. Moreover, the

Table 5: Recovery of Remogliflozin Etabonate and Metformin HCl.

Name of Drug	%Level of Recovery	Test Amount (µg/mL)	Amount of drug taken (µg/mL)	Spiked Std Amount (µg/mL)	Total amount Recovered (µg/mL)	% Recovery±S.D (*n*=3)
Remogliflozin Etabonate	50	2	1	3	2.98	99.33±0.330
	100	2	2	4	3.98	99.58±0.144
	150	2	3	5	4. 98	99.73±0.115
Metformin HCl	50	10	5	15	14.94	99.64±0.330
	100	10	10	20	19.96	99.83±0.076
	150	10	15	25	24.98	99.93±0.023

Table 6: LOD and LOQ for Remogliflozin Etabonate and Metformin HCl.

Parameter	Remogliflozin Etabonate	Metformin HCl
LOD (µg/mL)	2.218	0.582
LOQ (µg/mL)	6.724	1.764

Table 7: Robustness data for Remogliflozin Etabonate and Metformin HCl.

Sl. No.	Parameter	Variation	Area ± S.D (*n*=3)		% RSD	
			Remogliflozin Etabonate	Metformin HCl	Remogliflozin Etabonate	Metformin HCl
1	Flow rate (1 mL/min)	0.9 mL/min	1227635 ± 16027.18	2559145 ± 28379.0	1.30	1.10
2		1.0 mL/min	1227632 ± 15935.48	2559013 ± 29374.0	1.29	1.14
3		1.1 mL/min	1227636 ± 16293.68	2559028 ± 30349.0	1.32	1.18
1	Detection wavelength (230 nm) (± 2 nm)	228 nm	1227532 ± 16142.18	2558998 ± 29879.0	1.31	1.16
2		230 nm	1227629 ± 15674.18	2559088 ± 29379.0	1.27	1.14
3		232 nm	1227467 ± 16727.38	2557988 ± 29654.0	1.36	1.15

Table 8: Analysis of Pharmaceutical Dosage form.

Name of Drug	Amount taken (µg/mL)	amount Found (µg/mL)	%Assay± S.D (*n*=3)	% RSD
Remogliflozin Etabonate	2	1.98	99.16 ± 0.288	0.291
Metformin HCl	10	9.92	99.23 ± 0.251	0.253

Table 9: Summary Data of Degradation Study.

Metformin HCl			**2593994**	
Remogliflozin Etabonate			**1227630**	
Acid Degradation Study				
	Area		**% Degradation of Metformin HCl**	**% Degradation of Remogliflozin Etabonate**
	Metformin HCl	**Remogliflozin Etabonate**		
0.1 N HCl for 1 hr	2556221	1185914	1.45	3.39
0.1 N HCl for 3 hr	2493252	1165999	3.88	5.09
0.5 N HCl for 1 hr	2491742	1137877	3.94	7.31
0.5 N HCl for 3 hr	2419155	1121275	6.74	8.66
Base Degradation Study				
0.1 N NaOH for 1 hr	2121136	1164679	18.22	5.10
0.1 N NaOH for 3 hr	2118917	1154889	18.31	5.90
0.5 N NaOH for 1 hr	1959332	1007540	24.4	17.90
Oxidative Degradation Study				
3% H_2O_2 for 1 hr	2548618	1140124	1.74	7.12
3% H_2O_2 for 3 hr	2323844	1132512	10.41	7.74
Thermal Degradation Study				
After 1 hr	2584654	1185899	0.39	3.39
After 3 hr	2558618	1145927	1.36	6.65
After 5 hr	2519759	1125395	2.80	8.32
Photo Degradation Study				
After 3 hr	2568238	1209642	0.99	1.46
After 5 hr	2538739	1178635	2.10	3.99

positive recovery outcome achieved for the developed method suggests its suitability for routine quality control assays.

Detection Limit and Quantitation Limit

The LOD serves as a parameter for limit testing, which checks whether the analyte concentration falls within the specified limit or not. Conversely, LOQ which establishes the minimum concentration of analyte present in a sample serves as a quantitative assay parameter that is particularly beneficial for detecting impurities or degradation products. Table 6 tabulates LOD and LOQ for REMO, which were determined to be 2.218 µg mL^{-1} and 6.724 µg mL^{-1}, respectively, while for MET, LOD and LOQ were found to be 0.582 µg $mL^{-1}$and 1.764 µg mL^{-1}.

Robustness

Changes were made in the flow rate and detection wavelength, and the corresponding results were summarized in Table 7. The observations indicated that even minor variations in the method conditions did not significantly affect the results, thus confirming the robustness of the method.

Assay

The system recorded chromatograms from three independent injections of the sample solution from the same sample were made into the system. From the resulting % purity values, the % RSD was determined and reported. REMO showed 99.16% and MET showed 99.23%, which exhibited higher recovery (Table 8).[27]

Despite being subjected to stressful conditions, the sample exhibited minimal degradation, and the resulting well-resolved degradants provided satisfactory outcomes. Figures 3-5 display the corresponding chromatograms, while Table 9 summarizes the degradation study results.

CONCLUSION

Ensuring the quality of the Active Pharmaceutical Ingredient (API) is of paramount importance for the safety of patients. Any degradation impurities present in the API could potentially compromise the safety and efficacy of the drug product. To address this concern, RP-HPLC stability indicating method was established in this study.

When impurities from degradation were present, the technique validation revealed that it was linear, precise, accurate, and specific to the drug, while also demonstrating its shorter analysis time greater specificity, and greater accuracy. Based on these finding, the developed RP-HPLC method can be applied for monitoring the quality and detecting the content of Remogliflozin Etabonate and Metformin HCl in pharmaceutical formulations.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

ICH: International Council for Harmonization; **RP-HPLC:** Reverse phase High Performance liquid chromatography; **API:** Active Pharmaceutical Ingredient; **HCl:** Hydrochloric acid; **NaOH:** Sodium Hydroxide; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard deviation; **KH_2PO_4:** Potassium Dihydrogen ortho phosphate buffer; **REMO:** Remogliflozin Etabonate; **MET:** Metformin HCl.

SUMMARY

The RP-HPLC Method developed for Simultaneous Estimation of Remogliflozin Etabonate and Metformin HCl was validated in Compliance with ICH guidelines and was confirmed to be simple and economical in term of mobile phase. The Stability of Remogliflozin Etabonate and Metformin HCl in various stress condition remained unknown so far and no scientific report had reported till today. The evaluated stability of Remogliflozin Etabonate and Metformin HCl under various Forced degradation condition including Acidic, Alkaline, Oxidative, Photolytic, Thermal condition. The method gave linear regression value and simple recoveries in were in good agreement with their label claim. The drug showed no interference in degradation studies. The method can be easily adapted for routine analysis.

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